



ANNUAL REPORT

July 1, 2016 to June 30, 2017

Table of Contents

Introduction	Page 3
Annual Scientific Conference Agenda	Page 9
Institutional Information	Page 37
- Research Summaries	
- Key Personnel	
Project Progress Reports	Page 69
- Project Progress Reports by Institution	
2016-2017 Publications, Manuscripts, & Grants	
- Publications and Manuscripts	Page 221
- Current and Pending Grants	Page 246
Poster Abstracts	Page 278
Institutional Budgets and Justifications	See companion report



Introduction to the Annual Report

Background

The Arizona Alzheimer's Consortium is the nation's leading model of statewide collaboration in Alzheimer's disease (AD) research. It includes more than 150 researchers and staff from seven principal organizations, including Arizona State University, Banner Alzheimer's Institute, Banner Sun Health Research Institute, Barrow Neurological Institute, Mayo Clinic Arizona, the Translational Genomics Research Institute, and the University of Arizona, and from three affiliated organizations, including the Critical Path Institute, Midwestern University, and the University of Arizona College of Medicine, Phoenix. Established in 1998, the Consortium is intended to make a transformational difference in the scientific fight against AD, to engage Arizona's underserved and understudied Native American and Latino communities, to help address the unmet needs of patients and family caregivers, and to advance the understanding and promotion of healthy cognitive aging. The Consortium's major themes are the early detection and prevention of AD. Its primary goal is to find effective AD prevention therapies as soon as possible.

The Consortium is widely recognized as a model of multi-institutional collaboration in biomedical research. It capitalizes on complementary resources and expertise from different disciplines and organizations to address scientific problems in the most impactful way. Its researchers receive critical support from the state of Arizona (through the Arizona Department of Health Services [ADHS] and its Arizona Biomedical Research Commission [ABRC]), the participating organizations, a competitive Arizona AD Center (ADCC) grant from the National Institute on Aging (NIA), and numerous other grants and contracts.

Eric Reiman, MD, is the Director of the Consortium and the NIA-sponsored ADCC, Richard Caselli, MD, is the ADCC's Associate Director, and Carol Barnes, PhD, chairs the Consortium's 26-member Internal Scientific Advisory Committee. Mr. David Jerman is Administrative Director of the Consortium's state- and organizational-supported research program, Mrs. Andrea Schmitt is Administrative Director of its ADCC grant, and Executives from each of the seven principal organizations serve on the Consortium's Board of Directors. The Consortium's external advisors include Drs. Marilyn Albert, Zaven Khachaturian, Bruce Miller, and Thomas Montine, who are internationally recognized for their contributions and leadership roles in the study of AD and/or related disorders. They conduct annual site visits, review the progress and productivity of the Consortium and ADCC, and provide formal feedback and recommendations to the researchers, NIA, and state.

The Arizona Alzheimer's Consortium capitalizes on the state's strengths in brain imaging, genomics, the computational, mathematical and statistical analysis of complex data sets, the basic, cognitive and behavioral neurosciences, clinical and experimental therapeutics, and neuropathology research. It has made pioneering contributions to the scientific understanding, unusually early detection and tracking of AD, the accelerated evaluation of putative AD

prevention therapies, and the scientific understanding of the aging mind and brain. It has introduced new ways for different stakeholders to work together, it has provided data, biological samples and interested research participants for researchers inside the state and around the world, and it has introduced promising new care models for patients and family caregivers. It continues to attract new researchers and clinicians, and support other biomedical research developments in the state. Indeed, it has helped to make Arizona a destination center for the advancement of AD research and care.

State and organizational matching funds continue to provide the “glue” for this geographically distributed research program, the “fuel” needed to launch new research initiatives, and the framework needed to reach the Consortium’s over-arching goals. Funds are used to support dozens of research projects each year, almost all of which involve researchers from different scientific disciplines, and about half of which include researchers from different organizations. As one of our advisors observed, Arizona has become known around the world for its courage, groundbreaking organizational and scientific paradigms, and ability to make things happen in the fight against AD.

The Arizona ADCC has received continuous competitive NIA grant funding since 2001. The ADCC’s Administrative, Clinical, Data Management and Statistics, Neuropathology, and Education and Information Cores and its competitive Pilot Project Program have supported researchers and projects inside and outside of the state. In place of its previous Education and Information Core, the ADCC’s most recent renewal grant includes an Outreach and Recruitment Core and Research Education Component (REC).

ADCC’s most recent competitive renewal grant application to NIA received an Outstanding Impact Score and highly favorable reviewer comments. The Summary Statement noted our statewide programs’ “exceptional” track record, productivity and impact, its “outstanding scientific contributions, regional, national, and international initiatives, and impact,” its “effective” leadership and collaborative model, “impressive” commitments from the state and each of our participating organizations, and its leadership roles in the fight against AD. In July 2016, the ADCC received its fourth consecutive five-year renewal grant.

Productivity and Impact

The Arizona Alzheimer’s Consortium is not only the leading statewide AD Center in the nation, but one of the most productive AD research programs in the world. Since its inception in 1998, its researchers have generated more than 4,000 publications, 1,000 research grants and contracts, and \$1.5 billion in new investments, including more than half of those investments in the last 5 years.

Consortium researchers have made pioneering contributions to the study of AD, related disorders, and the aging mind and brain:

- They have helped clarify genetic and non-genetic risk factors and disease mechanisms, offered targets at which to aim new AD treatments, provided new insights about the pathological changes associated with AD and related disorders, and introduced promising ways to treat and prevent AD. They have also provided invaluable public resources of genetic, neuronal gene expression, longitudinal and neuropathological data and high-quality brain tissue, and introduced new endophenotypic approaches, and data-sharing and collaborative paradigms to assist researchers around the world in these endeavors.

- They have played leadership roles in the early detection and tracking of AD, including the detection and tracking of progressive brain imaging, other biomarkers, and cognitive changes—as well as the detection of neurodevelopmental changes—in cognitively unimpaired persons at genetic risk, and they have provided invaluable resources of data and volunteers from persons at three levels of genetic risk for AD in the Arizona APOE4 Gene Dose Cohort and in Colombian early-onset AD-causing mutation carriers from the world’s largest autosomal dominant AD kindred. They have introduced new experimental paradigms, image-analysis techniques and composite cognitive tests to help in this endeavor. Their work anticipated and helped to advance the conceptualization of preclinical AD, has informed the design of prevention trials in persons at increased genetic and/or biomarker risk, and helped to launch a new era in AD prevention research.
- They continue to clarify how different molecular processes and brain cells, regions, networks, and mental operations orchestrate memory and other thinking abilities, and how they are affected by AD and aging. They have developed, tested and used groundbreaking neuroscientific, experimental and behavioral paradigms to help in these endeavors; and they have played leading roles in the international study of the aging mind and brain.
- They have played leadership roles in the use of brain imaging in the detection, tracking, and scientific study of AD, and they have introduced methods to do so with improved power. They have played leadership roles in the effort to validate amyloid and emerging PET methods in persons at the end of life who subsequently donate their brains, supporting their current or future FDA approval for use in the clinical setting.
- They continue to provide a world-leading resource of longitudinal and neuropathological data, brain and body tissues for the study of AD, Parkinson’s disease, and related disorders in its Brain and Body Donation Program.
- With >\$500M in philanthropic, NIA and industry funding, they established the Alzheimer’s Prevention Initiative (API) to launch a new era in AD prevention research, establish the shared sense of urgency, scientific means, accelerated approval pathway, enrollment resources, public-private partnerships, and vetting mechanisms needed to rapidly test promising prevention therapies in unimpaired persons at genetic or biomarker risk. API includes a Colombian prevention trial of an anti-amyloid treatment in the world’s autosomal dominant AD kindred, an international prevention trial of two other anti-amyloid treatments in persons at highest genetic risk for AD in older persons, and other trials on the way. It includes exceptionally large enrollment registries (e.g., www.endALZnow.org), gene-matching, and genetic risk disclosure and impact assessment programs to help support interest and enrollment in prevention trials; and other emerging methods and strategies to help find and support the approval of an AD prevention therapy as soon as possible. It includes better tests of the amyloid hypothesis (the leading AD theory) than failed trials of anti-amyloid treatments in later stage of the disease, and ground-breaking clinical trial data and sample sharing agreements. These and related efforts have had a profound impact on researchers, policy makers, and other stakeholders around the world.

Consortium researchers continue to develop groundbreaking research methods and strategies, collaborative models and data, and biological sample-sharing paradigms to support these and other research endeavors. They continue to capitalize on their ADCC Cores, other shared resources and collaborations to assist in this effort. Furthermore, they continue to conduct state-

supported collaborative research studies to advance new ideas, find those that have the greatest impact, and generate the findings, publications and grants to have the maximum public impact.

The past two years have been marked by extraordinary progress, productivity and impact. Our researchers helped to secure grants for NIH's primary study of chronic traumatic encephalopathy (CTE) in former professional and college football players; NIH's primary study of AD in persons with Down syndrome (helping to set the stage for future prevention trials in this at risk group); and numerous other grants for the study of AD, related disorders and brain aging. They helped secure one of NIH's first Precision Medicine Initiative (PMI) grants to develop a resource of electronic medical records and related data, sequenced DNA, and biological samples in 150,000 persons from Arizona, including 90,000 Latinos and 7,500 Native Americans over the next five years; this development will introduce new ways in which to advance their inter-related AD, healthy cognitive aging, Latino and Native American research goals. They are now recruiting post-doctoral fellows with support from our statewide collaborative T32 grant to advance research careers in AD and aging research.

The Consortium continues to help support new research recruitments and the growth of AD-related research programs at many of our participating organizations. It helped to recruit Dr. Roberta Brinton to lead a neurodegenerative disease-oriented University of Arizona Center for Innovations in Brain Science. Efforts are underway to recruit a leader and other researchers for the Arizona State University-Banner Neurodegenerative Disease Research Center (NDRC), which is intended to become one of the world's largest basic and translational research programs for the study of AD and related disorders. This Center will leverage the push-pull relationship between use of a) large-scale RNA sequencing data from different brain cells and regions in expired brain donors with and without AD to discover molecular mechanisms, networks, and targets at which to aim new treatments and b) experimental studies in laboratory models to further clarify the nature of their involvement in the development and treatment of AD through data and samples from our researchers' extraordinary research cohorts, and collaborations with researchers from our other organizations.

Our researchers and clinicians continue to provide highly productive outreach and education conferences and services for affected persons, families and professional caregivers, including those in Arizona's underserved Latino and Native American communities. They continue to forge new partnerships to advance the study of AD in those under-represented communities. They also continue to develop, test and deploy new models of dementia care for affected persons and family caregivers, and hope to establish an affordable model of dementia care that more fully addresses both the medical and non-medical needs of affected persons and family caregivers in the emerging population-based healthcare financing system

Our researchers continue to make historic contributions to AD prevention research. As previously noted, they have introduced new trials, developed and begun to use large research enrollment registries, introduced new data sharing policies, and have begun to implement a program to disclose and assess the impact of genetic test results, starting in the era of AD prevention research. The impact of this effort is reflected in recent BBC, NOVA and other documentaries, numerous new articles and two recent *60 Minutes* reports, and growing support from national and international policy makers in the advancement of AD research. It is also reflected by their effort to assemble 84 researchers and federal officials from 72 international institutions in support of a multi-faceted proposal to help find, approve and support affordability and availability of prevention therapies by 2025—and to explore funding mechanisms to support

this effort. They have proposed a prevention trial that, if the treatment works, could provide the chance to find and approve an effective prevention therapy by 2023 and to establish the surrogate endpoints needed to rapidly test and support the approval and availability of prevention therapies in almost everyone who, based on their biological tests or genetic background, is at risk for AD.

During the next few years, the Consortium's organizations and researchers will continue to advance several new scientific and clinical initiatives. We will do everything we can to advance the scientific fight against AD, related disorders and the aging brain, and do so in ways that serve the needs of affected persons and families, including those from our underserved communities. We will try to set a new standard of dementia care for patients and family caregivers by the 2020s and try to find and support the approval of an AD prevention therapy as soon as possible.

In order to find an effective AD prevention therapy by 2025, one of the promising but unproven prevention therapies needs to work. We now have a chance to find out.

We are extremely grateful to the state of Arizona, NIA, our participating organizations, colleagues, collaborators, advisors, research participants, and other supporters. We are proud of our progress and excited about our plans. As we have said before, we are determined to make a transformational difference in the fight against AD, and do so together.

Arizona Alzheimer's Consortium
19th Annual Conference – Thursday May 18, 2017
Mayo Clinic (Host Institution)
Waugh Auditorium
5777 East Mayo Blvd.
Phoenix, AZ 85054

POSTER PRESENTATION SET-UP CONTINENTAL BREAKFAST	7:30 – 8:45AM
WELCOME Richard Caselli, M.D. Professor of Neurology Mayo Clinic Arizona Wyatt W. Decker, M.D. Vice President & Chief Executive Officer Mayo Clinic Arizona	8:45 – 9:00AM
INTRODUCTION Eric M. Reiman, M.D. Director, Arizona Alzheimer's Consortium	9:00 – 9:15AM
LEON THAL MEMORIAL LECTURE "Factors underlying progression in Alzheimer's disease" Bradley T. Hyman, M.D., Ph.D. Director, Massachusetts Alzheimer's Disease Research Center MassGeneral Institute for Neurodegenerative Disease Massachusetts General Hospital	9:15 – 10:30AM
ORAL RESEARCH PRESENTATIONS – SESSION I	10:30 – 11:50AM
POSTER SESSION I & LUNCH	12:00 – 1:00PM
POSTER SESSION II & LUNCH	1:00 – 2:00PM
ORAL RESEARCH PRESENTATIONS – SESSION II	2:00 – 3:30PM
CLOSING REMARKS Eric M. Reiman, M.D.	3:30 – 3:45PM

Arizona Alzheimer's Consortium

Oral Research Presentations

SESSION I Moderator: Heather Bimonte-Nelson, Ph.D.

- 10:30 – 10:42AM **ANK1 is up-regulated in laser captured microglia in Alzheimer's brain; the importance of addressing cellular heterogeneity.** Diego Mastroeni. Arizona State University; Arizona Alzheimer's Consortium.
- 10:43 – 10:55AM **Characterization of astrocytic circular RNAs in late-onset Alzheimer's disease brains.** Shobana Sekar. Translational Genomics Research Institute; Arizona Alzheimer's Consortium.
- 10:56 – 11:08AM **Evidence for necroptosis activation in Alzheimer's disease.** Salvatore Oddo. Arizona State University; Arizona Alzheimer's Consortium.
- 11:09 – 11:21AM **Amyloid-beta increases total tau by mediating Sirtuin 3 in Alzheimer's disease.** Jiong Shi. Barrow Neurological Institute; Arizona Alzheimer's Consortium.
- 11:22 – 11:34AM **Gender differences in Alzheimer's disease: brain atrophy, histopathology burden and cognition.** Geidy E. Serrano. Banner Sun Health Research Institute; Arizona Alzheimer's Consortium.
- 11:35 – 11:47AM **The brain's default network: relevance to aging and Alzheimer's disease.** Jessica Andrews-Hanna. University of Arizona; Arizona Alzheimer's Consortium.

Alzheimer's Consortium
Oral Research Presentations

SESSION II Moderator: Richard Caselli, M.D.

- 2:00 – 2:12PM **Histology informed probabilistic hippocampal atlases of young and old rhesus macaques.** Colin Kyle. University of Arizona; Arizona Alzheimer's Consortium.
- 2:13 – 2:25PM **Anticholinergics and cognitive function in midlife: a cross-sectional study.** Martin Limbaeck-Stokin. Mayo Clinic Arizona, Arizona Alzheimer's Consortium.
- 2:26 – 2:38PM **SSRIs are associated with less amyloid- β plaque deposition in persons with PTSD: preliminary PET findings from ADNI-DOD.** Kewei Chen. Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
- 2:39 – 2:51PM **Detailed retrieval of autobiographical events is impaired in healthy middle-aged and older adult e4 carriers.** Matthew Grilli. University of Arizona; Arizona Alzheimer's Consortium.
- 2:52 – 3:04PM **Blood based protein variant biomarkers for diagnosis of Alzheimer's disease.** Michael Sierks. Arizona State University; Arizona Alzheimer's Consortium.
- 3:05 – 3:17PM **The Alzheimer's Prevention Registry Genematch program.** David Gordon. Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Arizona Alzheimer's Consortium

Poster Presentations

1. **Antemortem-postmortem correlation of florbetapir (18f) PET amyloid imaging with quantitative biochemical measures of A β 40 and A β 42.** Beach TG, Maarouf CL, Intorcchia A, Sue LI, Serrano GE, Roher AE. Banner Sun Health Research Institute; Avid Radiopharmaceuticals; Arizona Alzheimer's Consortium.
2. **Staging Alzheimer's disease-like pathology in 3xTg-AD mice.** Belfiore R, Ferreira ET, Velasquez R, Oddo S. Arizona State University; Arizona Alzheimer's Consortium.
3. **Differential regional alterations of white matter integrity in healthy cognitive aging.** Bharadwaj PK, Nguyen LA, Haws KA, Hishaw GA, Trouard TP, Alexander GE. University of Arizona; Arizona Alzheimer's Consortium.
4. **Age-related cortical thickness differences in adults with autism spectrum disorder.** Braden BB, Riecken C. Arizona State University; Arizona Alzheimer's Consortium.
5. **An fMRI investigation of working memory in older adults with autism spectrum disorder: fronto-hippo-striatal-thalamic network differences.** Braden BB, Smith CJ, Thompson A, Glaspy TK, Wood E, Vatsa D, Baxter LC. Arizona State University; Southwest Autism Research & Resource Center; Barrow Neurological Institute; Arizona Alzheimer's Consortium.
6. **Mechanisms of neuronal loss in Alzheimer's disease.** Branca C, Caccamo A, Piras IS, Ferreira E, Huentelman MJ, Liang WS, Readhead B, Dudley JT, Spangenberg EE, Green KN, Belfiore R, Winslow W, Oddo S. Arizona State University; Translational Genomics Research Institute; Icahn School of Medicine at Mount Sinai; University of California, Irvine; Arizona Alzheimer's Consortium.
7. **Allopregnanolone as a regenerative therapeutic for Alzheimer's disease: Phase 1b/2a update.** Brinton RD, Schneider LS, Law M, Rodgers K, Shi Y, Irwin R, Rogawski M. University of Arizona; University of Southern California; University of California Davis; Arizona Alzheimer's Consortium.
8. **P62 improves AD-like pathology by increasing autophagy.** Caccamo A, Ferreira E, Branca C, Oddo S. Arizona State University; Arizona Alzheimer's Consortium.
9. **Progression from preclinical AD to MCI over a decade: cognitive and brain imaging trajectories.** Caselli RJ, Chen K, Chen Y, Thiyyagura P, Kuang X, Bauer III R, Stonnington CM, Reiman EM. Mayo Clinic Scottsdale; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

10. **Caveats when subtracting two serial measurements to estimate the number of participants needed for clinical trials that are longer or shorter than the observed measurement interval.** Chen K, Xiong C, Harvey D, Guo X, Weiner M, Jagust WJ, Reiman RM. Banner Alzheimer's Institute; University of Arizona; Arizona State University; Arizona Alzheimer's Consortium; Knight Alzheimer's Disease Research Center; Washington University in St. Louis School of Medicine; University of California, Davis; Beijing Normal University; San Francisco Veterans Administration Medical Center; University of California, San Francisco; Lawrence Berkeley National Laboratory; University of California Berkeley; Translational Genomics Research Institute.
11. **Differential pattern of altered gene expression among brain regions in aging and Alzheimer's disease.** Coleman PD, Mastroeni D, Delvaux E, Nolz J, Berchtold N, Cotman C. Arizona State University; Banner Sun Health Research Institute; University of California, Irvine; Arizona Alzheimer's Consortium.
12. **Intervention development for caregivers of people with ADRD and Down syndrome/ID.** Coon DW, Carll P, Goldman J, Montague R, Stotler K. Arizona State University; Arizona Alzheimer's Consortium.
13. **Updates from an innovative, community-level, music-based intervention for people with ADRD.** Coon D, Cortés M, McCarthy M, Rio R, Todd M, Bontrager V, Montague R, Rosas V, Carbajal L, Glinka A, Burlison M. Arizona State University; The Phoenix Symphony; Arizona Alzheimer's Consortium.
14. **Breaking barriers to Latino participation in dementia-related research & services.** Cortés M, Carbajal B, Rosas V, Coon DW. Arizona State University; Arizona Alzheimer's Consortium.
15. **Sensory vs. cognitive components of olfaction: target odor detection training with variable background odors enhances target detection against new background odors.** Daniels CW, Sanabria F, Smith BH. Arizona State University; Arizona Alzheimer's Consortium.
16. **Multivariate analysis of gene expression of peripheral blood leukocytes differentiates persons at risk for Alzheimer's disease from persons not at risk.** Delvaux E, Mastroeni D, Nolz J, Marshall F, Coleman PD. Arizona State University; Banner Sun Health Research Institute; University of Rochester Medical Center; Arizona Alzheimer's Consortium.
17. **Strategic Memory Alzheimer's Rehabilitation Training (SMART): cognitive protection and intervention for amnesic-type mild cognitive impairment (MCI).** DenBoer J, Valla J. SMART Brain Aging, Inc.; Grand Canyon University.
18. **Investigating the differences between ape and human Gfap proteins involved in neurodegeneration.** Eisemann R, Bae NS, Swanson MJ. Midwestern University; Arizona Alzheimer's Consortium.

19. **Identification of protein networks affected by ribosomal s6 kinase activity in the hippocampi of 3xTg-AD, a mouse model of Alzheimer's disease.** Ferreira E, Piras IS, Huentelman M, Dave N, Oddo S. Arizona State University; Translational Genomics Research Institute; Arizona Alzheimer's Consortium.
20. **Gender differences in Alzheimer's disease: brain atrophy, histopathology burden and cognition.** Filon JR, Intorcchia AJ, Sue LI, Vazquez Arreola E, Wilson J, Davis KJ, Sabbagh MN, Belden CM, Caselli RJ, Adler CH, Woodruff BK, Rapsack SZ, Ahern GL, Burke AD, Jacobson S, Shill H, Driver-Dunckley E, Chen K, Reiman EM, Beach TG, Serrano GE. Banner Sun Health Research Institute; Arizona State University; Barrow Neurological Institute; Mayo Clinic Arizona; University of Arizona; Banner Alzheimer Institute; Arizona Alzheimer's Consortium.
21. **Telomere protein Rap1 levels are affected by cellular aging and oxidative stress.** Gallas G, Chia J, Bentz G, Swanson MJ, Bae NS. Midwestern University; Mercer University School of Medicine; Arizona Alzheimer's Consortium.
22. **The safe and effective applications of essential oils in Alzheimer's dementia.** Geiger JL. Banner Desert Medical Center; Arizona Alzheimer's Consortium.
23. **Improved diagnosis of Parkinson's disease from a detailed olfactory phenotype.** Gerkin RC, Adler CH, Hentz JG, Shill HA, Driver-Dunckley E, Mehta SH, Sabbagh MN, Caviness JN, Dugger B, Serrano G, Belden C, Smith BH, Sue LI, Davis KJ, Zamrini E, Beach TG. Arizona State University; Mayo Clinic Scottsdale; Barrow Neurological Institute; Banner Sun Health Research Institute; University of California, San Francisco; Arizona Alzheimer's Consortium.
24. **SSRI use associated with reduced amyloid burden in persons with combat-related PTSD: preliminary findings from ADNI-DOD.** Goradia DD, Chen K, Chen Y, Snyder N, Harvey D, Landau SM, Jagust WJ, Weiner M, Reiman EM. Banner Alzheimer's Institute; Arizona Alzheimer's Consortium; University of Arizona; Arizona State University; University of California, Davis; University of California Berkeley; Lawrence Berkeley National Laboratory; University of California, San Francisco; San Francisco Veterans Administration Medical Center; Translational Genomics Research Institute.
25. **Alzheimer's Prevention Registry: lessons learned in developing a shared resource to the scientific community.** High N, Nichols J, Gordon D, Walsh T, Aggarwal R, Aisen PS, Albert MS, Comer M, Cummings JL, Manly JJ, Petersen RC, Sperling RA, Strobel G, Weiner MW, Reiman EM, Tariot PN, Langbaum JB. Banner Alzheimer's Institute; Provo; University of Southern California; Johns Hopkins University School of Medicine; Geoffrey Beene Foundation Alzheimer's Initiative; Cleveland Clinic Lou Ruvo Center for Brain Health; Columbia University; Mayo Clinic Rochester; Harvard Medical School; Alzforum; University of California San Francisco; Arizona Alzheimer's Consortium.

26. **A survey of microbial DNA present in the brain tissue of individuals with AD and MCI compared to non-demented high pathology and normal controls.** Jentarra G, Chu P, Chavira B, Tullo T, Kaufman J, Jones B, Vallejo J, Jones D, Potter P. Midwestern University; Arizona Alzheimer's Consortium.
27. **Effects of *Candida albicans* infection in 3x-Tg-AD mice.** Jones TB, Vallejo J, Gonzalez F, Kaufman J, Jentarra G, Kerry-Gnazzo A, Potter P, Tullo T, Jones D. Midwestern University; Arizona Alzheimer's Consortium.
28. **Histological tools for detecting infectious agents in neural tissue.** Kaufman JA, Castro MJ, Jones DC, Jones TB, Potter PE, Tullo T, Vallejo J, Jentarra GM. Midwestern University, Arizona Alzheimer's Consortium.
29. **Nuclear, but not mitochondrial encoded OXPHOS genes are altered in aging, mild cognitive impairment, and Alzheimer's disease.** Khdour OM, Delvaux E, Nolz J, Olsen G, Berchtold N, Cotman C, Hecht SM, Coleman PD, Mastroeni D. Arizona State University; Banner Sun Health Research Institute; University of California, Irvine; Arizona Alzheimer's Consortium.
30. **FDG-PET, neuropsychiatric symptoms and the risk of incident mild cognitive impairment.** Krell-Roesch J, Lowe VJ, Pink A, Stokin GB, Roberts RO, Mielke MM, Knopman DS, Christianson TJ, Machulda MM, Jack CR, Petersen RC, Geda YE. Mayo Clinic Scottsdale; Mayo Clinic Rochester; International Clinical Research Center, Brno, Czech Republic; Arizona Alzheimer's Consortium.
31. **Comparison of neuropsychological test scores before and after transition to mild cognitive impairment: a graphical approach.** Langlais BT, Caselli RJ, Dueck AC. Mayo Clinic Arizona; Arizona Alzheimer's Consortium.
32. **Integrative genomics analyses unveil downstream biological effectors of Alzheimer's disease polymorphisms buried in intergenic / non-coding regions.** Li H, Achour I, Bastarache L, Berghout J, Gardeux V, Li J, Lee Y, Pesce L, Yang X, Ramos KS, Foster I, Denny JC, Moore JH, Lussier YA. University of Arizona; University of Illinois at Chicago; University of Chicago; Vanderbilt University; Argonne National Laboratory; Dartmouth College; University of Pennsylvania; Arizona Alzheimer's Consortium.
33. **Age-related changes of structural brain network across the adult lifespan.** Liu K, Yao S, Chen K, Zhang J, Yao L, Guo X. Beijing Normal University; Banner Alzheimer's Institute; Arizona State University; Arizona Alzheimer's Consortium.
34. **Steps to H.O.P.E.: building health, optimism, purpose and endurance in palliative care for family caregivers of persons with dementia.** Long CO, Favaro S, Malek-Ahmadi M, Dougherty J. Capstone Healthcare; Palliative Care Essentials; Arizona State University; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

35. **Synaptosomal levels of phosphorylated alpha synuclein are correlated with, and are synergistic with QEEG in Parkinson's disease.** Lue L, Walker DG, Beach TG, Caviness JN. Arizona State University; Banner Sun Health Research Institute; Mayo Clinic Scottsdale; Arizona Alzheimer's Consortium.
36. **Interaction of cognitive reserve proxy measures with Alzheimer's disease neuropathology impacts episodic memory and executive function.** Malek-Ahmadi M, Lu S, Chan Y, Perez SE, Chen K, Mufson EJ. Banner Alzheimer's Institute; Barrow Neurological Institute; Arizona Alzheimer's Consortium.
37. **Increased 5-hydroxymethylation levels in the sub ventricular zone of the Alzheimer's brain.** Mastroeni D, Chouliaras L, Van den Hove DL, Rutten BPF, Nolz J, Delvaux E, Coleman PD. Arizona State University; Banner Sun Health Research Institute; Maastricht University Medical Centre, Maastricht, The Netherlands; University of Oxford; Arizona Alzheimer's Consortium.
38. **A paradigm shift in microglial expression profiles in human brain.** Mastroeni D, Sekar S, Delvaux E, Nolz J, Liang WS, Coleman PD. Arizona State University; Banner Sun Health Research Institute; Translational Genomics Institute; Arizona Alzheimer's Consortium.
39. **Long-term cyclic + tonic estradiol improves, and cyclic estradiol alone impairs, spatial working memory in ovariectomized middle-aged female rats.** Nishimura K, Koebele SV, Kemmou S, Ortiz JB, Judd JM, Bimonte-Nelson H, Conrad CD. Arizona State University; Arizona Alzheimer's Consortium.
40. **The Mtor/P70s6k pathway plays a key role in the pathogenesis of Alzheimer's disease.** Oddo S, Caccamo A, Branca C, Ferreira E. Arizona State University; Arizona Alzheimer's Consortium.
41. **Effects of a combined transcranial magnetic stimulation (TMS) and cognitive training in Alzheimer patients: results of medical device pivotal multi-center study.** Pascual-Leone A, Sadowsky C, Tousi B, Agronin ME, Alva G, Armon C, Bernick C, Keegan AP, Karantzoulis S. Barrow Neurological Institute; Arizona Alzheimer's Consortium.
42. **Increased expression of *AZGP1* in the middle temporal gyrus of Alzheimer's disease patients.** Piras IS, Krate J, Brokaw D, Delvaux E, Nolz J, De Both MD, Mastroeni D, Beach TG, Huentelman MJ, Coleman PD. Translational Genomics Research Institute; Arizona State University; Banner Sun Health Research Institute; University of Arizona; Arizona Alzheimer's Consortium.
43. **Region- and age-specific effects of APOE4 on murine microglial phenotype.** *Potter RM, *Jones TB, Jentarra G, Vallejo J. Midwestern University; Arizona Alzheimer's Consortium.

44. **Chemical and neuropathological analyses of an Alzheimer's disease patient treated with solanezumab.** Roher AE, Maarouf CL, Kokjohn TA, Belden C, Serrano G, Sabbagh MS, Beach TG. Midwestern University; Barrow Neurological Institute; Banner Sun Health Research Institute; Arizona Alzheimer's Consortium.
45. **Aging with traumatic brain injury: age-at-injury effects on behavioral morbidities and underlying neurovascular pathology.** Rowe RK, Ziebell JM, Morrison H, Vickers J, Lifshitz J. University of Arizona College of Medicine-Phoenix; Barrow Neurological Institute at Phoenix Children's Hospital; Phoenix VA Healthcare System; University of Tasmania; University of Arizona; Arizona Alzheimer's Consortium.
46. **Expectation of large rewards elicits bursts of beta-band oscillations in the aged rat amygdala.** Samson RD, Duarte L, Barnes CA. University of Arizona; Arizona Alzheimer's Consortium.
47. **Using clinical neuropsychological assessments to predict motor rehabilitative responsiveness in non-demented older adults.** Schaefer SY, VanGilder JL, Hengge CR, Duff K. Arizona State University; Creighton University; University of Utah; Arizona Alzheimer's Consortium.
48. **Sensitivity and specificity of the Mayo Sleep Questionnaire in predicting alpha-synuclein pathology.** Shprecher DR, Zhang N, Hentz JG, Dugger BN, Adler CH, Shill HA, Caviness JN, Driver-Dunckley E, Mehta SH, Sabbagh MN, Belden CM, Savica R, Serrano GE, Zamrini E, Sue LI, Beach TG. Banner Sun Health Research Institute; Mayo Clinic College of Medicine; University of California San Francisco; Barrow Neurological Institute; Arizona Alzheimer's Consortium.
49. **Development of advanced multiparametric MRI signatures of Alzheimer's disease.** Stokes AM, Baxter LC, McGee S, Sabbagh MN, Caselli RJ, Quarles CC. Barrow Neurological Institute; Mayo Clinic Scottsdale; Arizona Alzheimer's Consortium.
50. **Development of preclinical MRI biomarkers in mouse models of Alzheimer's disease.** Stokes AM, Velazquez R, Oddo S, Quarles CC. Barrow Neurological Institute; Arizona State University; Arizona Alzheimer's Consortium.
51. **Lorazepam challenge for individuals at varying genetic risk for Alzheimer's disease.** Stonnington CM, Harel B, Locke DEC, Hentz JG, Zhang N, Maruff P, Caselli RJ. Mayo Clinic Arizona; Cogstate, Ltd.; Arizona Alzheimer's Consortium.
52. **Elucidating tau's involvement in learning and memory during adulthood using an inducible tau shRNA.** Velazquez R, Tran A, Oddo S. Arizona State University; Arizona Alzheimer's Consortium.

53. **Characterization of RNA isolated from eighteen different human tissues: results from a rapid human autopsy program.** Walker DG, Whetzel AM, Serrano G, Sue LI, Lue LF, Beach TG. Arizona State University; Banner Sun Health Research Institute; Arizona Alzheimer's Consortium.
54. **Impact of *APOE* genotype on the sex-differentiated bioenergetic trajectories and ad risks in aging mouse brains.** Yin F, Wang Y, Mishra A, Mao J, Brinton RD. University of Arizona; University of Southern California; Arizona Alzheimer's Consortium.
55. **Amyloid-beta increases total tau by mediating Sirtuin 3 in Alzheimer's disease.** Yin J, Han P, Beach TG, Serrano GE, Song M, Nielsen M, Liang WS, Caselli RJ, Shi J. Barrow Neurological Institute; St. Joseph Hospital and Medical Center; Banner Sun Health Research Institute; University of Pennsylvania; Translational Genomics Research Institute; Mayo Clinic Arizona; Tianjin Medical University General Hospital; Arizona Alzheimer's Consortium.
56. **Patch-based sparse coding and multivariate surface morphometry for predicting progression to clinical stages of Alzheimer's disease.** Zhang J, Wang Y, Li Q, Shi J, Bauer III RJ, Reiman EM, Caselli RJ, Chen K, Stonnington CM. Arizona State University; Banner Alzheimer's Institute; Mayo Clinic Arizona; Arizona Alzheimer's Consortium.

STUDENT POSTER PRESENTATIONS

57. **Age-related attentional control and set shifting impairments arise independently in macaque monkeys.** Andersh KM, Gray DT, Smith AC, Burke SN, Gazzaley A, Barnes CA. University of Arizona; University of Florida; University of California, San Francisco; Arizona Alzheimer's Consortium.
58. **Improved associations between reduced cerebral glucose metabolism and elevated A β deposits with the use of a cerebral white matter reference region.** Ausdemore J, Bi R, Kramer H, Jing N, Chen Y, Kuang X, Luo J, Cary Savage, Reiman EM, Chen K. Desert Mountain High School; Brophy College Preparatory; University of California Berkeley; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
59. **An evaluation of the cognitive effects of clinically used combination hormone therapy.** Berns-Leone C, Prakapenka A, Hiroi R, Pena V, Northup-Smith S, Melikian R, Patel S, Ladwig D, Croft C, Bimonte-Nelson H. Arizona State University; Banner Neurological Institute; Arizona Alzheimer's Consortium.
60. **Associations between global and regional cerebral glucose metabolism measurements in normal aging and MCI.** Bi T, Ausdemore J, Jing N, Kramer H, Chen Y, Kuang X, Luo J, Cary Savage, Reiman EM, Chen K. Desert Mountain High School; University of California Berkeley; Brophy College Preparatory; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

61. **Norclozapine reduces beta amyloid1-42 levels and increases chat levels in 3xTg-AD female mice.** Burkart A, Potter P, Jones D. Midwestern University; Arizona Alzheimer's Consortium.
62. **Impact of A β positivity on whole brain atrophy in cognitively unimpaired ϵ 4 heterozygotes and related sample size estimation for clinical trials.** Chan Y, Lu S, Luo J, Malek-Ahmadi M, Cary Savage, Reiman EM, Chen K. Arizona State University; Williams College; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
63. **A novel drosophila model of dementia based on tar DNA binding protein-43 (TDP-43).** Chaung M, Mathieson D, Muñoz E, Kraft R, Zarnescu DC. University of Arizona, Arizona Alzheimer's Consortium.
64. **Effects of short-term oxidative stress on RAP1 expression in human glioblastoma (U251) cells.** Chia J, Gallas G, Bae NS. Midwestern University; Arizona Alzheimer's Consortium.
65. **Activation of neuronal populations in young and aged rat lateral entorhinal cortex during track-running behavior with odors.** Comrie A, Lister JP, Chawla MK, Barnes CA. University of Arizona; University of California Los Angeles; Arizona Alzheimer's Consortium.
66. **Elucidating the involvement of Rbbp7 in Alzheimer's disease.** Dave N, Ferreira E, Piras IS, Huentelman MJ, Oddo S. Arizona State University; Translational Genomics Research Institute; Arizona Alzheimer's Consortium.
67. **Bias correction method improves automatic brain extraction in rodent MR imaging.** Do L, Bharadwaj P, Bernstein A, Xiao J, Alexander GE, Barnes CA, Trouard TP. University of Arizona; Arizona Alzheimer's Consortium.
68. **Reading comprehension neural networks in aging.** Fitzhugh MC, Braden BB, Handley P, Connor D, Sabbagh MN, Caselli RJ, Baxter LC. Arizona State University; Barrow Neurological Institute; Banner Sun Health Research Institute; Mayo Clinic Arizona; Arizona Alzheimer's Consortium.
69. **Relation of physical sport activity to cognitive performance in older adults.** Franchetti MK, Bharadwaj PK, Nguyen LA, Haws KA, Fitzhugh MC, Hishaw GA, Raichlen DA, Alexander GE. University of Arizona; Arizona Alzheimer's Consortium.
70. **Age-related reduction in signal-to-noise ratio of sharp-wave ripple oscillations following behavior in aged rats.** Gray DT, Wiegand J, Schimanski LA, Cowen SL, Barnes CA. University of Arizona; Arizona Alzheimer's Consortium.

71. **Deep brain stimulation and cognitive function among patients with Parkinson's disease: a historical cohort study.** Hansen A, Mehta SH, Krell-Roesch J, Kirlin KA, Velgos SN, Roesler K, Limbaeck-Stokin M, Geda YE. Mayo Clinic Arizona; Arizona Alzheimer's Consortium.
72. **DNA methylation and gene expression pattern analysis for Parkinson's disease blood biomarker discovery.** Henderson-Smith AR, Meechoovet B, Siniard AL, Driver-Dunckley E, Huentelman MJ, Dunckley TL. Translational Genomics Research Institute; Mayo Clinic Scottsdale; Arizona Alzheimer's Consortium.
73. **Prediction of AD progression in patients with mild cognitive impairment using FDG PET biomarkers and neuropsychological assessment.** Jing N, Kramer H, Bi T, Ausdemore J, Chen Y, Kuang X, Luo J, Cary Savage, Reiman EM, Chen K. University of California Berkeley; Brophy College Preparatory; Desert Mountain High School; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
74. **AD-specific cerebral glucose metabolic declines in cognitively normal and MCI individuals prior to clinical progression.** Kramer H, Jing N, Ausdemore J, Bi T, Chen Y, Kuang X, Luo J, Cary Savage, Reiman EM, Chen K. Brophy College Preparatory; University of California Berkeley; Desert Mountain High School; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
75. **Longitudinal changes in cerebral metabolic rate for glucose in progressors to MCI.** Kutchey M, Xie J, Chen Y, Luo J, Cary Savage, Caselli R, Reiman E, and Chen K. University of Arizona; Emory University; Mayo Clinic-Scottsdale; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
76. **Histology informed probabilistic hippocampal atlases of young and old rhesus macaques.** Kyle CT, Bennett JL, Stokes JD, Permenter MR, Vogt JA, Ekstrom AD, Barnes CA. University of Arizona; M.I.N.D. Institute; University of California, Davis; Arizona Alzheimer's Consortium.
77. **Aged rats fail to integrate conflicting spatial reference frames.** Lester AW, Kapellusch AJ, Screen RT, Barnes CA. University of Arizona; Arizona Alzheimer's Consortium.
78. **Using bioengineering approaches to generate a three-dimensional (3-D) human induced pluripotent stem cell (hiPSC)-based model of Alzheimer's disease (AD).** Lundeen R, Bounds L. Arizona State University; Arizona Alzheimer's Consortium.
79. **Effects of APOE ϵ 4 on changes in white matter hyperintensity volume and cognition in older adults.** Matijevic S, Walther K, Ryan L. University of Arizona; University of Erlangen; Arizona Alzheimer's Consortium.

80. **3D Wasserstein distance as a univariate neuroimaging biomarker for FDG-PET analysis.** Mi L, Zhang W, Zhang J, Goradia D, Fan Y, Chen K, Reiman E, Gu X, Wang Y. Arizona State University; Stony Brook University; Banner Sun Health Research Institute; Arizona Alzheimer's Consortium.
81. **Sex differences in metabolic and neurological outcomes in humanized APOE-ε4 knock-in rat model.** Mishra A, Yin F, Mao Z, Brinton RD. University of Southern California; University of Arizona; Arizona Alzheimer's Consortium.
82. **Differential effects of hypertension status and white matter hyperintensity volume on white matter integrity in older adults.** Nguyen LA, Bharadwaj PK, Haws KA, Hishaw GA, Trouard TP, Alexander GE. University of Arizona; Arizona Alzheimer's Consortium.
83. **Contrasting effects of individual versus combined estrogen and progestogen regimens on cognitive function: one plus one does not equal two.** Prakapenka AV, Quihuis AM, Hiroi R, Carson C, Patel S, Croft C, Berns-Leone C, Fox C, Sirianni RW, Bimonte-Nelson HA. Arizona State University; Arizona Alzheimer's Consortium; Barrow Neurological Institute.
84. **Associations between cardiovascular risk factors and cognition in late middle age and older Hispanics compared to non-Hispanic whites.** Ryan L, Stickel A. University of Arizona; Arizona Alzheimer's Consortium.
85. **The impact of family history of AD on white matter tract health.** Ryan L, Stickel A, Gallegos N. University of Arizona; Arizona Alzheimer's Consortium.
86. **Characterization of astrocytic circular RNAs in late-onset Alzheimer's disease brains.** Sekar S, McDonald J, Cuyugan L, Mastroeni D, Liang WS. Translational Genomics Research Institute; Arizona State University; Arizona Alzheimer's Consortium.
87. **Tracking Alzheimer's disease progression by non-linear dimension reduction of brain mri features.** Seo K, Pan R, Chen K. Arizona State University; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
88. **3D-patch analysis based sparse coding system for Alzheimer's clinical group classification.** Srivastava A, Singh S, Zhang J, Mi L, Goradia D, Chen K, Reiman EM, Wang Y. Arizona State University; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
89. **Central insulin resistance precedes peripheral insulin resistance in two mouse models of Alzheimer's disease.** Tran A, Velazquez Ramon, Dave N, Ishimwe E, Denner L, Dineley KT, Oddo S. Arizona State University; University of Texas Medical Branch at Galveston (UTMB); Arizona State University; Arizona Alzheimer's Consortium.

90. **Estrogen regulation of mitochondrial respiration is cell type and ER subtype specific.** Wang Y, Brinton RD. University of Southern California; University of Arizona; Arizona Alzheimer's Consortium.
91. **Multi-task dictionary learning for predicting future cognitive decline.** Zhang J, Li Q, Caselli RJ, Thompson PM, Ye J, Wang Y. Arizona State University; Mayo Clinic Arizona; University of Southern California; University of Michigan; Arizona Alzheimer's Consortium.
92. **Genetic influence of APOE4 genotype on morphometric analysis of hippocampus and lateral ventricle in cognitively intact persons.** Zhang W, Li B, Wu J, Schron EH, McMahon T, Shi J, Gutman BA, Chen K, Baxter LC, Thompson PM, Reiman EM, Caselli RJ, Wang Y. Arizona State University; Wellesley College; University of Southern California; Banner Alzheimer's Institute and Banner Good Samaritan PET Center; Mayo Clinic Arizona; Arizona Alzheimer's Consortium.
93. **Deep learning based classification of PET imaging data for Alzheimer's diagnostic categories.** Singh S, Srivastava A, Mi L, Thompson P, Reiman EM, Chen K, Goradia D, Wang Y. Arizona State University; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.
94. **Uncinate fasciculus integrity assessed in young and aged bonnet macaque monkeys.** Umopathy L, Gray DT, Burke SN, Trouard TP, Barnes CA. University of Arizona; University of Florida; Arizona Alzheimer's Consortium.
95. **Enabling integration of Alzheimer disease (AD) Prevention Study data by application of CDISC standards: extension to biometric monitoring device (BMD) assessments.** Arneric SP, Kern VD, Neville J, Kaye J, Karlin D, Rhodes J, Hill D, Dorsey R, Spencer R, Nelson B, Ibara M, Mohler J, Ryan L, Barnett J, Hudson L. Critical Path Institute; University of Oregon; Pfizer; Biogen; IXICO; University of Rochester; University of Massachusetts; Clinical Data Interchange Consortium(CDISC); University of Arizona; Cambridge Cognition; Arizona Alzheimer's Consortium.
96. **Menopause and the aging brain: relationships among ovarian hormone levels, memory, and choline acetyltransferase-containing neurons in the basal forebrain.** Koebele SV, Mennenga SE, Patel S, Hiroi R, Hewitt LT, Quihuis AM, Mayer LP, Dyer CA, Demers LM, Bimonte-Nelson HA. Arizona State University; Arizona Alzheimer's Consortium; SenesTech, Inc.; The Pennsylvania State University College of Medicine.

2017 Oral Research Presentation

Abstracts

ANK1 IS UP-REGULATED IN LASER CAPTURED MICROGLIA IN ALZHEIMER'S BRAIN; THE IMPORTANCE OF ADDRESSING CELLULAR HETEROGENEITY.

Mastroeni D, Sekar S, Nolz J, Delvaux E, Lunnond K, Mill J, Liang WS, Coleman PD.

Biodesign NDRC Arizona State University; Banner Sun Health Research Institute; Translational Genomics Institute; University of Exeter; King's College London; Arizona Alzheimer's Consortium.

Background: Recent epigenetic association studies have identified a new gene, ANK1, in the pathogenesis of Alzheimer's disease (AD). Although strong associations were observed, brain homogenates were used to generate the data, introducing complications because of the range of cell types analyzed. In order to address the issue of cellular heterogeneity we isolated microglial, astrocytes and neurons by laser capture microdissection from CA1 of hippocampus in the same individuals with a diagnosis of AD and matched control cases. Using this unique RNAseq data set, we show that in the CA1, ANK1 is significantly ($p < 0.0001$) up-regulated 4-fold in AD microglia, but not in neurons or astrocytes from the same individuals.

Methods: In order to investigate disease and regional effects on gene expression we isolated microglia, astrocytes and pyramidal neurons by laser captured micro-dissection from CA1 of hippocampus in AD, normal control cases (NC), followed by RNA sequencing.

Results: ANK1 expression levels are increased in AD microglia, but not in AD Astrocytes or AD neurons from the same individuals. EWAS data shows significant hypermethylation and concordant expression changes of the ANK1 gene in AD cortex, but because homogenates were used no data exist on which classes of cells are associated with these changes. In order to address the effect of cellular heterogeneity, laser-captured microglia, astrocytes and neurons were analyzed in AD CA1 hippocampus. No significant difference in ANK1 expression was found between control neurons and AD neurons ($p > .1$), or control astrocytes and AD astrocytes ($p > .1$). In stark contrast, AD microglia from the same individuals, in the same brain region (CA1 of hippocampus) revealed a significant ($p < 0.01$) 4.1-fold increase in ANK1 expression compared to microglia isolated from control brain samples. These findings suggest that the insignificant change in homogenates between AD and control brain concealed highly significant alterations in ANK1 expression in microglia in AD. In order to determine disease specificity, laser captured CA1 microglia from PD cases were also analyzed. Similar differential expression patterns were identified in PD microglia for two of the eight genes analyzed (ANK1, and ANKLE2). These data show that ANK1 is significantly up-regulated in both AD and PD microglia. These data suggest that alterations in ANK1, at least in microglia, may not be disease specific, but rather a response, or phenotype associated with neurodegeneration.

Conclusions: Recent studies of human brain homogenates have identified hypermethylation of the ANK1 gene in Alzheimer's disease. Unfortunately, the interpretation of these data is obscured since brain homogenates produce data that result from many different classes of cells in many different disease states. Using identified cells we determined that ANK1 expression is up-regulated 4-fold in AD microglia, but not in pyramidal neurons or astrocytes from the same individuals. The role of increased expression of ANK1 in Alzheimer's microglia remains to be determined; but more importantly, these findings emphasize that expression profiling of defined classes of cells is required to clarify data from homogenate studies.

Acknowledgements: We are grateful to Brain and Body Donation Program for the provision of human brain samples. This work was supported by the Arizona Alzheimer's Consortium, New Investigator Research Grant-Alzheimer's Association NIRG-15-321390 and Arizona Department of Health ADHS16-104646 FY2015-16 to D.M.

CHARACTERIZATION OF ASTROCYTIC CIRCULAR RNAs IN LATE-ONSET ALZHEIMER'S DISEASE BRAINS. Sekar S, McDonald J, Cuyugan L, Mastroeni D, Liang WS. Translational Genomics Research Institute; Arizona State University; Arizona Alzheimer's Consortium.

Background: Circular RNAs (circRNAs) are a class of endogenous, non-coding RNAs that form covalently closed continuous loops and are pervasively expressed in the eukaryotic transcriptome. Although circRNAs have been found to possess potential microRNA regulatory roles and are enriched in the mammalian brain, they have not been widely characterized in the context of diseases. Given the previous evidence of astrocyte-specific contributions to Alzheimer's disease (AD), here we aim to characterize astrocytic circRNAs in the context of AD.

Methods: We laser capture microdissected astrocytes from the posterior cingulate (PC; N=10 AD, 10 controls), hippocampus (HIPP; N=6 AD, 6 controls) and substantia nigra (SN; N=6 AD, 6 controls) of clinically classified late-onset AD (LOAD) subjects and no disease (ND) healthy elderly controls. We then performed RNAseq on these samples on an Illumina HiSeq 2000. The raw fastqs generated from sequencing were run through 6 different circRNA prediction algorithms: find_circ, CIRI, DCC, Mapsplice, KNIFE and CIRCexplorer. CircRNAs unique and common to the AD and ND groups in each region were then parsed using custom bash, python and R scripts and annotated using bedtools and UCSC gene annotations.

Results: We sequenced an average of 192,081,648 reads for the PC samples, 54,121,880 reads for the HIPP samples and 62,854,856 reads for the SN samples. In total, 2,375 and 2,380 circRNAs were predicted across all tools in the AD and ND samples respectively. All 6 tools predicted 62 and 73 circRNAs in AD and ND PC samples, 35 and 15 circRNAs in AD and ND HIPP samples, and 15 and 20 circRNAs in AD and ND SN samples respectively. A widely reported circular RNA derived from the CDR1 (cerebellar degeneration-related protein 1) gene was detected across all 3 brain regions. CDR1 is overexpressed in the peripheral blood leukocytes of AD subjects, and the protein was identified in patients with paraneoplastic cerebellar degeneration. We also detected circRNAs generated from genes implicated in AD and other neurological diseases, such as insulin like growth factor 2 receptor (IGF2R), Fas apoptotic inhibitory molecule 2 (FAIM2) and bromodomain PHD finger transcription factor (BPTF). Overexpression of the receptor encoded by IGF2R can lead to an increase in the levels of amyloid precursor protein. FAIM2 confers neuronal protection in mouse models of ischemia and is regulated during the course of bacterial meningitis. In the brains of AD patients, the BPTF protein is localized in a subset of amyloid-containing plaques and its expression was found to be higher in neurodegenerative diseases.

Conclusions: In this study, we evaluated the abundance of astrocytic circRNAs in LOAD samples and healthy controls in 3 different brain regions, and demonstrate the feasibility of performing circRNA detection in whole transcriptome data using bioinformatics algorithms. Though the relevance of circRNAs in the context of AD is not well understood, we observe that certain key genes give rise to circRNAs to suggest that they could have a potential role in various cellular processes in AD. However, further functional studies are required to elucidate the role of circRNAs in AD pathogenicity and their relevance in other neurodevelopmental diseases.

EVIDENCE FOR NECROPTOSIS ACTIVATION IN ALZHEIMER'S DISEASE.

Caccamo A#, Branca C#, Piras IS, Ferreira E, Huentelman MJ, Liang WS, Readhead B, Dudley JT, Spangenberg EE, Green KN, Belfiore R, Winslow W, Oddo S. Arizona State University; Translational Genomics Research Institute; Icahn School of Medicine at Mount Sinai; University of California, Irvine; Arizona Alzheimer's Consortium. #These authors contributed equally.

Objectives: Neuronal loss is a cardinal feature of AD and invariably affects multiple brain regions. Despite this indisputable evidence, the precise mechanism by which neurons die is still unknown. Identifying the mechanisms leading to neuronal loss in AD is fundamental for the development of an efficient therapeutic strategy to treat or slow down the progression of AD.

Methods: We use postmortem human brains from multiple cohorts, two animal models of AD, primary neuronal cultures, and employed complementary genetic, histological, pharmacological, and behavioral approaches.

Results: We provide compelling evidence indicating that necroptosis contributes to neuronal loss in AD. To this end, necroptosis activation inversely correlates with brain weight and cognitive scores. Notably, in a gene regulatory network built from post-mortem brain tissue, the set of genes regulated by RIPK1, a key protein involved in necroptosis activation, overlap significantly with multiple, independent AD transcriptomic signatures, indicating that RIPK1 activity could explain a significant portion of described transcriptomic changes in AD.

Conclusions: We provide the first direct evidence of necroptosis activation in AD and provide a new avenue of drug development for this disorder. Given that the exact mechanisms of AD pathogenesis are unknown, and given the growing appreciation for the fact that multiple causes of the disease are extremely likely, targeting the mechanisms of neurodegeneration is critical as it may have beneficial effects independently of the initial events that trigger the onset of the disease. Alternatively, targeting necroptosis could be used in concomitance with other therapeutics that aim at blocking the neurotoxic insult. Our studies open new venues of research and interventions for this insidious disorder, which affects more than 40 million people worldwide. From a basic biology perspective, it will be fundamental to dissect the mechanisms underlying necroptosis induction in AD; such studies may uncover new and critical knowledge into the pathogenesis of this disorder. From a therapeutic perspective, these data strongly suggest that reducing necroptosis may be a valid therapeutic target for AD.

AMYLOID-BETA INCREASES TOTAL TAU BY MEDIATING SIRTUIN 3 IN ALZHEIMER'S DISEASE. Yin J, Han P, Beach TG, Serrano GE, Song M, Nielsen M, Liang WS, Caselli RJ, Shi J. Barrow Neurological Institute; St. Joseph Hospital and Medical Center; Banner Sun Health Research Institute; University of Pennsylvania; Translational Genomics Research Institute; Mayo Clinic Arizona; Tianjin Medical University General Hospital; Arizona Alzheimer's Consortium.

Background: Increasing evidence indicates that Sirtuin 3 (Sirt3) has neuroprotective effects in regulating oxidative stress and energy metabolism, both of which are involved in the pathogenesis of Alzheimer's disease (AD). However, it is unclear whether Sirt3 is associated with cognitive performance and pathological changes in AD.

Methods: We conducted a case-control study of the brains of late-onset AD, mild cognitive impairment and age matched cognitively normal (CN) subjects. We investigated Sirt3 expression, its association with cognitive performance and AD pathology.

Results: Sirt3 levels were reduced in the entorhinal cortex, the middle temporal gyrus and the superior frontal gyrus of AD subjects compared to CN. This reduction was associated with poorer cognitive test scores and the severity of tau pathology. Further study revealed that amyloid-beta increased total Tau protein expression via regulating Sirt3.

Conclusions: These data suggest that down-regulation of Sirt3 is critically involved in pathogenesis of AD.

GENDER DIFFERENCES IN ALZHEIMER'S DISEASE: BRAIN ATROPHY, HISTOPATHOLOGY BURDEN AND COGNITION. Filon JR, Intorcia AJ, Sue LI, Vazquez Arreola E, Wilson J, Davis KJ, Sabbagh MN, Belden CM, Caselli RJ, Adler CH, Woodruff BK, Rapsack SZ, Ahern GL, Burke AD, Jacobson S, Shill H, Driver-Dunckley E, Chen K, Reiman EM, Beach TG, Serrano GE. Banner Sun Health Research Institute; Arizona State University; Barrow Neurological Institute; Mayo Clinic Arizona; University of Arizona; Banner Alzheimer Institute; Arizona Alzheimer's Consortium.

Background: Multiple studies have suggested that females are affected by Alzheimer's disease (AD) more severely and more frequently than males. Other studies have failed to confirm this and there has been no clear resolution of the issue. Difficulties include differences in study methods and male versus female life expectancy. Another element of uncertainty is that the majority of studies lack neuropathological confirmation of diagnosis.

Methods: We compared clinical and pathological AD severity in more than 1000 deceased subjects with full neuropathological examinations.

Results: Age of dementia onset did not differ by gender but females were more likely to proceed to very severe disease, both clinically and pathologically, with significantly higher proportions of females having a Mini Mental State Examination score of 5 or less and Braak stage VI neurofibrillary degeneration. Median neuritic plaque densities were similar in AD females and males but females had significantly greater tangle density scores. In addition, we found that AD-control brain weight differences were significantly greater for females, even after adjustment for age, disease duration and comorbid conditions.

Conclusions: These suggest that, when affected by AD, females progress more often to severe cognitive dysfunction, due to more severe neurofibrillary degeneration and greater loss of brain parenchyma.

THE BRAIN'S DEFAULT NETWORK: RELEVANCE TO AGING AND ALZHEIMER'S DISEASE. Andrews-Hanna J, Gardiner C, Bryan A. University of Arizona; University of Colorado Boulder; Arizona Alzheimer's Consortium.

One of the brain's most metabolically active, yet least understood, large-scale networks is the brain's "default network" (DN). Recent years have brought growing interest in the DN, in part because resting state functional connectivity is now a widely used method in functional neuroimaging. The DN has also garnered interest from clinical neuroscience because the network is affected across a range of clinical populations, including Alzheimer's disease. In this talk, I will provide an overview of the anatomy and function of the default network – including its role in memory and spontaneous cognition – and discuss the its relevance to normal and pathological aging.

HISTOLOGY INFORMED PROBABILISTIC HIPPOCAMPAL ATLASES OF YOUNG AND OLD RHESUS MACAQUES. Kyle CT, Bennett JL, Stokes JD, Permenter MR, Vogt JA, Ekstrom AD, Barnes CA. University of Arizona; M.I.N.D. Institute; University of California, Davis; Arizona Alzheimer's Consortium.

Background: Identifying primate hippocampal subfields in vivo using structural MRI imaging relies on variable anatomical guidelines, signal intensity differences, and heuristics to differentiate between regions, and lack a clear anatomically-driven basis for subfield demarcation (Yushkevich et al., 2015).

Methods: Recent work, however, has begun to develop methods to use ex vivo histology or MRI to better inform subfield demarcations of in vivo images (Iglesias et al., 2015, Adler et al., 2014). For optimal results, though, ex vivo and in vivo images should be matched to the same subjects, with the goal to develop a neuroanatomically-driven basis for in vivo structural MRI images.

Results: Here, we address this issue in young and aging rhesus macaques (young n=2 and old n=2) using ex vivo Nissl-stained sections in which we identified the dentate gyrus, CA3, CA2, CA1, subiculum, presubiculum, and parasubiculum using morphological cell properties (30 μ m thick sections spaced at 240 μ m intervals and imaged at 161 nm/pixel). These were merged with in vivo structural MRIs (0.625 x 0.625 x 1 mm) from the same subjects via iterative rigid and diffeomorphic registration resulting in probabilistic atlases of young and old rhesus macaques.

Conclusions: These methods will inform subfield differentiation by identifying features of the MRI images that correspond to histological properties in the same animals, useful for work in both young and aging primates. Furthermore, we believe that this approach may be helpful in developing a phylogenetically-driven “ground truth” for more accurate identification of hippocampal subregions in human brains.

ANTICHOLINERGICS AND COGNITIVE FUNCTION IN MIDLIFE: A CROSS-SECTIONAL STUDY. Limbaeck-Stokin M, Krell-Roesch J, Hansen A, Roesler K, Temkit M, Caselli RJ, Geda YE. Mayo Clinic Arizona, Arizona Alzheimer's Consortium.

Background: Anticholinergic medications are associated with impaired memory and executive dysfunction in persons aged > 70 years. Our aim was to 1) investigate the association between anticholinergics and cognitive function in midlife; and 2) to further examine whether cognitively normal middle aged APOE e4 carriers might be differentially impacted by anticholinergic use.

Methods: We conducted a cross-sectional study derived from the Arizona APOE cohort and the Arizona Alzheimer's Disease Center cohorts at Mayo Clinic, Scottsdale, Arizona. All study participants were aged > 50 years and underwent an extensive neuropsychological test battery assessing four cognitive domains: memory, executive function, language, and visuospatial function. We used Anticholinergic Burden Scale to dichotomize the cohort in to individuals taking anticholinergics (AC+) versus not taking anticholinergics (AC-). Each participant in the AC+ group was matched by sex, age (+/- 5 years) and years of education (+/- 2 years) to participants in the AC- group (ratio of 1:4). We conducted t-tests, Wilcoxon-Rank-Sum tests, correlation tests and multiple regression analyses.

Results: 255 participants free of Alzheimer's Disease or any other form of dementia or MCI were included in the final analysis (51 AC+, 204 AC-). There were no significant differences between the two groups in age, education, sex or APOE e4 status. The AC+ group performed worse in all memory and executive function tests, except for Auditory Verbal Learning (AVLT) and Selective Reminding Test. Correlation analyses revealed none or weak correlations between cognitive test scores and anticholinergic burden scores; the strongest correlation was between AVLT and anticholinergic burden score ($R = -0.24$ and $R_s = -0.27$). In all memory and executive function tests, anticholinergic burden status did not have an effect on the multiple regression model ($p > 0.05$). Lastly, the regression model including APOE allele status showed that the effect of APOE status and the interaction between APOE status and anticholinergic burden on the model was not significant.

Conclusions: Anticholinergics are associated with cognitive impairment in late-life. However, our cross-sectional study showed that in midlife there was no significant association between anticholinergic burden and cognitive function. The findings remained the same when the data were stratified by APOEe4 status.

SSRIS ARE ASSOCIATED WITH LESS AMYLOID-B PLAQUE DEPOSITION IN PERSONS WITH PTSD: PRELIMINARY PET FINDINGS FROM ADNI-DOD. Chen K, Goradia D, Luo J, Thiyyagura P, Devadas V, Chen Y, Bauer R, Sheline Y, Jagust W, Landau S, Weiner MW, Reiman EM. Banner Alzheimer's Institute; Arizona State University; University of Arizona; Translational Genomics Research Institute; University of Pennsylvania; University of California, San Francisco; University of California, Berkeley; Arizona Alzheimer's Consortium.

Background: Florbetapir PET images were used to compare cerebral amyloid burden, calculated as standard uptake value ratios (SUVRs), in ADNI-DOD study participants with post-traumatic stress disorder (PTSD) against normal controls (NC) without PTSD. Since PTSD participants had suggestive evidence of lower SUVRs than NC, since antidepressants especially selective serotonin-reuptake inhibitors (SSRI) are commonly used in persons with PTSD, and since SSRIs have been shown to be associated with cerebrospinal fluid and PET evidence of lower amyloid accumulation (Cirrito, 2011; Sheline, 2014), post-hoc analyses were performed to investigate the possibility that these findings are attributable to SSRI use.

Methods: Mean cerebral-to-cerebellar SUVRs, an amyloid positive threshold of 1.18 consistent with moderate-to-severe amyloid plaques (Fleisher, 2011), and statistical brain maps (SPM) were used to compare florbetapir PET scans from 52 male participants with PTSD (68±4 years old) and 52 male controls (71±6 years old). Additional analysis was performed to determine whether regional SUVR differences are attributable to antidepressant use. Excluding other antidepressants, we then compared SUVR differences between 16 PTSD participants who were treated with SSRI (68.6±4.5 years) and 27 PTSD participants who were not (67.7±3.7 years). Based on the observed spatial pattern characteristics of lower SUVR in PTSD than in NC, we used an omnibus approach, free of multiple comparisons, to examine if the number of voxels with lower SUVR in the SSRI treated PTSD participants is higher than the number of voxels in the opposite direction (lower SUVR in the non-treated PTSD participants) via 1000 Monte Carlo simulations.

Results: The PTSD group had a significantly lower percentage of amyloid positive individuals compared to controls (PTSD: 3.8%, NC: 19.2%; $P=0.01$) though PTSD and control groups did not differ in their mean cerebral SUVRs after correcting for age and education ($P=0.18$). Additionally, brain maps revealed significantly lower regional SUVRs in the PTSD group, primarily in the frontal cortex ($P<0.005$, corrected for age and education, uncorrected for multiple comparisons). SUVR differences between PTSD patients and controls in the superior medial frontal region were no longer apparent after correction for antidepressant use ($P=0.10$). Comparison of SSRI users and non-users with PTSD revealed a lower percentage of amyloid positive users, though not statistically significant (users: 0%, non-users: 5.4%; $P=1.00$). Moreover, SSRI users had 55 times more cerebral voxels with lower than higher mean SUVR (13536 versus 245 voxels, type-I error <0.001 based on 1000 Monte-Carlo simulations) with or without controlling for effects of age, education, presence or absence of APOE4 allele, MMSE scores and depression ratings.

Conclusions: While preliminary, our findings suggest that adults with PTSD have less fibrillar amyloid and that this reduction may be at least partly attributable to SSRI use. They also support the possibility that SSRIs are associated with reduced amyloid plaque burden in PTSD.

DETAILED RETRIEVAL OF AUTOBIOGRAPHICAL EVENTS IS IMPAIRED IN HEALTHY MIDDLE-AGED AND OLDER ADULT E4 CARRIERS. Grilli MD, Wank AA, Bercel JJ, Ryan L. University of Arizona; Arizona Alzheimer's Consortium.

Cognitive neuroscience has established that the retrieval of autobiographical (i.e., personal) events recruits a neural network that overlaps with regions affected early in Alzheimer's disease (AD), including the medial temporal lobe (MTL) and prefrontal and posterior cortical regions. However, it is not known whether impaired retrieval of autobiographical events might be a preclinical sign of AD. In this study, we investigated autobiographical event retrieval in seemingly healthy middle-aged and older adult carriers of the e4 allele of the APOE gene, and we compared their performance to non-e4 carriers who were matched on age, education, IQ, and performance on standard neuropsychological tests of memory and cognition. Participants verbally recalled autobiographical events in as much detail as possible. Their responses were scored for episodic details (i.e., details unique to these specific events) and semantic details (i.e., other content not unique to the specific events). The results revealed that the e4 carriers were impaired in the ability to retrieve episodic details but not semantic details. This impairment was exhibited across multiple types of episodic detail and was not attenuated by cueing. These findings indicate that the ability to retrieve autobiographical events that are rich in episodic detail is impaired in many seemingly healthy e4 carriers. Aspects of memory that tax the functional limits of the MTL network, such as autobiographical event retrieval, may be particularly sensitive measures of abnormal cognition in adults at increased genetic risk of AD.

Supported by: Arizona Alzheimer's Consortium P30 AG #019610

BLOOD BASED PROTEIN VARIANT BIOMARKERS FOR DIAGNOSIS OF ALZHEIMER'S DISEASE. Sierks M. Arizona State University; Arizona Alzheimer's Consortium.

Oligomeric forms of beta-amyloid, tau, and TDP-43 play important roles in Alzheimer's disease (AD) and other neurodegenerative disorders. Therefore presence of disease specific variants of these proteins are promising biomarkers for these diseases. We have previously generated antibody based reagents that selectively bind disease related variants of beta-amyloid, tau and TPD-43. Analysis of human pathologically verified post-mortem tissue, CSF and sera samples from AD, PD and healthy control cases showed that we could readily distinguish AD tissue, CSF and sera samples by detecting the presence of beta-amyloid variants. We then analyzed a small set longitudinal human AD plasma samples from four AD and two control cases where the samples included time points taken prior to initial diagnosis of MCI as well as time points after conversion to AD. As observed with the post-mortem samples, the cognitively normal control samples did not show reactivity with any of the reagents against the beta-amyloid, tau or TDP-43 variants. In contrast, all the samples taken from patients that eventually converted to AD showed the presence of multiple biomarkers at every time point, even samples taken years before initial diagnosis of MCI. The biomarker profile showed distinct patterns as the samples progressed from pre-MCI, to initial diagnosis of MCI, to conversion to AD. These results suggest that disease related protein variants have great promise as blood based biomarkers for diagnosing and staging AD.

THE ALZHEIMER'S PREVENTION REGISTRY GENEMATCH PROGRAM. Gordon D, Walsh T, High N, Langlois C, Nichols J, Reiman EM, Tariot PN, Langbaum JB. Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: The Alzheimer's Prevention Initiative (API) is a collaborative funded by the NIH, philanthropy, and industry to conduct preclinical Alzheimer's disease (AD) trials in people who, based on age and genetics, are at elevated risk of developing AD symptoms. Given that enrollment is one of the biggest obstacles faced by research studies, there is a need to establish a registry database that includes genetic information in order to more efficiently match interested individuals to studies. The API Generation Study is currently enrolling apolipoprotein E (APOE) ϵ 4 homozygotes age 60-75. API established a trial-independent, internet-based APOE genetic testing program, known as GeneMatch, to enrich referrals to the API Generation Study while also serving as the basis for an enduring recruitment infrastructure for the API program. GeneMatch aims to enroll tens of thousands of participants.

Methods: GeneMatch is a trial-independent program performing APOE genotyping in individuals age 55-75 to enrich referrals to prevention studies. Participants review a brief, online education video providing an overview of Alzheimer's disease and the APOE gene prior to electronically signing an informed consent. Participants use a buccal swab kit for collection of DNA; APOE genotyping is done by a CLIA-certified lab. To help facilitate enrollment into GeneMatch, the protocol was modified in Q2 2016 to become a multi-site program to allow partner sites to enroll individuals into GeneMatch onsite, rather than waiting to receive the buccal swab kit in the mail. Based in part on APOE genotype, participants may be contacted to complete additional online questionnaires, learning modules, surveys, or to notify them about new research studies. GeneMatch does not disclose APOE results to participants, either directly or inadvertently through referral to studies. Recruiting studies, however, may ask or invite individuals to learn their APOE results. The API Generation Study is the first trial to recruit from GeneMatch; discussions are underway with other studies to use GeneMatch as a recruitment tool.

Results: GeneMatch launched in November 2015; as of February 20th, 2017, 31,961 people have enrolled. 3.8% of participants are APOE ϵ 4/ ϵ 4, 28.5% APOE ϵ 3/ ϵ 4, 55.2% APOE ϵ 3/ ϵ 3, 9.4% APOE ϵ 2/ ϵ 3, 2.6% APOE ϵ 2/ ϵ 4 and 0.5% APOE ϵ 2/ ϵ 2. Participants have a mean age of 64 years old, 80% are female. In July of 2016, GeneMatch began referring participants to the Generation Study. Participants are sent both electronic and mailed invitations to participate in the study. As of February 2017, 308 participants have been invited to participate in the Generation Study at a nearby site and 143 have accepted their invitation.

Conclusions: GeneMatch is a key element of the API, facilitating enrollment into a range of research studies, including the Generation Study, and serving as a resource to the Alzheimer's scientific community. During the initial pilot phase, several barriers to enrollment were observed and modifications to the enrollment process are being made on a rolling basis. Preliminary results and lessons learned to date from GeneMatch will be presented.

Institutional Information

**Research Summaries and Key Personnel
from Each Participating Institution**

ARIZONA STATE UNIVERSITY

Institutional Abstract

Over a decade ago, ASU set forth to redefine higher education, focusing on a model of the New American University. With swift momentum, ASU has led the world with innovative ideas to student-centric public higher education, honing in on academic excellence, the highest quality education and training, inclusiveness to a broad demographic, and maximum societal impact. Underscoring this exemplary new path, ASU has been ranked number one for innovation by U.S. News and World Report for the last two years (2016 and 2017). With Alzheimer's disease affecting roughly one in nine people 65 years old and over, and one in three people 85 years old and over, research on Alzheimer's disease exemplifies the type of endeavor that ASU seeks to promote, and a focus on innovative understandings and therapies is most certainly critical to research and treatment efforts.

For the Arizona Alzheimer's Consortium, ASU provides the Data Management and Biostatistics Core, the new Research Education Component, as well as the Education and Information Transfer Core. This serves researchers throughout the state as part of the Consortium's NIA-sponsored Arizona Alzheimer's Disease Center. The ASU team includes leaders in the development of novel animal models of Alzheimer's disease to study new treatments as well as mechanisms and trajectory of pathology (Oddo laboratory), in antibody and novel compound strategies for the treatment of Alzheimer's and other neurodegenerative diseases (Sierks, Hecht, and Johnson laboratories), in epigenetics, transport mechanisms, and pathophysiology of Alzheimer's disease (Coleman), in the development and use of animal models to characterize the influence of reproductive senescence and hormonal influences on brain aging and cognition (Bimonte-Nelson laboratory), in the development of mice as a model for odor learning and discrimination to understand pathologies and novel markers of neurodegenerative disease (Smith laboratory), in the development and implementation of computational image analysis and biomathematical techniques to increase the power to detect and track Alzheimer's disease (Chen and Wang laboratories), and in the development of improved care models for patients and family caregivers, including the HOPE memory partner program to explore the role of community health workers in Alzheimer's disease research and clinical practice (led by David Coon). It is noteworthy that ASU has numerous scientific research domains that are being further developed and strengthened to further bolster the impact on Alzheimer's disease and aging research, with a focus on discovery and action to move forward for trajectories, diagnosis, and treatment. These include, but are not limited to, the neurosciences, health outcomes research, and focused translational research realms that pose hypothesis-driven questions approached from a systems and interdisciplinary perspective. Collectively, ASU has a solid framework and wide-ranging strengths that are poised to make great strides in the scientific fight against Alzheimer's disease, as well as to optimize the trajectory of brain aging, using both preclinical and clinical approaches. Moreover, it is noteworthy that the assets in the research programs at ASU within the Arizona Alzheimer's Consortium represent a range of colleges and institutes across ASU.

A strength of ASU is the training, mentoring, and education of future generations of aging and neurodegenerative disease researchers and academicians, spanning high school students, to undergraduate students, to graduate students, to postdoctoral fellows. The approach to training is hands-on, multifaceted, and interdisciplinary, with the goal to engage future scientists in aging

and neurodegenerative research to yield maximal impacts on research discovery and translational outcomes. The new Research Education Component, Co-Directed by Dr. Heather Bimonte-Nelson (ASU) and Dr. Yonas Geda (Mayo) reflects this strong and extensive training commitment. Notably, ASU offers graduate degrees in Statistics and Biomedical Informatics, the Behavioral Neuroscience Program within the Department of Psychology, as well as the Interdisciplinary Graduate Program in Neuroscience . The latter two training programs focus upon approaches that integrate multiple levels of analysis using systems and interdisciplinary approaches – cellular, behavioral, and cognitive – to address preclinical, clinical, and translational questions about brain and behavior relationships.

¹<https://psychology.clas.asu.edu/content/psychology-behavioral-neuroscience-phd>

²<http://neuroscience.asu.edu/>

ARIZONA STATE UNIVERSITY

Key Personnel

Name (last, first)	Degree	Role on project
Bimonte-Nelson, Heather	PhD	PI
Northup-Smith, Steven	BS	Lab manager
Hiroi, Sheri	PhD	Postdoctoral Fellow
Pena, Veronica	MS	Graduate student
Koebele, Stephanie	MS	Graduate student
Prakapenka, Alesia	MS	Graduate student
Stonebarger, Gail	BS	Undergraduate
Granger, Steve	BS	Undergraduate
Berns-Leon, Claire		Undergraduate
Poisson, Mallori		Undergraduate
Palmer, Justin		Undergraduate
Patel, Shruti	BS	Undergraduate
Carson, Catie		Undergraduate
Strouse, Isabel		Undergraduate
Neeley, Rachel		Undergraduate
Fox, Carly		Undergraduate
Croft, Corissa		Undergraduate
Hadder, Bryanna		Undergraduate
Melikian, Ryan		Undergraduate
Plumley, Zachary	BS	Undergraduate
Kirby, Destiney		Undergraduate
Ladwig, Ducileia		Undergraduate
Ahmed, Kinza		Undergraduate
Bulen, Haidyn		Undergraduate
Woner, Victoria		Undergraduate
Baier, Emma		High school student
Anbar, Nathaniel		High school student
Polster, Julien		High school student
Mastroeni, Diego	PhD	PI
Coleman, Paul	PhD	PI
Delvaux, Elaine	MS	Research Technologist
Nolz, Jennifer		Technician

Coon, David	PhD	PI
Carbajal, Berta		Research specialist
Rosas, Victoria		Research technician
Oddo, Salvatore	PhD	PI
Belfiore, Ramona	BS	International Graduate
Branca, Caterina	PhD	Postdoctoral fellow
Caccamo, Antonella	PhD	Assistant Research
Dave, Nik		High School Student
Ferreira, Eric	MS	Research Technician
Haque, Rizwan	PhD	Postdoctoral fellow
Rodin, Alexis	BS	Research Technician
Sarette, Patrick		Undergraduate
Shukla, Prakriti		Undergraduate Student
Silva, Casey		Undergraduate Student
Surendra, Likith		Undergraduate Student
Tran, An		Undergraduate Student
Vartak, Rasika	PhD	Postdoctoral Fellow
Velazquez, Ramon	PhD	Postdoctoral fellow
Winslow, Wendy	BS	Laboratory Manager
Sierks, Michael	PhD	PI
Venkataraman, Lalitha		Graduate Student
Smith, Brian	PhD	PI
Gerkins, Richard	PhD	Research Assistant
Daniels, Carter	MS	Graduate Student
Ma, Jason		Undergraduate Student
Sanabria, Federico	PhD	Collaborator
Decourt, Boris	PhD	Lab director
D'Souza, Gary	PhD	Postdoctoral Fellow
Prinzhorn, Bree	BS	Lab research assistant

BANNER ALZHEIMER'S INSTITUTE

Institutional Abstract

The Banner Alzheimer's Institute (BAI) has three goals: To find treatments to prevent Alzheimer's disease (AD) without losing a generation, to set a new standard of care for patients and families, and to promote a model of multi-institutional collaboration in biomedical research. BAI is intended to accelerate the evaluation, approval and availability of treatments to postpone, reduce or completely prevent the clinical onset of AD as quickly as possible; leverage its brain imaging resources and expertise to advance the scientific study, early detection, tracking, diagnosis, treatment and prevention of AD and related disorders; address the medical and non-medical needs of affected persons and families to the fullest extent possible, and help to establish a new standard of dementia care in the emerging population-based healthcare financing system. Finally, it is intended to complement, enhance, and benefit from close working relationships with its organizational partners inside and outside of the Arizona Alzheimer's Consortium (AAC).

BAI's Stead Family Memory Center includes a Memory Clinic, Family and Community Services Program and Clinical Trials Program. It offers a wide range of services for the evaluation and care of affected persons and family caregivers, helping to address their medical and non-medical needs throughout the illness. It provides educational, outreach and research enrollment programs for Arizona's Native American and Latino communities, evaluates and follows Native Americans in the NIA-sponsored Arizona AD Center's Clinical Core, and oversees an Annual Conference on AD and Dementia in Native Americans. Its Banner Dementia Care Initiative is seeking to demonstrate ways in which to optimize the identification and evaluation of cognitive problems, address a broad range of the affected person's and family's medical and non-medical needs, reduce unnecessary hospitalizations, and is affordable to payers in the emerging healthcare financing system. BAI conducts numerous clinical trials of investigational treatments, including those in the Alzheimer's Prevention Initiative (API). Its researchers also help oversee prospective an NIA-sponsored cohort study of cognitively unimpaired persons with two, one and no copies of the APOE4 allele, which has helped to conceptualize the preclinical stages of AD, an NINDS-sponsored study of chronic traumatic encephalopathy (CTE) in former National Football League and college football players, and one of the Precision Medicine Initiative's (PMI's) first healthcare provider-led cohort programs in a partnership between University of Arizona and Banner Health.

Its state-of-the-art NIH-supported Imaging Center includes two PET systems, a 3T MRI, cyclotron, radiochemistry laboratory, and computational image analysis laboratory. It provides imaging resources and expertise, research PET tracers, image-analysis methods, data and biological samples for researchers inside and outside of Arizona. In collaboration with Mayo Clinic, it includes a longitudinal brain imaging study of cognitively unimpaired persons with two copies, one copy, and no copies of the APOE4 allele, reflecting three levels of genetic risk for late-onset AD, and image-analysis techniques with improved power to characterize subtle brain changes over time. In collaboration with the University of Antioquia and a Harvard post-doctoral student, it also includes a study of PSEN1 E280A mutation carriers and non-carriers from the world's largest autosomal dominant AD kindred in Colombia. It is a member of the AD Neuroimaging Initiative (ADNI) PET Core, where it is responsible for the development, testing

and use of voxel-based image analysis techniques with improved power to detect and track AD. It has played pioneering roles in the study of preclinical AD.

AARC funds complement research activities supported by competitive grant awards from several NIA-sponsored research grants, private foundation grants, and clinical trials. In conjunction with our NIA-sponsored ADCC, subjects, images, other data, and image-analysis techniques from our study of cognitively normal APOE ϵ 4 carriers and non-carriers provide a core resource for interested investigators inside and outside of Arizona.

With several hundred million dollars in NIH, philanthropic and industry support, API has helped to launch a new era in AD prevention research. In its partnership with the University of Antioquia, Genentech and the NIH, the API Autosomal Dominant AD (ADAD) trial is evaluating a passive amyloid- β ($A\beta$) immunotherapy in 300 cognitively unimpaired 30-60 year-old PSEN1 E280A mutation carriers and non-carriers in the world's largest ADAD kindred, located in Antioquia Colombia. In its partnership with Novartis and the NIH, the international API APOE4 ("Generation") trial will evaluate an active $A\beta$ immunotherapy and BACE-1 inhibitor in 1,340 60-75 year-old APOE4 homozygotes. The 5-year trials are intended to evaluate the investigational treatments in potentially license-enabling prevention trials; to provide a better test of the amyloid hypothesis than trials in the later preclinical or clinical stages of AD; establish the extent to which a treatment's different biomarker effects are associated with a clinical benefit and provide evidence to support their use as reasonably likely surrogate endpoints in future 24-month prevention trials; provide a shared resource of data and biological fluids for the research community after the trial is over; complement, support and providing a foundation for other prevention trials; to help clarify the benefits, risks and role of APOE genetic test disclosure in the era of Alzheimer's prevention trials; support the advancement of Alzheimer's prevention research in the Collaboration for Alzheimer's Prevention CAP); and empower persons at highest risk in the scientific fight against AD.

API also includes exceptionally large registries to support interest and possible enrollment in prevention studies. In partnership with the University of Antioquia, the API Colombian Registry, in collaboration now includes >5,700 members of the PSEN1 E280A mutation kindred, including nearly 1,200 mutation carriers, who have provided their DNA and had clinical and neuropsychological evaluations. The web-based Alzheimer's Prevention Registry (www.endALZnow.org) now provides information about advances in prevention research and opportunities to enroll in prevention trials to >260,000 people and continues to grow rapidly; our GeneMatch Program (www.endALZnow.org/genematch) has enrolled >30,000 persons and aims to enroll 100,000 persons 55-75 years of age, match interested participants to relevant prevention trials, including but not limited the extremely large number of APOE ϵ 4 homozygotes in the Generation Trial, and to begin to clarify what it means to learn about one's APOE test results; and these programs continue to grow. It continues to champion new ways to identify and support enrollment in prevention trials and to address the logistical, ethical, and scientific issues involved in this endeavor.

During the past year, we received a top priority score for the API ADAD Colombia Trial's renewal grant and notification and a large administrative supplement to the Arizona APOE4 Gene Dose grant, enabling us to incorporate tau PET and support our data sharing infrastructure. We have contributed to the development of new API Trials, at least one of which is anticipated

to begin in late 2017. We also developed a proposal to help address ~24 scientific, regulatory, economic, data sharing and policy challenges needed to find, support the accelerated approval, affordability and widespread availability of effective prevention therapies by 2025. The proposal generated the interest of 84 leaders from 72 international organizations, and venues to support as many of these activities as possible is now underway.

BAI has several specific aims:

1. To leverage our imaging resources in the early detection, tracking, and diagnosis of AD, the clarification of genetic and non-genetic risk factors, and other collaborative research studies inside and outside of Arizona.
2. To leverage our imaging resources in the early detection and tracking of related diseases (e.g., chronic traumatic encephalopathy [CTE] and AD in patients with Down syndrome)
3. To implement, test and use PET radiotracer techniques (e.g., for the assessment of amyloid and tau pathology) in the study of AD and related disorders
4. To develop image analysis techniques and composite cognitive test scores with improved power to detect and track AD and evaluate AD-modifying and prevention therapies.
5. To accelerate the evaluation of AD prevention therapies through API's preclinical AD trials and enrollment registries.
6. To share data and biological fluid samples with the research community, and advance the complementary research goals of our partners inside and outside Arizona.
7. To provide a care model that more fully address the needs of patients and families and BAI, and to develop and test the cost-effectiveness of a dementia care program that better addresses the needs of patients and family caregivers in the Banner Health Accountable Care Organization in the Banner Dementia Care Initiative.
8. To support the clinical research and Native American outreach, education and enrollment goals of the Arizona ADCC.
9. To promote the further development, productivity, and close working relationships of research programs involved in the fight against AD and related disorders.

BANNER ALZHEIMER'S INSTITUTE

Key Personnel

Name (last, first)	Degree	Role on project
Reiman, Eric	MD	Executive Director, Banner Alzheimer's Institute (BAI) Director, Arizona Alzheimer's Consortium (AAC)
Tariot, Pierre	MD	Director, BAI
Bandy, Dan	MS, CNMT	PET Technical Director and Sr. Scientist
Batchuluun, Dawn	BA	Clinical Research Coordinator
Boker, Connie	BS, MBA	Imaging Center Operations Director
Brand, Helle	PA	Physician Assistant, Memory Disorders Center
Burke, Anna	MD	Dementia Specialist
Burke, Bill	MD	Director, Stead Family Memory Center
Chen, Kewei	PhD	Director, Computational Image Analysis Laboratory Director, ADCC Data Management & Statistics Core
DeMarco, Kathryn	BS	Clinical Research Program Manager
Dougherty, Jan	RN, MS	FAAN FCS Special Projects Consultant
Goradia, Dhruvan	PhD	Associate Scientist
Hall, Geri	PhD, ARNT, CS	Clinical Nurse Specialist, Family & Community Services
High, Nellie	MS	Research Project Coordinator
Jakimovich, Laura	RN	Multi-Center Clinical Trials Manager
Langbaum, Jessica	PhD	Associate Director, Alzheimer's Prevention Initiative
Langlois, Carolyn	MA	Clinical Research Program Manager
Lee, Wendy	MS	Assistant Director, Computational Brain Imaging
Lomay, Nicole	BS	Native American Outreach Representative
Lopez, Ashley	MS	Clinical Trials Operations Director
Nisson, Lori	MSW/LCSW	Director, Family & Community Services
Perrin, Allison	MD	Physician Dementia Specialist
Protas, Hillary	PhD	Associate Scientist
Saner, Don	MS	Data Management & Statistics Core Co-Director
Savage, Cary	PhD	Senior Scientist/Lab Head, Image Analysis Laboratory
Seward, James	PhD	Neuropsychologist
Weidman, David	MD	Physician Dementia Specialist

BANNER SUN HEALTH RESEARCH INSTITUTE

Institutional Abstract

Banner Sun Health Research Institute (BSHRI) was established in 1986 in the heart of Sun City, Arizona, the nation's first planned retirement community, including more than 100,000 older adult residents in the area, and intended to make a profound difference in the scientific study of Alzheimer's disease (AD), Parkinson's disease (PD), other age-related brain disorders, and healthy aging. BSHRI has historically included: a) the state's largest number of productive basic scientists in the fight against AD, who are well known for their major contributions to the study of amyloid processing, brain inflammation, epigenetics, and the roles of cholesterol and cerebrovascular disease in AD that have now completed relocation to ASU; b) a world-class Brain and Body Donation Program (BBDP) for the study of AD, PD, related disorders and normal aging; c) clinical, family and community service, wellness, clinical research, and extensive clinical trials programs for AD, PD, and related disorders; d) a Center for Healthy Aging, with an additional longitudinal cohort of nearly 1,000 research participants, including nearly 200 in their 90s and 100s, for the study of aging and age-related brain disorders; e) an extensive outreach, education, and volunteer programs, including a highly productive summer research internship program for under-represented and other college and high school students, and partnerships with Sun Health Foundation and other stakeholders in this highly concentrated community of active older adults; and f) close working relationships with researchers throughout Arizona and around the world. From July 2001 to June 2016, BSHRI served as the applicant organization for the Arizona ADCC on behalf of the organizations in the Arizona Alzheimer's Consortium, and it remains home to the ADCC's Administrative Director, Andrea Schmitt.

Directed by Dr. Thomas Beach, the BBDP includes >800 clinically characterized and longitudinally assessed participants, including patients with AD, PD, and related disorders, and older adults who are cognitively and neurologically unimpaired at the time of their enrollment, all of whom have consented to donate their brains after they die. It is internationally recognized for: a) its unusually rapid autopsy program, with a median 3-hour post-mortem interval for the approximate 1,700 expired brain donors, comprehensive neuropathological assessments, and the unusually high tissue quality needed to support certain kinds of post-mortem studies; b) the unusually large number of brain donors who are cognitively and neurologically unimpaired at the time of their clinical enrollment, advancing the study of preclinical AD and PD and providing numerous clinically and neuropathologically normal control subjects for genetic and other research studies; c) whole body donation, banked organs and tissues from about 900 expired donors since 2005, and the opportunity to relate brain pathology to biological features of other body organs; and d) an extraordinary number of annual tissue distributions to advance research in Arizona and around the world. The BBDP includes many research participants in the Arizona ADCC's Clinical and Ancillary BBDP Cores, the ADCC's Neuropathology Core, and the NINDS's National Brain and Tissue Resource for PD and Related Disorders (NBTR-PD, in partnership with Mayo Clinic Arizona and BNI). In addition, it continues to play critical roles in the neuropathological validation of amyloid PET, tau PET, and other ante-mortem biomarker measurements in end-of-life (e.g., hospice) patients, helping contribute to FDA approval for use of some of these measurements in the clinical setting. It continues to provide a tissue resource for genome-wide genetic, transcriptomic and proteomic data from different brain regions and cell

types, and to contribute to numerous research studies, collaborations, grants, publications, and findings each year.

Since 2016, BSHRI has been undergoing a period of significant change, setting the stage for BSHRI and its organizational partners further develop its AD, PD related disorders, and aging programs. These changes include the following: a) continued harmonization of Banner Alzheimer's Institute's AD-related clinical, family and community services, clinical research and clinical trials programs on its downtown Phoenix and BSHRI campuses; b) further development of our Memory and Movement Disorders Center; a formal partnership with the emerging Arizona State University-Banner Neurodegenerative Disease Research Center (NDRC), including ASU's commitment to support 20 basic/translational research laboratories, construction of state-of-the-art laboratory space for NDRC researchers at ASU's BioDesign Institute (to be completed in late 2017), the ongoing recruitment of an international leader to direct what is intended to become the largest basic/translational research programs in the fight against neurodegenerative diseases, and new research collaborations among all of the participating organizations in the Arizona Alzheimer's Consortium; e) a developing plan to enhance BSHRI's longevity cohort, harmonize some of the elements in the longevity and BBDP programs, and support the study of AD and resilience to cognitive decline in the oldest old; and f) ongoing development of a strategic plan for the development and further growth of clinical and clinical research programs on the BSHRI campus. In addition to BSHRI's large clinical, family and community services, PD-related "Neurowellness", and clinical trials programs, its outreach efforts include >100 community presentations per year.

BANNER SUN HEALTH RESEARCH INSTITUTE

Key Personnel

Name (last, first)	Degree	Role on project
Beach, Thomas	MD, PhD	BBDP & Neuropathology Core Director, Neuropathologist
Belden, Christine	PsyD	Neuropsychologist
Davis, Kathryn	BA, CSP, CRC	Clinical Core Coordinator
Liu, Ming-Jai	M.D., PharmD	Neurologist, Movement Disorder Neurologist
O'Connor, Kathy	M.S.	Outreach Program Manager/Longevity Program Coordinator
Powell, Jessica	PsyD	Neuropsychologist
Schmitt, Andrea	B.S., CRA	ADCC Administrative Director
Serrano, Geidy	Ph.D.	Anatomist, BBDP
Sue, Lucia	B.S	Coordinator, Neuropathology Core, BBDP
Shprecher, David R	D.O., M.D.	Movement Disorders Program Director, Movement Disorders Neurologist
Zamrini, Edward	M.D.	Director, Memory Clinic Director; ADCC Clinical Core Site PI, Interim Director, Center for Healthy Aging Dementia Neurologist

BARROW NEUROLOGICAL INSTITUTE

at St. Joseph's Hospital and Medical Center

Institutional Abstract

The Barrow Neurological Institute focuses on human and animal research that can translate to clinical care. The BNI focus in Alzheimer's Disease and aging is in prevention, early detection and defining mechanisms of AD. On the cellular level, the Cellular Metabolism laboratory (Dr. Jiong Shi) studies the role of energy metabolism and, more specifically, mitochondrial function, in brain aging and age-related neurological disorders, primarily Alzheimer's disease. Dr. Elliot Mufson has an internationally known molecular neuroanatomy program in the area of dementia in the aged and diseased brain, and is one of the hundred most cited authors in the ISI Web of Knowledge. His focus is on gene expression patterns in Alzheimer's disease and has conducted a study this year examining tau protein in the nucleus basalis of Meynert in autopsy brains. Dr Sabbagh joins the faculty in the department of neurology at the Barrow Neurological Institute at Director of the Alzheimer's and Memory Disorders Division after 15 years at the Banner Sun Health Research Institute. His focus is development of the clinical trials program which will include prevention, MCI, and treatment trials as well as imaging and biomarker studies. His research interest is longitudinal assessment of adults with Down Syndrome to detect AD changes which occur prior to the onset of cognitive decline. Dr. Leslie Baxter studies longitudinal cognitive and brain changes in aging in Autistic Spectrum Disorders (ASD), one of the first studies of the interaction of aging and ASD.

Alzheimer's and Memory Disorders Program

With the recruitment of Dr. Marwan Sabbagh as our Director of the Alzheimer's and Memory Disorders Division in the Department of Neurology, Dr Sabbagh is expanding the flow of the clinic and our clinical trial footprint in AD. We are capitalizing on the strengths of the Behavioral Neurology team in clinical trials and the state-of-the-art imaging platforms at the BNI to expand our work in AD, imaging and prevention. We are an active contributor to the Arizona Alzheimer's Disease Core Center study, and with our expansion, will increase our inclusion of Hispanics in the Phoenix area. We have 1 full-time bilingual/bicultural staff member who participates in the ADCC to recruit and assess Hispanic patients. We continue to partner with the Latino community through a Promotore program and outreach activities. About one third of the BNI's ADCC participants are Hispanic, with Hispanics enrolled for as long as 12 epochs. In the past year, the number of subjects enrolled into the clinical core of the ADCC has increased. Additionally, the clinical research footprint has expanded significantly with 8 additional research staff and the number of enrolling studies increased from two to 15 including IDEAS and A4.

Alzheimer's disease biomarker studies in the Cellular Metabolism Lab

The focus of the laboratory is to study and identify biomarkers in brain aging and age-related neurological disorders, primarily Alzheimer's disease. Presently, our efforts are aimed at understanding the role of the PACAP-AMPK-Sirtuin3 pathway in the pathogenesis of Alzheimer's disease, at characterizing the neuroprotective properties and at identifying underlying molecular mediators that will be amenable to pharmacological intervention. We rely on a variety of techniques, including cognitive testing, recording of electrical brain activity,

anatomy and microscopy studies, magnetic resonance imaging, biochemical energy measurements and genetic manipulations using specialized viruses to introduce desired DNA into neurons.

Alzheimer's Disease Research Laboratory

The focus of Dr. Mufson's Alzheimer's disease Research Laboratory is to study the mechanisms underlying the selective vulnerability of neuronal populations which degenerate in people with mild cognitive impairment, Alzheimer's disease and Parkinson's disease.

Translational Bioimaging Laboratory

The Translational Bioimaging lab pursues and leverages advances in imaging technology, contrast mechanisms, and contrast agents to develop targeted bioimaging methods that are specifically sensitive to a tissue's underlying biological, microstructural, and molecular features. With the additions of Drs. Chad Quarles and Ashley Stokes, there are now a series of imaging projects funded through the AARC funds and institutional match. We are currently evaluating imaging-based signatures of normal and pathological aging, including measures of blood volume and flow, molecular species, and iron deposition. We are also exploring the sensitivity and specificity of advanced MRI and PET metrics in preclinical models of AD.

BARROW NEUROLOGICAL INSTITUTE

at St. Joseph's Hospital and Medical Center

Key Personnel

Name (last, first)	Degree	Role on Project
Baxter, Leslie	PhD	Neuropsychologist, Associate Principal Investigator
Sabbagh, Marwan	MD	Principal Investigator Karsten Solheim Chair Professor of Neurology Director, Alzheimer's and Memory Disorders Program
Shi, Jiong	MD, PhD	Neurologist
Mufson, Elliot	PhD	Neuroscientist
Wu, Jie	MD, PhD	Neuroscientist
Lukas, Ronald	PhD	Neuroscientists
Braden, Blair	PhD	Post-Doctoral Fellow
Yin, Junxiang	PhD	Post-Doctoral Fellow
Han, Pengcheng	PhD	Post-Doctoral Fellow
Mendoza, Perla	MS	Clinical Coordinator
Zhuang, Ninging	BS	Lab technician
Arnold, Lisa	BS	Program Administrator
Rowcliffe, Stacey	BS	Psychometrist
Martinez Lujan, Lazaro	BS	Study Coordinator
Thomas, George	BS	Study Coordinator
Chacon, Bianca	BS	Study Coordinator
Jensen, Allyson	BS	Psychometrist
Perez, Sylvia	PhD	Associate Professor
Liu, Qiang	PhD	Staff Scientist, Asst Prof
Pipe, James	PhD	Director, Keller Center for Imaging Innovation
Debbins, Josef	PhD	MR Engineer, Keller Center for Imaging Innovation
Stokes, Ashley	PhD	MR Research, Keller Center for Imaging Innovation
Quarles, Chad	PhD	MR Research, Keller Center for Imaging Innovation

CRITICAL PATH INSTITUTE

Institutional Abstract

The Critical Path Institute (C-Path) is an independent, nonprofit organization that brings together pharmaceutical, academic, government, patient and nonprofit organizations to work on important drug and other medical product development challenges. C-Path's global consortia work to decrease the length of time, cost, and risk for developing safe, effective medical products.

C-Path teams advance regulatory science by developing tools and scientifically valid processes. With a failure rate of 95 percent and over \$1 billion dollars spent on average for every drug that is approved, there is a significant need to enhance the process. Our work makes a lasting difference in treatment options for physicians and patients.

To deliver solutions to limitations in regulatory science, C-Path staff and consortia members develop biomarkers, clinical outcome assessment instruments, disease progression models and clinical trial simulation tools, in vitro tools, data standards, and databases. C-Path leads teams with scientific rigor and operational excellence to facilitate the sharing of data, expertise, and knowledge.

Coalition Against Major Diseases (CAMD)

CAMD is one of fourteen public-private partnerships of Critical Path Institute, a nonprofit organization dedicated to deliver on the vision of the US Food & Drug Administration's (FDA) Critical Path Initiative. CAMD aims to convene diverse stakeholders (academia, advocacy groups, industry, & regulatory agencies) to create new tools and methods that can be applied during the drug development process that advance new treatments for various stages of Alzheimer disease (AD), and neurodegenerative diseases with related cognitive and functional impairments.

CAMD has a mission to develop new technologies and methods to accelerate the development and review of medical products for neurodegenerative diseases by advancing Drug Development Tools for evaluating the foundational nature of disease progression, designing and conducting clinical trials, evaluating drug efficacy, and streamlining the process of regulatory review.

The consortium serves as a neutral third party and focuses on three key areas supporting regulatory science:

- Sharing anonymized, precompetitive patient-level data from the control arms of legacy clinical trials
- Developing consensus data standards
- Developing new regulatory-endorsed tools (e.g., use of clinical outcome assessment instruments, prognostic imaging biomarkers and digital biomarker measures from wearables & remote monitoring devices, as well as quantitative drug development platforms).

CRITICAL PATH INSTITUTE

Key Personnel

Name (last, first)	Degree	Role on project
Arnerić, Stephen	PhD	Principal Investigator; Executive Director, Coalition Against Major Diseases (CAMD) Consortium
Romero, Klaus	MS, MD	Alzheimer's Clinical Expert; Director for Clinical Pharmacology
Stafford, Bob	MA	Data Manager
Neville, Jon	PSM	Standards Developer
Kern, Volker	PhD	Project Manager
Economou, Sonia	MA	Grants Administrator

MAYO CLINIC ARIZONA

Institutional Abstract

The main goal of this research program is to determine the correlation between genetic risk for Alzheimer's disease (apolipoprotein E [APOE] genotype) and the effect of normal aging on certain measures of cognitive function, brain volume, brain metabolism, cerebral amyloid deposition, and potential plasma biomarkers (APOE fragments and others). The principal institutions involved in this collaborative research effort are Mayo Clinic Arizona (primary site), Banner Alzheimer Institute, Barrow Neurological Institute, Arizona State University, and Translational Genomics Research Institute though as the program has matured, it has evolved into a core resource for investigators from these and other institutions as well. Our research program capitalizes on the clinical and neuropsychological expertise of the Behavioral Neurologists and Neuropsychologists at Mayo Clinic Arizona, in conjunction with the genetic expertise of Dr. Rosa Rademakers at Mayo Clinic Jacksonville.

During the initial phase of our program, data were analyzed in cross sectional correlations between APOE genetic status, physical and psychological stressors, cognition, and brain imaging measures. Since then, the bulk of our efforts have been dedicated to longitudinal analyses, and we have shown the neuropsychologically defined onset of Alzheimer's disease begins during our 50's in APOE e4 carriers, it is confined to memory during the early preclinical phase but there is an increase in self-awareness of decline that is not mirrored by informant observation. In later stages of preclinical Alzheimer's disease, as patients get within a few years of incident MCI conversion, executive measures begin to decline and informant observations begin to parallel self-reports of decline. Finally, by the time MCI emerges, memory, executive skills, and in some cases visuospatial skills begin to decline. Although missing from this preclinical profile is any indication of depression as a preclinical harbinger, we have most recently shown that subtle personality changes herald the transition from preclinical AD to MCI and may provide the substrate for the frequent depression and related behavioral issues that complicate the clinical course of AD.

To date we have:

1. analyzed the longitudinal trajectories of all our measures, identified those showing significantly greater acceleration of decline in APOE e4 carriers relative to noncarriers, and developed a cognitive profile of APOE e4 driven pathological aging that defines the cognitive profile of preclinical Alzheimer's disease.
2. compared our incident cases of mild cognitive impairment (MCI) to a clinical (prevalent) group of matched patients to further define an early and late preclinical/early clinical phase in which we begin to see decline in non-memory measures, especially those sensitive to executive functions.
3. characterized the significance of subjective impairment as voiced by one's self as well as by one's informant and showed that both reflect an early stage of decline in a small subset, but that stress related symptoms overshadow the cognitive changes so that subjective impairment alone is an unreliable indicator of imminent decline.

4. showed that personality traits that increase one's proneness to stress further speed up age-related memory decline, and this effect is more apparent in APOE e4 carriers reflecting their inherent predilection for Alzheimer's disease.
5. In contrast we found that the developmental sex-based cognitive advantages of women over men regarding verbal memory and men over women regarding visual memory do not buffer the rate of decline associated with APOE e4.
6. evaluated the sensitivity of a computer-based cognitive task developed by Mario Parro sensitive to memory "binding" of different stimulus properties (e.g., shape and color), but we did not find this to be more sensitive than current gold standard neuropsychological measures of declarative memory.
7. completed a survey both online as well as among members of our cohort examining attitudes about predictive testing for Alzheimer's disease (genetic and biomarker based) and found there is considerable interest in having such testing even in the absence of definitive therapy, but that roughly 12% and 6% respectively envision suicidal ideation should they be found at high risk for Alzheimer's disease. These results are informing the design of test disclosure methods in forthcoming trials. This has contributed to Dr. Caselli serving as DSMB chair for the most recent iteration of the REVEAL study.
8. showed for the first time that personality changes very early in the clinical course of AD and likely contributes to the behavioral symptoms that follow.
9. combining the detailed neuropsychological and behavioral profiles of preclinical AD with the imaging results obtained in a subset of this cohort by Dr. Reiman and his BAI team, we have together demonstrated that preclinical AD mirrors clinical AD in all aspects, implying it is distinct from normal aging despite the lack of symptoms. These results have informed the design of current secondary prevention trials.
10. collaborated with Dr. Yalin Wang at ASU whose novel MRI analytics are proving to be more sensitive to the early detection of structural change in MCI and preclinical AD than current volumetric methods.
11. started a genomic exploration of unexpectedly young onset dementia.
12. completed a pilot study that has found reduced plasma levels of eosinophil peroxidase activity in patients with AD
13. completed the establishment of lymphocyte derived iPS cells differentiated in vitro to cortical neurons by Dr. David Brafman at ASU who is continuing to explore intraneuronal pathophysiology related to Alzheimer's disease.

These types of analyses will continue well into the future permitting us to achieve our longer term goals of:

1. correlating changes in brain function with structure, metabolism, and pathology
2. determining rates of symptomatic conversion from preclinical Alzheimer's disease to MCI, and from MCI to dementia
3. developing a predictive model based on presymptomatic parameters for the timing of symptomatic conversion
4. develop prevention strategies
5. provide a core resource to all our collaborative partners
6. correlating nontraditional but potentially modifiable measures of health factors and neuropsychiatric status such as intellectual achievement, sleep patterns, and personality factors with presymptomatic cerebral amyloid levels

Specific goals for this fiscal year include:

1. continue to expand our biobanking efforts to include all those with young onset Alzheimer's disease
2. expand last year's pilot project of whole exome sequencing and bioinformatics analysis of a 55 gene panel in a clinical cohort of patients with biomarker supported young onset Alzheimer's disease (or frontotemporal dementia) to another 20 patients, and, in collaboration with Dr. Li Liu from ASU explore novel correlations (related to evolutionary genomics)
3. publish our analysis of personality changes during the transition from preclinical AD to MCI
4. analyze and publish our longitudinal MRI and FDG-PET correlations with neuropsychological trajectories that comprehensively characterize preclinical AD
5. publish the results of a study evaluating a cognitive "stress test" based upon APOE and TOMM40 genotype
6. Extend our exploration of plasma eosinophil products in patients with AD and those with vascular contributions to dementia
7. Exploration of a partnership with Adelante Health Center to address disparities in dementia care and research opportunities in a large Latino population.

This research proposal has been peer reviewed and approved by the Mayo Clinic Institutional Review Board (IRB #259-99).

MAYO CLINIC ARIZONA

Key Personnel

Name	Degree	Role on Project
Caselli, Richard	MD	Principal Investigator, Clinical Core Director, Associate Director, Behavioral Neurologist
Woodruff, Bryan	MD	Co Investigator, Behavioral Neurologist
Locke, Dona	PhD	Co Investigator, Neuropsychologist
Stonnington, Cynthia	MD	Co Investigator, Psychiatrist
Geda, Yonas	MD	Co Investigator, Psychiatrist
Hoffman-Snyder, Charlene	DNP	Nurse Practitioner
Henslin, Bruce	BA	Study Coordinator
Johnson, Travis	BA	Study Coordinator

MIDWESTERN UNIVERSITY

Institutional Abstract

Midwestern University offers a diverse group of scientific researchers and clinicians from a variety of disciplines opportunities to contribute to the efforts of the Arizona Alzheimer's Consortium. These contributions can be broadly characterized as efforts to understand the genetic contributions to Alzheimer's disease in regard to the APOE4 allele and TREM2 variants, potential treatments for Alzheimer's disease and associated disorders, as well as explorations of the mechanisms by which Alzheimer's disease pathology develops. The goals of Midwestern University are to leverage this diversity of expertise and establish a common core of investigators that contribute to our understanding of neurodegenerative disorders and aging, to inspire collaboration within Midwestern and with investigators at other institutions, and to complement and enhance the efforts of other Consortium institutions and investigators around the state.

The Midwestern Alzheimer's Advisory Committee (MAAC) was established to lead the efforts of the Alzheimer's Consortium at Midwestern University. The MAAC holds an annual open intramural competition for the Consortium funding allocated to Midwestern to enhance the diversity of thought while contributing productively to the Consortium. The MAAC currently consists of investigators from 15 different departments across six different colleges. Midwestern University has recently engaged in a major expansion of its research programs and capability, including the opening of a College of Veterinary Medicine and the concomitant addition of many new research faculty. We will leverage this expansion to further the goals of the Consortium. Future goals for Midwestern University's Consortium efforts include broader roles in basic science understanding, patient evaluation and treatment mechanisms, education and outreach, and clinical recruitment.

Midwestern University investigators can capitalize on support mechanisms that enhance their ability to conduct Consortium-relevant research. For instance, faculty salaries are not dependent on extramural support, most of the research technicians are University-funded, the University provides generous funding for capital equipment, and multiple intramural funding mechanisms are available. This allows the MAAC to focus funds on enhancements, productivity, and new directions for research and to meaningfully fund a larger group of investigators than would otherwise be possible.

The overall Consortium-related research program at MWU has several specific aims:

- 1) Continue to analyze the effects of the APOE4 genotype and other emerging risk factors in human carriers of the genes, as well as in transgenic animals, to deduce mechanisms and modes for the increase in risk that each presents for neurological disorders.
- 2) Establish and enhance new neuroscientific techniques, such as CLARITY and tissue expansion, and leverage these to increase our understanding of disease mechanisms and pathology.

- 3) Support the development and validation of new pharmacological treatments that could have a positive impact on Alzheimer's disease and other neurological conditions, and support research on the cellular and subcellular targeted delivery of relevant treatments.
- 4) Continue to evaluate the dysfunction within and contribution of various neurotransmitter systems in Alzheimer's disease and related disorders, such as Parkinson's disease, prominently including the nicotinic and muscarinic receptor systems of the brain.
- 5) Continue work in human tissues provided by the BSHRI, to assess the potential role of infectious organisms in the induction of Alzheimer's disease pathology, including amyloid and tau pathology.
- 6) Continue work in the 3xTG mouse models of Alzheimer's disease to determine if infection can exacerbate the development of AD pathology.
- 7) Support research in the involvement of inflammatory molecules in the pathophysiology of Alzheimer's disease, related disorders, and CNS injury.
- 8) Support ongoing efforts in the psychological evaluation of and intervention within the aging population.
- 9) Enhance the ability of the Midwestern Clinics to recruit, evaluate, and intervene in geriatric populations; assist in the efforts of diverse specialties to contribute to the treatment and care of patients suffering from neurodegenerative disorders and the well-being of their caregivers.

MIDWESTERN UNIVERSITY

Key Personnel

Name (last, first)	Degree	Role on project
Jentarra, Garilyn	PhD	Administrative PI, Assistant Professor, Biochemistry
Kaufman, Jason	PhD	Associate Professor, Anatomy
Olsen, Mark	PhD	Associate Professor, Pharmaceutical Sciences
Jones, Doug	PhD	Assistant Professor, Pharmacology
Vallejo, Johana	PhD	Associate Professor, Physiology
Jones, T.B.	PhD	Associate Professor, Anatomy
Bae, Nancy	PhD	Assistant Professor, Biochemistry
Hernandez, Jose	PhD	Associate Professor, Biochemistry
Gadagkar, Sudhindra	PhD	Assistant Professor, Biomedical Sciences
Jones, Carleton	PhD	Associate Professor, Biomedical Sciences
Amin, Kiran	PhD	Professor, Clinical Psychology
Flint, Melissa	PsyD	Acting Director, Clinical Training, Clinical Psychology
Kokjohn, Tyler	PhD	Professor, Microbiology & Immunology
Potter, Pam	PhD	Chair, Pharmacology
Yevseyenkov, Vladimir	OD, PhD	Assistant Professor, Optometry
Veltri, Charles	PhD	Assistant Professor, Pharmaceutical Sciences
Knudsen Gerber, Dawn	PharmD	Associate Professor, Pharmacy Practice
Eckman, Delrae	PhD	Assistant Professor, Biomedical Sciences
Nithman, Robert	DPT	Assistant Professor, Physical Therapy
Myers, Kent	MD	Associate Professor, Podiatry
Tullos, Tony	MD	Chair, Pathology
Swanson, Mark	PhD	Assistant Professor, Biochemistry
Powell, Jessica	PsyD	Assistant Professor, Clinical Psychology
Al-Nakkash, Layla	PhD	Professor, Physiology

TRANSLATIONAL GENOMICS RESEARCH INSTITUTE

Institutional Abstract

The Translational Genomics Research Institute (TGen) is a non-profit biomedical research institute whose mission is to make and translate genomic discoveries into advances in human health. TGen is dedicated to bringing the breakthroughs in genomics research to the bedside and benefit of patients. Its focus on translational research involves coupling, in novel ways, basic and clinical research with emerging molecular technologies to accelerate the development of therapeutics for human disease. Part of the unique nature of TGen is its partnering relationships with academic institutions, clinical practices and corporate entities, each aimed at accelerating the movement of discovery-based research toward clinical application.

TGen is organized into several research Divisions including: Cancer and Cell Biology, Clinical Translational Research, Computational Biology, Genetic Basis of Human Disease, Integrated Cancer Genomics, Neurogenomics, and Pathogen Genomics. The Neurogenomics Division is the home of Alzheimer's disease (AD) and aging research within TGen. AD and aging has been a focus of the Division since its inception and every laboratory within the Division performs research related to aging or AD.

The Neurogenomics Division is subdivided into several disease-oriented research clusters. Each cluster represents a unique cross-pollination between basic researchers and clinicians with the endgame being successful clinical trials that ultimately lead to improved treatments. These clusters include geneticists, molecular and cellular biologists, brain imaging researchers, proteomics researchers and other experts. The Division has accomplished several milestones in AD research including: (1) the first high-density genome screen to identify common heritable risk factors for AD, (2) the identification of a key genetic driver of episodic memory function in healthy individuals, (3) the first large-scale study identifying the genes differentially expressed in pathology-containing and pathology-free neurons in the brains of AD patients and control donors, (4) the identification of protein kinase targets responsible for phosphorylation of the tau protein which contributes to AD pathology, and (5) the collaborative discovery of a novel cognitive enhancing agent based on the genetic finding in episodic memory. Recently the focus within several laboratories in the Division is in the area of biomarker development for the early assessment of AD and/or dementia risk.

**TRANSLATIONAL GENOMICS
RESEARCH INSTITUTE
Key Personnel**

Name (last, first)	Degree	Role on project
Adkins, Jonathan	BS	Research Associate
Balak, Chris	BS	Research Associate
Bleul, Christiane	MS	Research Associate
Cuyugan, Lori	MS	Research Associate
De Both, Matthew	BS	Bioinformatician
Geiger, Philipp	MS	Research Associate
Henderson-Smith, Adrienne	BS	Research Associate
Huentelman, Matthew	PhD	Principal Investigator
Jespen, Wayne	BS	Research Associate
Lechuga, Cynthia	MBA	Sr. Grants & Contract Administrator
Liang, Winnie	PhD	Co-Investigator
Piras, Ignazio	PhD	PostDoc Fellow/Bioinformatician
Reiman, Eric	MD	Consultant
Richolt, Ryan	BS	Research Associate
Siniard, Ashley	BS	Research Associate
Sekar, Shobana	MS	Bioinformatician, PhD student
Van Keuren-Jensen, Kendall	PhD	Co-Investigator
Wolfe, Amanda	BS	Research Associate

UNIVERSITY OF ARIZONA

Institutional Abstract

Researchers at the University of Arizona (UA) are engaged in collaborative, multi-disciplinary programs of research focused on advancing our understanding of the major risk factors for brain aging and age-related neurodegenerative disease, their underlying neural substrate, and ways to delay or prevent cognitive aging and dementia. To accomplish these goals, UA investigators representing 12 departments and institutes, including researchers in the fields of neuroimaging, cognitive and behavioral neuroscience, neuropsychology, psychiatry, neurology, pharmacology, and statistical analysis are involved in these research programs. Projects apply a range of scientific approaches from basic neuroscience to cognitive science to clinical intervention, in studies that translate across species with humans and non-human animal models of aging and age-related disease. A major component of this research uses magnetic resonance imaging (MRI) as a cross-cutting method to measure brain function, structure, and connectivity in aging and age-related, neurodegenerative disease.

A translational approach to research is undertaken that spans multiple laboratories and methodologies to address clinical and basic research aims concerning the effects of healthy and pathological aging, including 1) to investigate the neural systems and associated cognitive processes that are altered in the context of aging and age-related disease, 2) to track brain changes and cognitive abilities during aging, 3) to evaluate how genetic and other health risk factors influence brain aging and cognitive decline, 4) to develop and test new imaging methods to aid early detection and the tracking of brain changes due to aging and disease, 5) to develop and test strategies to improve cognitive function during aging, and 6) to provide information to the community to advance understanding about aging, cognitive decline, and age-related neurodegenerative disease.

Program-related activities at the UA include three major areas of research:

1. Imaging methods development. Our researchers are developing and implementing new MRI techniques and statistical analysis methods that may prove useful in examining brain structure, function, connectivity, and pathology in both human and non-human animal models of aging and age-related disease. Methods are developed with high resolution MRI for quantitative, non-invasive measurements in humans, non-human primates, and wild-type and transgenic rodents.

2. fMRI studies of memory and aging. These studies utilize functional MRI in order to better understand the neural basis of memory and other cognitive changes across the normal adult lifespan, and compensatory or adaptive strategies that lead to better memory function.

3. Early detection of healthy and pathological aging. The application of several MR methods including high-resolution anatomical imaging, diffusion MRI, perfusion MRI, and MRI measures of functional connectivity for the early detection, diagnosis, and treatment of cognitive and psychological impairments associated with cognitive aging and Alzheimer's disease (AD). The projects focus on identifying early neurocognitive and biological markers that may signal the early effects of AD prior to the onset of cognitive symptoms. MR methods are also being applied to understand factors that increase risk for AD, including genetics, familial risk, health

factors such as hypertension, head injury, and obesity, and those that may decrease risk for AD, such as exercise, education, and the use of anti-inflammatory drugs.

This program of research is complemented by interactions with other UA investigators and programs. Other complementary areas of activity at the UA include research on the underlying biological mechanisms of normal age-related alterations in memory as part of the Arizona Evelyn F. McKnight Brain Institute, studying the longitudinal effects of aging on memory processes in older adults with and without increased risk for AD, investigating the cognitive effects of Down syndrome as a cohort with increased genetic risk for the development of AD pathology, and the development of novel radiotracer imaging methods to detect pathology in transgenic animal models of AD. In addition, UA researchers participate in complementary efforts to support the Arizona ADC with recruitment and longitudinal follow up of individuals with mild cognitive impairment, AD, and other forms of dementia, with administrative support for a pilot grant program and the center Internal Scientific Advisory Committee, with an Annual Conference on Successful Aging to support education and outreach in the Tucson community and with a Diversity Outreach Program to enhance community outreach, education, and research participation by underserved minority groups in Arizona.

UNIVERSITY OF ARIZONA

Key Personnel

Name (last, first)	Degree	Role on project
Ahern, Geoffrey	MD	Investigator; Neurology, Psychology, Psychiatry, Evelyn F. McKnight Brain Institute
Alexander, Gene	PhD	Investigator; Psychology, Psychiatry, Neuroscience, Evelyn F. McKnight Brain Institute
Barnes, Carol	PhD	Investigator; Psychology, Neurology, Neuroscience, Evelyn F. McKnight Brain Institute
Billheimer, Dean	PhD	Investigator; Epidemiology and Biostatistics
Brinton, Robbie	PhD	Investigator, Center for Innovation in Brain Science, Pharmacology, Neurology, Psychology, Evelyn F. McKnight Brain Institute
Edgin, Jamie	PhD	Investigator; Psychology
Erickson, Robert	MD	Investigator, Pediatrics
Fernandez, Fabian	PhD	Investigator; Psychology, Neurology, Psychology, Evelyn F. McKnight Brain Institute
Furenlid, Lars	PhD	Investigator; Optical Sciences, Medical Imaging
Glisky, Elizabeth	PhD	Investigator; Psychology, Evelyn F. McKnight Brain Institute
Gmitro, Art	PhD	Investigator, Biomedical Engineering, Medical Imaging, Optical Sciences
Hay, Meredith	PhD	Investigator; Physiology, Psychology, Evelyn F. McKnight Brain Institute
Hishaw, G. Alex	MD	Investigator; Neurology, Psychiatry
Klimentidis, Yann	PhD	Investigator, Epidemiology and Biostatistics
Konhilas, John	PhD	Investigator, Physiology
Koshy, Anita	MD	Investigator; Neurology, Immunobiology, Evelyn F. McKnight Brain Institute
Lussier, Yves	MD	Investigator, Medicine
Matsunaga, Terry	PhD	Investigator, Medical Imaging
Mohler, Jane	PhD	Investigator, Arizona Cancer Center
Nadel, Lynn	PhD	Investigator; Psychology, Cognitive Sciences, Evelyn F. McKnight Brain Institute
Raichlen, David	PhD	Investigator; Anthropology
Rapcsak, Steven	MD	Investigator; Neurology, Psychology, Speech/Language and Hearing, Evelyn F. McKnight Brain Institute
Romanowski, Marek	PhD	Investigator, Biomedical Engineering
Ryan, Lee	PhD	Investigator; Psychology, Neurology, Neuroscience Program, Evelyn F. McKnight Brain Institute
Su, Judith	PhD	Investigator, Optical Sciences, Chemistry and

		Biochemistry
Sweitzer, Nancy	MD, PhD	Investigator, Sarver Heart Center
Trouard, Theodore	PhD	Investigator; Biomedical Engineering, Medical Imaging, Evelyn F. McKnight Brain Institute
Watts, George	PhD	Investigator, Genomics Shared Service, Cancer Center

UNIVERSITY OF ARIZONA

COLLEGE OF MEDICINE – PHOENIX

Institutional Abstract

The University of Arizona (UA) has the only allopathic medical schools in the state of Arizona, with two campuses: the Tucson campus located at the Arizona Health Sciences Center and University Medical Center, and the Phoenix campus located on the Phoenix Biomedical Campus (PBC). The UA College of Medicine – Phoenix is part of the University of Arizona, and is governed by the Arizona Board of Regents. UA College of Medicine – Phoenix shares the PBC site with the UA College of Pharmacy, College of Public Health, Eller College of Management, several Allied Health programs from Northern Arizona University, and Arizona State University, and the Translational Genomics Research Institute.

The UA College of Medicine – Phoenix mission is to inspire and train exemplary physicians, scientists and leaders to optimize health and healthcare in Arizona and beyond. Founded in 2007, UA College of Medicine – Phoenix is a full, four-year program. It was initially established as a branch campus of the long-standing UA College of Medicine in Tucson, and now is approaching full, independent accreditation by the Liaison Committee on Medical Education (LCME). At its present size, the college matriculates and graduates approximately 80 new doctors each year. Enrollment at the Phoenix campus will ultimately be increased to 120 students per class. The UA College of Medicine – Phoenix also provides graduate training opportunities in Masters and PhD programs via the Clinical Translational Science program, and combined MD/PhD and MD/MPH degrees.

To inspire life-long learning and critical thinking, the UA College of Medicine – Phoenix requires that each student conduct a Scholarly Research Project over their four years of training. Students are paired with a physician/scientist mentor to conduct this project and culminates with a thesis as part of the graduating requirement.

The college is positioned uniquely to accelerate the biomedical and economic engines in Phoenix and the State by leveraging our relationships with key clinical and community partners. As part of this goal, the UA College of Medicine – Phoenix has developed and continues to reinforce a number of cooperative agreements and collaborations with local institutions. Examples of these interactions have resulted in the development of the Translational Neurotrauma Research Program between the UA College of Medicine – Phoenix, Phoenix Children’s Hospital, Barrow Neurological Institute of St. Joseph’s Hospital and Medical Center, and the Phoenix Veterans Administration to become the destination for neurotrauma research, training and clinical care. In the area of wellness for women, a grant funded by the Flinn Foundation based on collaborative efforts between St. Joseph’s Hospital and Medical Center, UA College of Medicine – Phoenix will evaluate the vaginal microbiome that may be responsible for pathological conditions such as cancer or sexually transmitted diseases. More recently this program is engaging with other partners at Scottsdale Healthcare Shea Medical Center, Maricopa Integrated Health System, and Banner University Medical Center Phoenix.

**UNIVERSITY OF ARIZONA
COLLEGE OF MEDICINE – PHOENIX
Key Personnel**

Name (last, first)	Degree	Role on project
Lifshitz, Jonathan	PhD	PI
Rowe, Rachel K.	PhD	Co-PI
Ziebell, Jenna M.	PhD	Investigator
Tallent, Bret R.	LATG	Laboratory Manager

Project Progress Reports

Project Progress Reports

Arizona State University

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Pulses of estrogen as a transient hormone therapy in young versus old age. Heather Bimonte-Nelson, PhD. Arizona State University; Arizona Alzheimer's Consortium.

Specific Aim: The specific aim of this study is to evaluate age differences in responsiveness to cyclic pulses of estrogen for cognitive efficacy.

Background and Significance: It is estimated that by the year 2050 there will be 88.5 million people in the United States over the age of 65; over half of these individuals will be postmenopausal women (U.S. Census Bureau, 2010). The onset of menopause has been associated with symptoms that affect quality of life, and hormone therapy is given to attenuate menopause-induced symptoms (Curtis & Martins, 2006; Sherwin, 1988). A NIA-sponsored workshop was held in 2010 to elucidate the impact of the menopause transition on cognition and mood (Maki et al., 2010). Outcomes were several-fold, including that "identifying a cognitively neutral or beneficial combination therapy for the treatment of menopausal symptoms in naturally menopausal women is an important goal for future research" (Maki et al., 2010, p2). A critical step toward this goal is defining the hormone regimens to be used in hormone therapy treatments during menopause. This is important now more than ever given recent controversies driving many new hypotheses regarding personalization of hormone therapy, and finding hormone therapies that provide the health benefits without the risks.

We have shown that estrogens have beneficial effects when given tonically in young adulthood or middle age (Bimonte and Denenberg, 1998; Bimonte-Nelson et al., 2006; Talboom et al. 2008). However, we showed that in old age estrogen was no longer beneficial when given tonically (Talboom et al., 2008). Recent evidence from our laboratory has shown profound benefits of estrogens in middle age when given cyclically via a daily injection, in a pulsatile manner (unpublished observations). These findings build on our prior work showing similar beneficial effects with estrogen injections given every other week during middle age (Bimonte-Nelson et al., 2006). Based on data evaluating estrogen receptor recycling in cyclic versus tonic treatments (Bimonte-Nelson et al., 2010; Blaustein, 1993; Kassis and Girski, 1981), we anticipate that cyclic injections in old age could yield beneficial effects. In fact, cyclic estrogen treatment could provide many benefits of hormone therapy, but with less overall exposure, thereby potentially attenuating some of the risks associated with hormone treatments. The current study will directly evaluate the impact of cyclic, pulsed estrogen treatment on memory performance in young versus aged animals.

Experimental Design and Methods: Rats will be young or aged at study initiation. Fisher-344CDF rats born and raised at the National Institute on Aging colony will be used. All procedures are approved by the Arizona State University Institutional Animal Care and Use committee and adhere to National Institutes of Health standards. All rats will receive surgery to remove ovaries (Ovx). The E2 dose per injection will be based on previous studies used in our laboratory, resulting in levels within the range of what circulates endogenously (Bimonte-Nelson et al., 2010). Rats will be randomly assigned to treatment group, and a battery of maze tests will be given.

Proposed One-Year and Long-Term Outcomes: Our long-term overarching goal is to understand how ovarian hormone loss and replacement affects cognitive and brain aging in the rat model. The ultimate goal is to translate effects to humans so that we can optimize women's health during aging. The one-year outcome will be a manuscript submitted for publication by the end of the year of funding. These data will also allow considerations for future hormone mechanism and/or pharmaceutical studies. We anticipate that these data will be incorporated into a larger RO1 application.

Progress to Date: The experiment is ongoing and final data will be analyzed within the next few months. Manuscript write-up and submission will follow shortly thereafter.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Testing a putative mechanism of menopause and ovarian steroid effects on cognition.
Heather Bimonte-Nelson, PhD. Arizona State University; Arizona Alzheimer's Consortium.

Specific Aim: The specific aim of this study is to evaluate whether the IGF1 signaling pathway drives menopause- and estrogen therapy- induced effects on cognition.

Background and Significance: It is estimated that there will be 88.5 million people in the United States over the age of 65 by the year 2050, and, notably, over half of these individuals will be postmenopausal women (U.S. Census Bureau, 2010). Alterations and an eventual drop of ovarian hormone levels characterize menopause. Menopause onset has been associated with symptoms that affect quality of life, and estrogen-containing hormone therapy is given to attenuate menopause-induced symptoms (Curtis & Martins, 2006; Sherwin, 1988). Ascertaining a cognitively beneficial (or even neutral) hormone therapy for the treatment of menopausal symptoms is critical (Maki et al., 2010). An important step toward this goal is understanding the underlying mechanistic effects of how the hormone milieu impacts cognitive and brain changes during menopause, including effects of estrogens and progestogens. This is important now more than ever given recent controversies driving many new hypotheses regarding personalization of hormone therapy, and finding hormone therapies that provide the health benefits without the risks.

We have shown that estrogens, including the most potent estrogen in women and rats, 17 β -estradiol (E2), exert beneficial effects on cognition in rats during young adulthood or middle age (Bimonte and Denenberg, 1998; Bimonte-Nelson et al., 2006; Talboom et al. 2008). E2 triggers signal transduction cascades in cells along various pathways. One more recently identified pathway, whereby E2 signals intracellularly, is via the IGF1 receptor. IGF1 receptor activation signals AKT/PI3K downstream (Wang et al., 2015). These signaling events then lead to a host of downstream effects that have been implicated in various cellular and systems processes, including cognition (Talboom et al., 2015). Collectively, this model suggests that E2 can signal via IGF1 receptors, leading to intracellular signaling cascades resulting in changes in cognition. Here, the contributions of IGF1 receptor signaling will be methodically tested for its effects on cognition in our rat surgical menopause model with and without E2 and a progestin commonly used in hormone therapy and oral contraceptives.

Experimental Design and Methods, and Progress to Date: Forty female middle-aged Fisher-344CDF rats from the National Institute on Aging colony were ordered and used. All procedures adhered to National Institutes of Health standards and were approved by the Arizona State University Institutional Animal Care and Use committee. Rats received ovariectomy (Ovx) whereby ovaries were surgically removed. The Ovx model simulates surgical menopause in rodents, and provides a 'blank' slate of circulating ovarian hormone milieu that can be utilized to assess specific effects of exogenously administered hormones. Animals were then administered daily subcutaneous injections of either vehicle, E2 (E2-Only), Levonorgestrel (Levo-Only), or E2 plus Levo (E2+Levo). The treatment manipulations were systematically designed to assess

the effects of *chronic* estrogen-based hormone therapy where each clinically available component is administered individually (E2-Only and Levo-Only) and in combination (E2+Levo). All animals were tested on behavioral tasks to assess spatial working and reference memory (water radial arm maze, WRAM) and spatial reference memory (Morris water maze, MWM). Following behavior, animals were euthanized and brain regions involved in cognitive function, including the frontal cortex, dorsal hippocampus, ventral hippocampus, entorhinal cortex, and perirhinal cortex, were dissected and immediately frozen for western blot analysis. **The entire behavioral portion of the study has been completed, and brains are being processed at this time.** Specifically, western blot analyses are being conducted to probe for IGF-1 β receptor expression to determine how chronic treatment of E2 alone, Levo alone, and the combination of E2 plus Levo impact the expression of this receptor. Once western blots are completed for IGF-1 β receptor expression, in addition to probing main effects of treatment, Pearson r correlation analyses will be run in order to assess whether there is a relationship between cognitive performance on the WRAM and the MWM and IGF-1 β receptor levels in brain regions involved in cognitive function.

Proposed One-Year and Long-Term Outcomes: *Our long-term overarching goal* is to understand how menopause and hormone milieu affects cognitive and brain aging using rodent models. Our ultimate goal is to translate effects to humans so that we can optimize women's health during aging. *The one-year+ outcome* will be a manuscript submitted for publication by 18 months after initiation of funding. These data will additionally allow considerations for future hormone mechanistic and/or pharmaceutical studies to understand the underlying mechanisms of menopause, hormone, and aging effects on the brain and cognition. We anticipate that these data will be incorporated into a large competitive grant application to be submitted in the summer of 2017.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Development & evaluation of social media-based testimonials targeting potential ADRD research participants within the Latino/Hispanic community. David W. Coon, PhD. Arizona State University; Arizona Alzheimer's Consortium (Note: This project will be conducted in coordination with *Leslie Baxter, PhD, Barrow Neurological Institute*).

Specific Aims:

- 1) **Create 7-8 full length testimonials related to ADRD and aging research that target the Latino/Hispanic community.** These testimonials will be designed to help the Arizona Alzheimer's Consortium (AAC) in developing its relationship with and outreach to the Latino community. Testimonials will be combined into an overall "supercut" as well as spliced into various clip lengths for mainstream media and social media channels such as:
 - a) Twitter with a quote and photograph/link to short clip (10-sec);
 - b) Facebook with an auto play (viewers click to hear the audio) with medium sized clip (15-sec);
 - c) Instagram with 30-sec clip (although AAC doesn't currently have Instagram; this seems a good time to start using that visual, "no language barrier" medium);
 - d) YouTube with all those clips (plus a potentially longer "full story" ~90sec clip) and allow viewers to embed them on other sites;
 - e) Create an active social media window on the AAC website to highlight each post.
- 2) **Develop and launch a campaign using the testimonial clips and track views on the various social media platforms (e.g., Facebook, Twitter, YouTube).**
- 3) **In tandem with Aims 1 & 2, use focus groups with participants from the Latino community to provide a formative and summative evaluation at key project time points through the following:**
 - a) Focus groups would be held three times: prior to creating/filming of testimonials to determine what would be compelling to see/hear about within testimonials to encourage Latino families to participate in research; after development of a "beta version" of testimonials to help refine the final clips and supercut; and, prior to distribution via the project's campaign to gather feedback on ways to optimize reach (e.g., key social media platforms and mainstream media venues);
 - b) Transcribe and code the data collected in "a" through an established thematic analysis approach to identify key themes and sub-themes to support publication(s);
 - c) Collect quantitative sociodemographic and work-life data through a structured questionnaire distributed to participants prior to the focus group; and
 - d) Combine the information gathered and analyzed in Aim 3 a-c to complement the tracked views collected in Aim 2 and serve as preliminary data for publications and grant applications.

Background and Significance: A recent review and meta-analysis of ethnic/racial differences in dementia treatment, care, and research found consistent evidence that minority groups, including Latinos, accessed dementia diagnostic services later than their non-Hispanic whites (NHWs). After diagnosis, they were also less likely to access research trials. Hispanics, in particular, reported longer duration of memory loss at referral to dementia services. The authors concluded that this was due to later presentation to services. Latino/Hispanic family caregivers and other members of the family are critical “cultural brokers” in accessing treatment, care, services and research opportunities for people with ADRD. As part of another project, we recently ran six focus groups in Spanish and English with 51 self-identified Latino participants to uncover ways to recruit Latino families earlier in ADRD’s progression. Three focus groups included 26 Latino family/informal caregivers (mean age = 50.9; $SD = 17.5$) who had been caregiving for an average of 2.6 years ($SD = 2.5$) and were currently spending an average of 5.9 hrs a day ($SD = 4.0$) actually doing things for their care recipient (CR) and 18.4 hrs a day ($SD = 8.2$) “being there” or feeling “on duty.” Caregivers were predominantly women (80.8%) who were the CR’s spouse (11.5%), adult child (42.3%), other relative (15.2%), or friend (30.8%), with over half (52%) reporting less than a high school education. The remaining focus groups involved 25 Latino staff/professionals (mean age = 49.7 years; $SD = 10.3$; 64% high school graduates, 16% college graduates) who were experienced in working with Latino families of older adults ($M = 8.4$ years; $SD = 4.2$) and were currently employed full- (36%) or part-time (64%) in various positions (e.g., outreach coordinator, certified nursing assistant, *promotora*, senior companion). Results suggested a number of strategies that were effective with Latino participants (e.g., remove language barriers and provide conceptual translations; emphasize use of *memory loss* vs. *AD* to break down stigma; value interpersonal relations that provide the opportunity to get to know one another and the project team; engage *promotores*; and use social media to reach across generations. According to 2013 and 2015 reports from the Pew Research Center, Hispanics/Latinos use mobile and social networking technologies in proportions similar to those of other ethnic and racial groups. Moreover, Hispanics/Latinos of all ages use social media, especially Facebook, as much as if not more than other racial/ethnic groups. Finally, a higher percentage of Hispanics/Latinos use Instagram than their non-Hispanic White counterparts.

Preliminary Data and Plan: This is a collaboration between ASU (Dr. Coon) and BNI (Dr. Baxter). Both Drs. Coon and Baxter have bilingual-bicultural staff to conduct the study. Dr. Coon’s group has expertise in organizing and executing studies with Latino caregivers, and Dr. Baxter has access to Latino patients and caregivers who will serve as the subjects of the videos. Funds will also provide services of Michael Terrill of Off Melrose, who has experience with the Alzheimer’s Consortium and will produce the products. The methodology for this project is: (1) Recruit and enroll up to 30 professionals/staff working with Latino families with impaired older adults and 30 family members caring for Latino older adults. (2) Conduct a series 6 audiotaped focus groups across the project with 2 conducted prior to development of the testimonials; 2 after development of the “beta version” of the testimonials; and 2 prior to distribution via the project’s campaign. (3) Transcribe and verify the transcriptions and double entry verify the structured data. (4) Conduct qualitative and mixed method analyses to integrate the data collected. Our approach encompasses both constant comparative analysis using grounded theory and content analysis. To do this, we will assemble and compare all text references to a concept. Four types of analysis will be conducted: a) *descriptive* analyses that helps differentiate among groups (e.g., spouse/non-spouse); b) *thematic* analyses that elaborate the structures of the basic constructs (e.g., mistrust of research); c) *comparative* analyses that clarify differences among subgroups;

and d) *theory building* that occurs once the conceptual scheme is suitably differentiated. (5) Integrate the focus group data with tracked views from the campaign to serve as preliminary data for publications and grant applications.

Proposed One-Year and Long-Term Outcomes: 1) Data analyses would yield both professional presentations at meetings like the Gerontological Society of America, the American Society on Aging or American Psychological Association as well as the submission of a publication related to the integration of the data to venues like the *Clinical Gerontologist* or *The Gerontologist (Practice Concepts Section)*. 2) Results of this project will help in the recruitment sections of grants for multiple PIs, not just Drs. Coon and Baxter. 3) Based on the project's findings, the team will also explore submitting either an R21 or an R01 and/or explore opportunities with other ADC sites for funding comparing various outreach/recruitment strategies utilizing social media platforms.

Year-End Progress Summary: The project is on track for completion 6/30/17. Focus groups have been conducted in Spanish and English with family caregivers and professionals assisting older adults with memory impairment and their family caregivers. These focus groups are integral to the project assisting with testimonial development and refinement. Final focus groups to support product dissemination will be conducted after completion of an overall "supercut" and other clip lengths for mainstream media and social media channels. All focus group data is transcribed, verified, and integrated with quantitative data. Critical analyses of the focus group data used constant comparative analysis and content analysis in four types of analysis: *descriptive* analyses to differentiate among groups; *thematic* analyses to elaborate on basic constructs; *comparative* analyses to clarify subgroup differences; and *theory building*. The project data will serve as preliminary data for publications and grant applications, and will be augmented with tracked views from future media campaigns utilizing the testimonial clips.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

The effect of hormone status on synaptic transcripts in aging and Alzheimer's disease.
Diego Mastroeni, PhD, Paul D. Coleman, PhD. Arizona State University; Arizona Alzheimer's Consortium.

Specific Aims:

Aim 1: Isolate cholinergic neurons from the basal forebrain in an aging female cohort and in AD female brain.

Aim 2: Quantify expression of selected genes related to the synapse and to hormone synthesizing and receptor systems as a function of age in laser captured neurons from the basal forebrain. We will then use ANOVA to determine significant expression changes affected by age and gender followed by regression analyses (including multiple regression) to quantify the relation between hormone status and expression of synaptic genes.

Background and Significance: It is known that women have a higher incidence of Alzheimer's disease (AD) than men, even after correction for the greater longevity of women. It is also known that Alzheimer's disease on the maternal side increases risk for offspring more than does AD on the paternal side. Considerations such as these have led to intense interest in potential molecular and other differences between the male and female brain, with special emphasis on aging in many studies. The brief evidence cited above supports the concept of gender related brain regional differences in expression of a variety of molecules important to synaptic structure and function and, consequently, behavior especially cognition. However, important evidence testing this concept is lacking. In this proposal, we address this lack with brain regional studies at the level of single neurons defined as to systemic and local hormonal status and expression of genes related to the synapse. Earlier, we described age-related gender differences in gene expression. Other, unpublished data from this study showed that the human male hippocampus exhibited an age-related increase in expression of synaptic genes in the 60s, while females showed no such increase. On the other hand, females in the 60s showed an increase in expression of synaptic genes in the primary somatic sensory cortex while males showed no such increase. Our hypothesis is that the age-related sex/brain region differences in expression of synaptic genes are a function of brain regional differences in hormonal systems. It has been postulated that hormonal changes in post-menopausal women directly affects the cholinergic system, of the basal forebrain, which are selectively vulnerable during aging and age related diseases. The cholinergic basal forebrain (CBF) connectome consists of four subfields which innervate select cortical regions in a topographical pattern; and all of which may be affected by hormones. Cortical cholinergic denervation can undermine not only the physiological integrity of synaptic transmission but also the capacity for structural neuroplasticity. Loss of this capacity most likely would interfere with the acquisition of new knowledge. We would test this hypothesis by profiling the expression of laser captured cholinergic neurons in basal forebrain in an aging human cohort and in AD brain. We will quantify expression of genes related to hormone synthesizing and hormone receptor systems and correlate these data to expression profiles of synaptic genes.

Preliminary Data / Experimental Design and Methods:

Overall Design: Snap-frozen AD and ND basal forebrain frozen blocks will be obtained from the Banner Sun Health Research Institute's NIA AD Center Brain Bank. The brain bank at BSHRI is one of the world's best, dedicated to the highest standards (average RNA integrity; RIN 8.5) and postmortem interval (2.8 hours). A minimum of 10 AD females and 10 ND female cases (n=5 55-65; n=5-66-76) and 5 AD males and 5 ND females, all with detailed clinical history including hormonal drug history, if any, and pathological annotation required by NIA AD Centers will be obtained. PMIs will be well-matched and will not exceed 4 hours in any case. All samples will also be matched for gender, race, age, PMI and APOE status. Blocks will be sectioned at 15um and a minimum of 200 neurons per case per brain region will be selected by single cell laser capture micro dissection. Cholinergic neurons in the basal forebrain will be recognized on the basis of location, cell size and shape. Material from laser capture will then be assayed for synaptic, and hormonal genes by quantitative PCR (qPCR). We expect that hormonal alterations will be significantly correlated with synaptic alterations, suggesting that age effects on hormonal pathway play an important role in the expression of synaptic genes.

Analysis and Anticipated Results: To demonstrate potentially causal relationships between Hormonal and synaptic transcripts as a function of age and brain region, we will perform 2-way ANOVA, with sex as the first factor, and and brain region as the as the second factor. The dependent variable will be age. A significant main effect of age, followed by specific comparisons (by t-test) would confirm our initial observations and hypothesis that hormonal status influences the expression of synaptic transcripts differentially in males compared to women of similar ages in different brain regions. In order to evaluate how these hormonal transcripts may influence the expression the aforementioned genes, we will perform regression analyses, including multiple regression analyses. We expect that the analyses will show that hormonal transcripts and expression of synaptic transcripts will be significantly related. We further predict that the most significant relationships will be between hormone receptors and synaptic transcripts. These findings will therefore provide specific therapeutic targets for potentially reorganizing a causal mechanism for aging differences between males and females. Translational contexts that can be moved swiftly forward experimentally to directly manipulate hormone profiles in a sex-specific fashion in future experiments.

Innovation/Significance/One-year long term goal: The proposed studies are the first to address the impact of expression of hormone regulating genes on the expression of synapse genes in the basal forebrain. The multidimensional approach proposed here will lay the foundation for future directions taking an unprecedented translational perspective. After the completion of the study this will allow enough preliminary data for a subsequent R21/RO1. In addition to grant opportunities these findings will lead to a manuscript in a respected peer-reviewed journal. By focusing on gene expression of individual or small numbers of cells more precision becomes possible. Thus, the work proposed here will allow more precise definition of relationships between selected hormonal properties, and the effects they have on the expression of synaptic genes during aging and disease.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Assessing the role of necroptosis in Alzheimer's disease. Salvatore Oddo, PhD, Antonella Caccamo, PhD. Arizona State University; Arizona Alzheimer's Consortium.

Project Description: Alzheimer's disease (AD), the most common neurodegenerative disorder, is characterized by severe neuronal loss. Despite the tremendous progress made towards the understanding of the pathogenesis of AD, the mechanisms underlying neuronal loss remain elusive. Necroptosis, a programmed form of necrosis, is executed by the activation of the mixed lineage kinase domain-like 1 (MLKL-1) protein, which is activated by receptor-interactive protein kinases (RIPK) 1 and 3. Our preliminary data indicate that necroptosis is activated in human AD brains. This novel and exciting finding may answer a key, and yet unresolved question: which mechanisms govern cell loss in AD? To address this question we propose the following Specific Aim: Determine the involvement of necroptosis in Alzheimer's disease

Progress to date: We have successfully completed all of the experiments proposed in this grant application. We found that genetically increasing necroptosis in a mouse model of AD induces neuronal degeneration to a greater degree than in non-transgenic controls, indicating that mice with AD-like pathology are more prone to necroptosis-induced cell loss. Given these exciting data, we performed additional experiments and found that necroptosis is activated in postmortem human AD brains, where it positively correlates with Braak stage and inversely correlates with brain weight and cognitive scores. Together our results provide compelling evidence that necroptosis is activated in AD and thus may contribute to neurodegeneration in this insidious disorder. We anticipate that our findings will spur a new area of research in the AD field focused on further detailing the role of necroptosis in AD and developing new therapeutic strategies aimed at blocking RIPK1 and/or MLKL activation. These data are currently under review in Nature Neuroscience. Additionally, we have leveraged these novel and exciting data in two ways: (i) we wrote a R01 where we focus on dissecting the mechanisms underlying the role of necroptosis in AD (this grant application was submitted on February 5, 2017); (ii) we filed a provisional patent application titled "Necroptosis Signaling as a Therapeutic Target for Alzheimer's disease". Provisional Application No. 62356983.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Protein variants as blood based biomarkers for neurodegenerative disease and brain injury. Michael Sierks, PhD. Arizona State University; Arizona Alzheimer's Consortium.

Specific Aims: The specific aims of this project are:

- 1) Assay sera samples of AD and traumatic brain injury patients for presence of disease related variants of beta-amyloid, tau and alpha-synuclein using novel sandwich ELISA
- 2) Correlate the levels of protein variants with disease

Background and Significance: Biomarkers that can facilitate presymptomatic diagnosis of Alzheimer's disease (AD) and distinguish it from other dementias would be extremely valuable clinical tools. The primary constituents of the two major pathological features of AD, amyloid plaques and neurofibrillary tangles, are respectively, the amyloid beta (A β) and tau proteins. Substantial efforts have been expended to identify biomarkers for AD and other neurodegenerative diseases, where the most promising biomarkers for AD to date are variants of A β and tau, in particular the 42 amino acid variant of A β (A β 42) and phosphorylated variants of tau [1-4]. While A β and tau biomarkers suffer from a relatively low sensitivity and specificity for diagnosing AD, they still hold great promise for early detection of AD as changes in CSF levels of A β 42 and tau have been shown to occur well before symptoms develop, up to 25 years earlier for A β 42 [5]. While CSF Protein Variants as Blood Based Biomarkers for Neurodegenerative Disease and Brain Injury and tau levels correlate with AD, the vast majority of studies have focused on detection of non-toxic monomeric forms of A β 42 and phosphorylated tau rather than on detection of the actual toxic protein species responsible for neurodegeneration. Both A β and tau can exist in a variety of different forms and aggregate morphologies and numerous studies indicate that specific oligomeric forms of both A β [6-9] and tau [10-12] are involved in neuron degeneration and spread of toxicity, and can interfere with important functions such as long term potentiation. Therefore a more powerful and sensitive diagnosis for AD and other dementias would be to specifically detect the individual protein species that are involved in disease onset and progression. Because misfolded and aggregated variants of A β and tau are intimately involved in the progression of AD, detection of specific variants of these proteins in CSF and/or serum has great promise for an early definitive diagnosis of AD and for following progression of the disease or effectiveness of different therapeutic regimens. In a parallel manner, misfolded toxic oligomeric variants of the protein alpha-synuclein (a-syn) have been correlated with the onset and progression of Parkinson's disease (PD) and related synucleinopathies [13-15] so detection of the relevant toxic oligomeric a-syn species should facilitate early diagnosis of synucleinopathies such as PD and Dementia with Lewy Bodies (DLB) and help distinguish these diseases from AD. Recent evidence has suggested that cytoplasmic misfolding and aggregation of TAR DNA binding protein 43 (TDP-43) associates with the pathology observed in a high percentage of FTD and ALS cases [16] and more recently in other neurodegenerative diseases including AD and traumatic brain injury. TDP-43 is also prone to misfold and form aggregate species, where disease associated TDP-43 mutations similar to A β and a-syn aggregate more readily [17]. Therefore selected toxic aggregated variants of A β , tau, a-syn and TDP-43 all have potential value as early and sensitive diagnostic biomarkers

to distinguish different neurodegenerative diseases, and also as promising therapeutic targets. In addition, we have shown that levels of these protein variants are present in sera of patients that have suffered traumatic brain injury. The presence of the different protein variants may account for the increased incidence of neurodegenerative disease in patients that suffer brain trauma.

Quantification of serum levels of the actual toxic protein species involved in disease onset and progression should provide a much more sensitive and powerful set of biomarkers for early detection and staging of neurodegenerative diseases such as AD and for evaluating damage following brain injury. Detection of different toxic variants of A β , tau, a-syn and TDP-43 in serum should provide a powerful tool to facilitate early diagnose of different neurodegenerative diseases, for example presence of toxic oligomeric A β aggregates could be indicative of early AD, oligomeric A β and tau aggregates could indicate a later stage AD, only oligomeric tau could indicate a tauopathy such as Frontal Temporal Dementia, oligomeric a-syn could indicate PD, and oligomeric A β and a-syn could indicate DLB. While detection of these specific protein aggregate species has great promise, such studies have not been feasible due to the low concentrations of the target aggregate species in sera samples and the poor specificity of reagents for the different aggregated species. To overcome this problem, we have developed novel protocols in our lab that enable us to generate reagents that very selectively recognize individual protein morphologies and we also developed a simple novel sandwich ELISA that enables femtomolar or better detection of target antigens in biological samples[18]. Here we will utilize a panel of such antibody based (nanobody) reagents generated in our lab that selectively recognize different toxic aggregated species of A β , tau, a-syn and TDP-43 to demonstrate that the presence of specific toxic aggregate proteins species in serum are very selective biomarkers for distinguishing AD from other dementias.

Progress to Date:

Aim 1). Assay sera samples of AD and TBI patients. We are continuing to assay samples of control, AD and TBI patients with a panel of antibodies that we generated that selectively bind AD related variants of A β , tau and a-syn. TBI and AD samples are significantly different than control samples. Still collecting data with additional tau variant nanobodies.

Aim 2). Correlate the levels of protein variants with disease. We are still analyzing data to correlate different protein variants with disease.

Proposals: R01 awarded from NIH (R01 AG054048-01) in collaboration with Dr. Caselli. Additional proposals to NIH to study structural conformations of beta-amyloid aggregates and ADDF and ALS foundations to develop biomarkers for neurodegenerative diseases have not been funded to date.

Commercialization potential: We have started a company, Bloom Biotherapeutics to use our nanobodies as potential therapeutic and diagnostics for neurodegenerative diseases. Pat Mallon (CEO) and partners at AZTe and La Jolla Institute of Allergy and Immunology are developing a business plan. In addition, we are in discussions with Abbvie and Lilly to study the potential therapeutic and diagnostic value of our antibodies.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Plasticity of odor coding in the mouse olfactory bulb. Brian H Smith, PhD. Arizona State University; Arizona Alzheimer's Consortium.

Specific Aims: NIH-funded research in the PI's laboratory using the honeybee as a model for olfactory processing has led to the identification of the effects of nonassociative and associative plasticity on the spatiotemporal olfactory code. The guiding hypothesis of this work is that this plasticity is critical to enhance detection and discrimination of odors. However, in the honey bee the critical modifications to circuit and cellular components that give rise to identified changes in population-level coding of the output have not been identified, except by computational modeling. There are limitations in the honeybee in regard to molecular manipulation of cellular components to test hypotheses from the modeling. Therefore, development of population coding studies in an animal model in which cellular components can be more easily studied and manipulated via pharmacological and/or molecular means is necessary to test the function and mechanism of olfactory plasticity.

Use of a mouse model for these studies will have the capacity to unite well understood cellular mechanisms of plasticity in the olfactory bulb (OB) with changes in the population-level code relayed by mitral and tufted cells (M/Ts) to higher brain centers. Mechanisms of non-associative and associative plasticity in early processing of the honeybee brain are identical to plasticity identified to date in identified cellular components of the OB and piriform cortex. However, the consequences of changes in these cellular components for population codes have not been investigated like they have in the honey bee. The first step in this research will be to evaluate changes in population spatiotemporal codes across M/T outputs as a result of different types of associations with odors. Specifically, from the honeybee work we predict that the neural representations of outputs from the OB should be pushed apart (made more distinct) or collapsed (made less distinct) as odors are associated, or not, with specific meanings, such as reward.

Experiments combining standard behavioral conditioning protocols with electrophysiological recordings from the mouse OB, both of which are now established in the PI's laboratory, will test these hypotheses in the mouse. Experiments will first establish whether, like in the honeybee, the neural representations for a panel of pure odors and binary mixtures have specific multidimensional representations in the OB. For example, the representation of a binary mixture of odor A and X may fall between the representations for the pure odors in this multidimensional state space defined by M/T spiking. Then two specific hypotheses from the honeybee research will be tested:

Aim 1: Hypothesis – Long-term associative plasticity separates neural representations of odors.

Animals will be conditioned to discriminate two odors, one associated with water reinforcement and the other with nothing. Experiments will be modeled on a recent publication from the PI's laboratory. *The specific prediction is that associative differential conditioning will push the neural representations for the odors farther apart in the OB coding space.* Furthermore, we will evaluate, in more detail than has been done in the honey bee, specifically how these representations move relative to a third – neutral – odor that is easily discriminated from the other two.

Aim 2: Hypothesis - Long-term non-associative plasticity collapses neural representations of odors. Animals will first be exposed to an odor without explicit exposure to a conditioning context. This exposure has been shown in both the honey bee and mammals to produce odor-specific habituation. Recent work from the PI's laboratory leads to the specific *prediction that the representation of the binary mixture of a habituated pure odor will become less like that of the habituated odor and more like that of the novel odor in the mixture.* That is, the habituated odor will be to some extent filtered out.

Background and Significance: Olfactory coding occurs by way of broadly similar mechanisms in both insects and mammals. In the epithelium, in spite of molecular differences in the transduction mechanisms across phyla, each olfactory sensory neuron responds to a subset of odor ligands, and each ligand is encoded by a combinatorial pattern of activity across a sensory neuron subset. Input from sensory neurons drives a spatiotemporal pattern of activity across mitral cell outputs of the mammalian Olfactory Bulb (OB) and from projection neurons in its insect analogue, the Antennal Lobe (AL). These output patterns are shaped by a variety of local excitatory and inhibitory circuits in both OB and AL. Furthermore, both networks are modulated by odor experience. Cellular mechanisms underlying both nonassociative and associative plasticity in the OB and AL are beginning to be elucidated. In insects these mechanisms serve to distinguish spatiotemporal output patterns corresponding to differentially reinforced odors, and also to filter a novel odor from a habituated background. However, although some plasticity rules have been established for specific OB cell types, the mechanisms and implications for plasticity of population-based spatiotemporal coding across stages of learning have not been identified for mammals with the clarity that they have for insects.

NIH-funded research in the PI's laboratory has used the honeybee as a model for understanding behavioral plasticity using spatiotemporal population codes for odors. Smith's work has linked behavioral studies to imaging and electrophysiological analyses, and coupled those techniques to pharmacological and molecular genetic manipulations. Several studies from the Smith lab have identified spatiotemporal activity patterns that are critical for discrimination of odors, particularly when the discrimination problem is difficult. Moreover, associative plasticity modifies spatiotemporal patterns of output neurons such that representations for differentially reinforced odors are made more distinct. Nonassociative plasticity (odor habituation) serves to filter out irrelevant odors from a mixture such that more informative odor components are more clearly processed. These studies have identified octopamine, an invertebrate analogue to norepinephrine, as a neuromodulator that is important for driving plasticity in the AL. This research has shown that an important receptor for octopamine is expressed on inhibitory interneurons in the AL. Finally, Smith and colleagues have used computational modeling to generate testable predictions about changes in circuit connectivity that might produce changes in odor coding observed using imaging and electrophysiological studies. This computational work has revealed how modulation of inhibition changes AL circuitry to enhance odor discrimination and detection of relevant odors against an odor background.

NIH has funded this basic research on the premise that the results would be applicable to mammals because of the anatomical and functional similarities between the OB and AL, which are mirrored by similarities between mammals and honeybees in detection, discrimination and learning about odors in the natural environment. The OB network is also modulated by biogenic amines. Centrifugal modulation of the bulb occurs via serotonin, acetylcholine, and

norepinephrine, alternatively exciting or inhibiting mitral or tufted cells (M/Ts, the output neurons), typically by targeting local inhibitory interneurons. These neuromodulators underlie changes in M/T odor responses resulting from association with reinforcement or association of odors with important life events. Noradrenergic fibers from the locus coeruleus project into the OB. Gamma oscillations, believed to be important for olfactory learning, are enhanced by norepinephrine. Norepinephrine has also been implicated in reduced odor detection thresholds, which simulation suggests is due to enhanced signal-to-noise ratio of M/Ts. Stimulation of noradrenergic β -receptors by activation of these fibers is an essential component of associative memory in the OB.

All of these effects must be in some way reflected in the activity of the M/T ensemble, as this is the final common output to higher brain areas. Information about odors can be encoded in the temporal evolution of this ensemble activity during odor sampling. In invertebrates this has been represented as a trajectory of firing rates through a high-dimensional “state space”, showing dissimilar odors diverging as they are sampled. This analytic method has been infrequently used in mammalian olfaction. Instead, investigators have shown that M/T activity can be used to decode odor identity and predict behavioral discrimination, that the correlation structure of the M/T cell activity affects this code, and that the respiration cycle underlies its precision. There is also a gross chemotopic organization across the bulb, but at the local circuit level nearby M/T cells can still encode distinct odorants and local ensembles can be highly informative.

The study of how plasticity is encoded in the OB has important translational implications for understanding the onset and progression of neurodegenerative diseases. Olfactory impairment is one of the earliest clinical manifestations of Alzheimer’s (AD) and Parkinson’s (PD) diseases. These impairments directly correlate to early manifestation of inclusions in the OB such as neurofibrillary tangles (NFT) or Lewy bodies (LB), diagnostic of AD and PD, respectively. Inclusions in the OB are strongly associated with the earliest stages of AD and PD. Accumulation of the human form of alpha-synuclein, the soluble protein that forms LB’s, in mice leads to deficits in olfactory performance. The percentage of humans with NFT’s in the OB increases linearly after approximately 50 years of age. Indeed, OB inclusions and olfactory deficits are among the earliest indicators of neurodegenerative diseases.

Damage to the OB from early manifestations of these inclusions, and the resulting decline in olfactory acuity in behavioral tests, contrasts sharply with results from lesioning studies. There is now an extensive literature showing that substantial lesioning of the OB produces little or no decline in detection or discrimination of odors. These studies have used mechanical, chemical, and genetic lesioning of the OB. The conclusion has generally been that olfactory coding for any odor is robust and distributed across large areas of the OB. Presumably, lesions in one area can be compensated by activity in areas left intact.

How then can early manifestation of inclusions disrupt olfaction when extensive lesioning cannot? Clearly there must be differences in how inclusions and lesions affect components of the OB network. Lesions disrupt all of the components of the OB network, but they do so only in a specific part of the OB. The network components in the remaining part of the OB remain intact. These intact portions are still capable of encoding odors because of the highly distributed nature of olfactory coding. In contrast, inclusions may target specific network components in all areas of the OB, such that the full OB network is not intact at any location.

To answer this question, we must understand the computations occurring in the OB at the population level. This requires a read-out of activity in ensembles of neurons at perceptual timescales. This read-out must include M/T cells, as these constitute the output of the OB. And

we must have working models that can distinguish the effects of specific changes to olfactory circuits on this read-out. For example, most of the manifestation of NFTs in AD occurs in tufted and outer granule cells, whereas there is no loss of M/T cells. Meanwhile, during PD progression, mitral cells and neurons in the inhibitory components (e.g., granule cells) of the network label for alpha-synucleins, whereas modulatory units such as OB dopaminergic cells do not. Thus, there appear to be several paths to OB disruption, focusing on different circuit components, each of which may be essential for olfactory function. Without the appropriate read-out of OB output in animals representing these disease models, and in healthy controls, meaningful impacts on OB function can neither be adequately assessed empirically nor explained via simulation experiments.

This award will for the first time enable tests of OB plasticity in a mammal during olfactory learning using specific predictions about spatiotemporal coding at the population level. The work also enables subsequent proposals for how inclusions could interfere with early olfactory processing to produce the well-documented deficits in olfaction observed with AD and PD patients. These diseases could prevent, for example, the improved separation of familiar odors that have differential associations; such as in honeybee discrimination conditioning using differentially reinforced odors. Or disease conditions could interfere with filtering irrelevant odors out of mixtures with more informative odors, which is the proposed function for odor habituation.

Experimental Designs and Methods: The focus of this work will be to behaviorally condition mice and make chronic electrophysiological recordings to test hypotheses derived from the honeybee research. The behavioral work will be performed by a graduate student RA working under the direct supervision of Dr. Richard Gerkin in Smith's lab. Gerkin's expertise is in electrophysiological recordings from the mammalian OB in mice *in vivo*. The work will be performed in a new laboratory available to Smith. This laboratory is shared with Dr. Janet Neisewander. The shared space will be mutually beneficial, as my lab will be able to draw on the expertise of Neisewander's group in behavioral conditioning. And Neisewander's group is

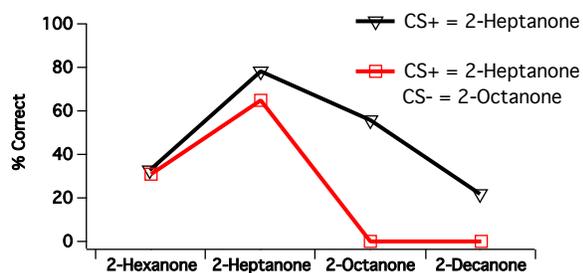


Fig 1. Mice reinforced on the CS+ (2-heptanone) respond preferentially to 2-heptanone over other ketones varying only in carbon chain length. When a CS- is added, varying by only one carbon from the CS+, the preference for the CS+ is further sharpened.

interested in adding chronic electrophysiological recordings to their NIH-funded work on drug abuse. Furthermore, a new faculty member – Dr. Jason Newbern - in neighboring space brings expertise in use of transgenic mouse models.

C.1 General methods
C.1.1 Animals and Behavioral Protocol. Wild-type mice (*Mus musculus*) of the C57bl/6 strain aged P40-75 will be selected for behavioral training, and water-restricted to 90% of free body weight. Training and testing will occur singly in a “Slotnick” olfactometer from Knosys, Inc. This consists of a transparent operant chamber in which a mouse can obtain water reward by inserting its snout into an odor stream and licking a water port. Odors reinforced by delivery of sucrose solution at the port are here indicated “A+”, odors unreinforced are indicated “B₀”, and a ‘novel’ odor delivered only in a testing context is indicated “X”. Success consists of licking the water port immediately following presentation of “A+” and withholding lick for “B₀” or “X”. Following a pre-training protocol to

acclimate mice to the olfactometer, training sessions consist of ten blocks of 10 trials of random sequences containing A+ or B₀. Mice will train once per day, and among the 14 mice we have subjected to this protocol so far, 13/14 reached behavioral criterion (<10% type 1 + type 2 error) after 4 sessions (days) or fewer using easily discriminated odorants. Behavior is not inhibited by connection of the recording apparatus (below), as we have verified using post-surgical mice.

Testing and training are simultaneous, following the pre-training acclimation phase. In addition to testing on the trained odorants (A+ and B₀), testing will also include up to 4 other odorants that we use to assess generalization. The test procedure will be similar to one used by Smith in an earlier collaboration to study olfactory learning in the rat. It is also possible to train and/or test using mixtures, which would permit us to evaluate other hypotheses motivated by the honeybee literature. The identities of A, B, and X are chosen to keep A and B initially difficult to distinguish in state space, and to use X as a reference point to determine if state space separation of A and B corresponds mostly to movement of A, movement of B, or both. In preliminary studies we have used alcohols or ketones of varying carbon chain length (0.1% dilution in mineral oil). An example using ketones is shown in Fig. 1, which indicates that mice can learn to

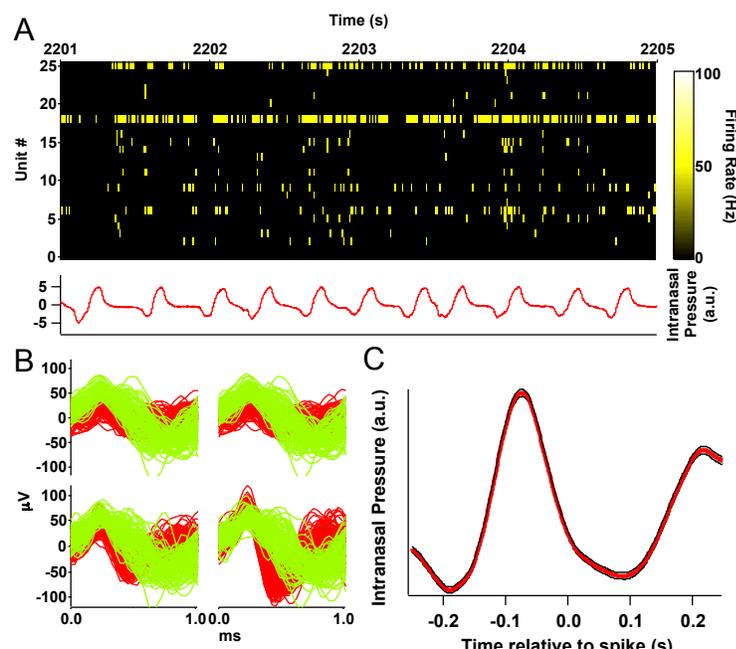


Fig. 2. A) Firing rate profile (top) of 25 simultaneously recorded single units (putative M/T cells) in the OB during free exploration in an awake, behaving mouse in our lab. The respiration rhythm (bottom) is measured simultaneously using a nasal cannula. **B)** Two random single units shown to illustrate typical waveform differences. **C)** The spike-triggered average (red) of the respiration cycle for one representative neuron, with standard error shown in black, indicating the typical strong respiration phase preference.

We will use procedures similar to those used by Gerkin for his postdoctoral research to record from M/Ts in the mouse OB and sort units for statistical analysis (Fig. 3). Prior to behavioral training, mice will be anaesthetized with 2% isoflurane and placed in a stereotaxic frame (Kopf) on a homeothermic blanket where they will remain anaesthetized and thermally regulated. Pre-analgesia with buprenorphine/meloxicam (0.1/1 mg/kg) will be delivered IP. A sagittal incision will be made to expose the skull, and connective tissues scraped and removed. A 0.2 mm hole

respond selectively to a given odor A (the CS+), varying by only one carbon from the others, but that the presence of B during training (the CS-) helps to sharpen this discrimination ability. The total number of mice subject to the training/testing paradigm will be determined by the number that reach behavioral criterion on pre-training, and for which a sufficient number of well-isolated single units (>10) can be regularly obtained during recording. Based on preliminary work and communications with the lab of Dmitry Rinberg, who uses identical recording technology, we expect this to hold for >80% of subjects. Power analysis (below) indicates that n=11 mice should be sufficient to test the hypotheses put forth here.

C.1.2 Surgery and Olfactory Bulb Electrophysiology.

We will use procedures similar to those used by Gerkin for his postdoctoral research to record from M/Ts in the mouse OB and sort units for statistical analysis (Fig. 3). Prior to behavioral training, mice will be anaesthetized with 2% isoflurane and placed in a stereotaxic frame (Kopf) on a homeothermic blanket where they will remain anaesthetized and thermally regulated. Pre-analgesia with buprenorphine/meloxicam (0.1/1 mg/kg) will be delivered IP. A sagittal incision will be made to expose the skull, and connective tissues scraped and removed. A 0.2 mm hole

will be drilled in the left nasal cavity, and a steel canula inserted from which respiratory pressure can be measured. 0.2 mm holes will be drilled over the left and right occipital lobes and used to secure a headpost with skull screws. A 1 mm hole will be drilled over the right olfactory bulb, 1 cm lateral to midline and 1 cm rostral to the rhinal fissure. Dura will be carefully removed. A custom microdrive attached to a probe will be lowered into the craniotomy until probe shank tips have entered 0.5 mm into the bulb. The tetrode will be covered with a plastic half-pipe and the microdrive and headpost will be secured with dental cement. Incision margins will be collected and bonded together. Mice will recover over 5 days with an analgesia and antibiotic regimen under supervision of university veterinary staff.

Probes will be model H4x2-tet-5mm-150-200-312-H32 (Neuronexus). These probes are used routinely in mouse OB, and a typical yield is 10-30 well-isolated single units (Fig. 2). Each day of testing, if there is a need to isolate new single units mice will be briefly anaesthetized with isoflurane and the probe will be advanced slowly (< 1 micron/sec) along the microdrive to identify single unit activity. A typical advancement is 20 microns. Single units recorded across days will only be considered to be the same units if they have sufficiently similar shapes, inter-spike-interval histograms, respiration phase preferences, and odor response functions. Recording the same units across days has the advantage of enabling longitudinal investigations of the same neurons; however if entirely new units are isolated each day, general features of M/T ensembles, such as their correlation structure and within-session plasticity, should be conserved. Only the spikes of mitral/tufted cells can be identified using these probes, and these subtypes can be further distinguished by respiration phase preferences. Activity is recorded using a Neuralynx Digital Lynx system. Samples (acquired at 30 kHz) will be stored raw, with offline filtering at 600-6000 Hz to identify spikes and 0.1-200 Hz to identify LFPs. We record respiration using a tube attached to the steel canula in the nasal cavity and to a pressure sensor (Fig. 2).

C.1.3 Statistical Analysis of M/T Cell Activity. Automated spike-sorting will be performed using Klustakwik, with manual clustering to refine results. Single units with isolation distance > 25 and ISI histograms indicating refractoriness will be retained for further analysis. We will use Igor Pro (Wavemetrics) for basic spike statistics, and for computation and visualization of subsequent analyses. For each of a variety of bin sizes (1 ms, 10 ms, 100 ms, etc.), we will denote as $\mathbf{s}_{t,A,i}$ the vector consisting of spike counts in time bin \mathbf{t} (relative to odor onset) for each recorded cell during a trial \mathbf{i} using odor \mathbf{A} . The trajectory $\mathbf{s}_{A,i}$ is the path taken by vector $\mathbf{s}_{t,A,i}$ across one trial. The mean trajectory across repeated trials is $\mathbf{s}_{A,i}$. The Euclidean distance between neural representations for odors \mathbf{A} and \mathbf{B} is a function of time, and is given by $\mathbf{d}_i(\mathbf{A},\mathbf{B},\mathbf{t}) = |\mathbf{s}_{t,A,i} - \mathbf{s}_{t,B,i}|$ for one trial, where $|x|$ denotes vector magnitude, and by $\mathbf{d}(\mathbf{t}) = |\mathbf{s}_{t,A} - \mathbf{s}_{t,B}|$ for mean trajectories. Ideal observer discriminability can be calculated parametrically (assuming single trial trajectories normally distributed about a mean for each odor) using $\mathbf{D}(\mathbf{t}) = |\mathbf{s}_{t,A} - \mathbf{s}_{t,B}| / \det(\text{cov}(\mathbf{s}_t))^{1/2}$, where \det is the determinant and $\text{cov}(\mathbf{s}_t)$ is the covariance matrix (at time \mathbf{t}) across trials for a pair of odors. Alternatively, discriminability can be computed non-parametrically using bootstrap resampling and simulation. All analyses using time bin \mathbf{t} can be applied using respiration phase bin Θ by aligning trials to respiration onset and computing respiration phase using any of several methods, including complex wavelet transform. There will be variability in trajectories across presentations due to random alignments between respiration phase and presentation onset, which is under control of the animal. This variability will be controlled by accounting for the influence of respiration using generalized linear models.

In order to observe changes ($\Delta\mathbf{D}(\mathbf{t})$) in discriminability $\mathbf{D}(\mathbf{t})$ as small as size 0.1 (note that discriminability is dimensionless), let us assume $\Delta\mathbf{D}(\mathbf{t})$ has across mice a standard deviation of

0.05. We then want to use enough mice that the false negative rate is less than 5%, and the false positive rate is less than 0.1%. Therefore in a sample of N data distributed normally with mean 0.1, SD 0.1, we require the sample mean to be significantly different from zero at $p < 0.001$ 95% of the time. Thus we require the sample size N for which random samples of size N will have sample means satisfying that criterion. We also require a multiple comparisons correction; since there are up to 6 pairwise odors combinations to discriminate, the correction is to divide the p -value by ~ 6 . Solving this by simulation yields a minimum N of 10. The failure rate for experiments is estimated to be 10%, so a conservative criterion would be to require $N=11$ mice. These 11 mice plus 9 for student training purposes will be used for a total of 20.

C.2 Research Design

C.2.1 Hypothesis: Long-term associative plasticity separates the neural representations of odors. We will train mice on a go/no-go discrimination task using two similar but distinct monomolecular odors, the reinforced odor, $A+$, and the un-reinforced odor, B_0 (Methods). Testing will occur on these odors, and unfamiliar but related odors, X , to assess generalization and provide a neutral reference point. It is expected that neural representations of odors, defined by activity in populations of M/T cells, will become significantly more separated as a result of associative (discrimination) conditioning. Fig. 1 illustrates hypothetical spatiotemporal trajectories for the two odors during training. At the start of odor presentation near the origin, changes from baseline activity begin to separate the odors along different spatiotemporal trajectories. The trajectories reach peak separation (d) measured via Euclidean distance just before odor termination. Continued presentation of odors would likely reach a fixed orbit (with

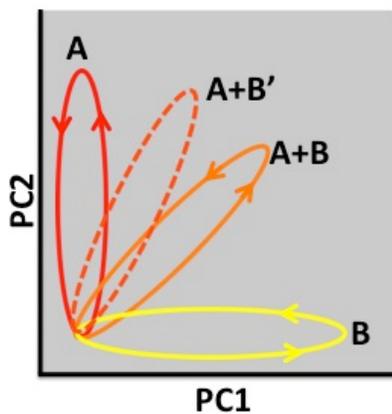


Fig. 3. Prediction from spatiotemporal representations of odor A, odor B and the 50:50 mixture $A+B$. Initially the representation of $A+B$ lies between that of A and B . After unreinforced (habituation) training with B , the representation of the mixture becomes more like A ($A+B'$). This figure represents patterns observed in data from Fig. 1 and computational modeling from Fig. 2 in Locatelli et al (2013).

periodicity determined by sniffing) at which the trajectory ceases to evolve. After odor termination (withdrawal of the nose from the odor port) the trajectories relax back to baseline. After $A+/B_0$ training (dashed lines) the trajectories evolve greater separation (d') at corresponding time points along each trajectory.

Predictions: The experimental design described here will allow us evaluate a variety of outcomes for

to whether and how representations of odors move apart in state space in the OB. The test phase will allow for replication and expansion of what has been done in the honeybee. We initially tested in the honeybee only whether the state space representations of A and B become more distinct as a result of learning. However, there are several plausible ways in which this separation could occur, including plasticity of the representation of A , or B , or of both. For example, 1) The reinforced odor ($A+$) may be pulled away from *all* other odors (the unreinforced B and various untrained X 's); in this case B is neutral, its distance from X remains the same, and there is no plasticity induced by lack of reinforcement. Or 2) A and B may each move apart (Fig. 1), and the distances to X increase for each odor. It is unclear whether mixtures (AB , AX , or BX) would have state space representations more or less similar to their individual components after training. Other honeybee work suggests that non-associative plasticity drives mixture

representations (e.g. BX) to become more similar to their unfamiliar components (e.g. X). However, the presence of reinforcement may result in a different outcome, for example driving all mixture representations towards the representations of their reinforced components. This remains an open question across species. The work here will allow us to establish precisely how the state space representation of odors remaps as a result of plasticity.

C.2.2 Hypothesis: Long-term non-associative plasticity also separates neural representations of odors.

We recently showed how non-associative plasticity (unreinforced odor exposure) changes the processing of odors in the honey bee AL. ‘Habituation’ to odors is well-documented in mammals, and both short- and long-term plasticity have been shown in the piriform cortex and OB, respectively after unreinforced exposure. But no one has established the consequences of non-associative odor exposure for population coding in awake animals.

Method: We will adopt a standard protocol for non-associative odor exposure (used by the PI in rat behavioral studies) that produces changes at one or more levels in olfactory processing. This protocol consists of odor-enriched cotton inside plastic tubing placed in the home cage for 20 seconds at 1 minute intervals. Investigation duration, defined as sniffing within 1cm of the presented odor, is used as the behavioral measure, and decreases with habituation.

Predictions: Based on outcomes in the honeybee (hypothetical trajectories in Fig. 3), I expect that a mixture of a preexposed (habituated) odor with a novel odor will cause the mixture representation to become more like the novel odor.

Overall outcomes: Cumulatively, the behavior and electrophysiology experiments in C.2.1 and C.2.2 will characterize OB plasticity underlying olfactory learning and help us make testable predictions using computational models -- such as we have done for the honeybee -- about how changes in neuromodulation or other circuit elements drive plasticity. For example, a computational model by Smith and colleagues in the honey bee AL has predicted that plasticity at synapses that transmit feedback inhibition of projection neurons (M/T analogues in the AL) would be the most reliable way to produce observed non-associative changes in AL processing of odors and odor mixtures. That prediction was recently tested and confirmed for odor habituation in the fruit fly. Other models from Smith’s research have predicted that modulation would need to target inhibitory processes – more than excitatory transmission through projection neurons such as M/Ts – in order to change odor representation. These predictions were confirmed in recent empirical studies showing receptors for a biogenic amine (that represents reinforcement) on local inhibitory interneurons. Subsequent studies would also be informed by present understanding of where inclusions target circuitry in the OB, including modulatory feedback from cortex, to constrain a search for mechanisms by which neurodegenerative disease affects OB output via M/Ts, the plasticity of this output, and ultimately behavioral outcomes.

Proposed One-Year and Long-Term Outcomes: In a subsequent proposal we plan to expand the work to mouse models for neurodegenerative disease. Robustness of olfactory function to partial OB lesion -- but not to AD/PD-related OB pathology -- indicates that specific OB circuit components are likely targeted by AD/PD, and that these components are essential to shaping the representation and retention of olfactory percepts in the OB. The longitudinal electrophysiological assessment of neuronal population dynamics in awake, behaving mice that we propose here provides an objective, longitudinal, and high temporal resolution window into olfactory perception and learning, and helps to identify critical OB circuit components using established computational models. We expect to use this tool in the future to show how

pathologies can impact the critical state-space separation shown in Fig. 1, and how specific pharmacological interventions might mimic pathological effects on this separation, or recover it when it is disrupted. The decision to use mice, and specifically the C57bl/6 strain, is motivated by the ability to apply the research to transgenic mouse models. We expect that the support of the researchers and clinicians in the Arizona Alzheimer's Consortium will help us transition to AZ and PD mouse models in the future.

Year End Progress Summary: In 2016 I implanted new research that was funded under new awards from NIH, NSF and HFSP as well as implementing a new trajectory under the AZ Alzheimer's consortium. We developed two new behavioral protocols for studying electrophysiological representations in the mouse olfactory bulb under Aims 1 and 2 (listed below and described in detail in the proposal). First, we evaluated the effect of variable background odors on detection of a target odor (Aim 1). We found that, when trained on variable background, mice were less confused when in detecting a target odor in comparison to mice trained on a constant background. Second, we developed a protocol for studying habituation to natural (strawberry) odors (Aim 2).

A second research tack involved developing an analysis of UPSIT data from human patients and relating scores on individual tests answers to post-mortem data on clinical hallmarks of Alzheimer's disease.

In the remaining part of the current award, and continuing into the next FY pending renewal, we expect to use electrophysiology to more directly test hypotheses related to the aims below. We expect to extend the new behavioral protocols to study performance of mouse models of Alzheimer's disease with Prof S. Oddo at ASU. We may (pending IRB approval) expend behavioral studies - modeled on the odor background problem described above – to healthy human patients. The long-term objective of this study, if we implement it, would be to develop more detailed and reliable studies of olfaction in Alzheimer's patients that stress cognitive performance rather than sensory deficits.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Identification of the amyloidogenic targets altered by lenalidomide. Gary D'Souza, PhD, Boris Decourt, PhD. Arizona State University; Arizona Alzheimer's Consortium.

Specific Aims: For our 2016-2017 project, we have a single Specific Aim which is to test the hypothesis that lenalidomide lowers amyloidogenesis by altering gliosis. For this, we are culturing human macrophagic (THP-1) and neuroblastoma BE(2)M17 cell lines, with and without the drug, and exchange the culture media between cell types. We are using receptor-blocking antibodies to analyze the molecular pathways activated in BE(2)M17 cells. We anticipate that lenalidomide will decrease the expression of TNF α in THP-1 cells, which should reduce amyloidogenesis in neuroblastoma cells.

Background and Significance: Chronic low-grade inflammation is often associated with aging and Alzheimer's disease (AD). Inflammatory markers were found elevated in the central nervous system (CNS) and blood of AD patients. Interestingly, the pro-inflammatory cytokine TNF α was shown to stimulate A β synthesis in vitro. Thus, a treatment that would target multiple neuropathogenic mechanisms of AD, e.g. inflammation and amyloidogenesis, both in the periphery and CNS, might have a higher chance of success in the clinic than targeting a single disease mechanism. Thus, our project has the potential to make a significant impact on the field of AD because it will assess the ability of lenalidomide to reduce chronic inflammation, amyloidogenesis, and tau pathology by using a single, pleiotropic therapeutic agent, as well as start deciphering the molecular pathways regulated by the drug.

Preliminary Data and Plan: Lenalidomide (100 mg/kg) was administered to 9- and 11- month old transgenic APP23 mice, which were then terminated at 12 months of age (i.e. 12 and 4 weeks treatment duration, respectively). The drug significantly improved learning and memory, and decreased the number of plaques. Quantitative PCR (qPCR) analysis confirmed that the drug inhibited brain TNF α synthesis and, very interestingly, reduced BACE1 mRNA levels. Western blotting showed that lenalidomide normalized BACE1 to WT control levels. To explore the possible mechanism underlying the lenalidomide reduction of BACE1 expression, the BE(2)-M17 neuroblastoma cells were treated with TNF α . This led to a significant increase in the transcription of TNF α and TNFR2 genes in BE(2)-M17 cells, and a minor increase in BACE1 mRNA levels (qPCR detection). Lenalidomide, however, did not directly inhibit the expression of BACE1. Based on these results, we hypothesize that glial cells are the main target of lenalidomide.

Aim 1: THP-1 cells will be stimulated for 2 to 6 hours with lipopolysaccharide (LPS) to induce TNF α expression. We will create dose-response curves for TNF α mRNA (qPCR) and protein levels (ELISA) to determine what dose of LPS causes maximum TNF α production. We will similarly create dose-response curves for lenalidomide to identify the optimal TNF α -inhibiting dose. We will then culture THP-1 and neuroblastoma cells in separate wells, in parallel. THP-1 cells will be treated with either vehicle, LPS, or LPS + lenalidomide for 6 hours, while BE(2)-M17 cells are unstimulated. We will collect the conditioned THP-1 media from all treatments, centrifuge them to remove all cells, and apply the THP-1 media to the BE(2)-M17

cells for 24 hours. We will assess BACE1, APP and PS1 mRNA levels (qPCR), and BACE1 (Western blot) and total A β (ELISA) protein levels to determine whether the BE(2)-M17 cells cultured in LPS + lenalidomide media have lower amyloidogenesis than cells cultured in LPS media without drug.

Aim 2: BE(2)-M17 cells will be pre-treated with anti-TNFR1 and anti-TNFR2 (R & D Systems) - functional blocker antibodies against the two major TNF α receptors. The cells will then be stimulated with TNF α for 1 to 24 hours, to determine which receptor type drives the response to TNF α . The efficacy of these antibodies will be assessed by measuring BACE1 expression and A β levels as above. To identify the underpinning pathways through which the response to TNF α is mediated, we will carry out Western blots of cell lysates for proteins involved in three intracellular pathways which are activated by TNFRs: NF- κ B, p38, and p42/44 Mitogen-Activated Protein Kinases (MAPK). The activation of each pathway will be assessed by the relative signal intensity of the phosphorylated protein subunits, compared to the signal intensity of the unphosphorylated subunits.

Proposed One-Year and Long-Term Outcomes: This project was used to apply for a postdoctoral fellowship for Dr. D'Souza. All data obtained from this project will be compiled in a manuscript submitted in May or June 2017 for review. In addition, we will apply for additional grant programs to pursue the identification of the molecular targets affected by lenalidomide.

Year End Progress Summary:

Aim 1: We have identified the optimal concentrations of both LPS and lenalidomide to stimulate and inhibit TNF- α production in THP-1 cells. We are currently optimizing the Western blot procedures to complete the analysis of the surrogate molecules we are testing.

Aim 2: Similarly to Aim 1, we are currently optimizing the Western blot detection of NF- κ B, p38, and p42/44 Mitogen-Activated Protein Kinases (MAPK) and their phosphorylated forms. We anticipate that all results will be collected by the end of May 2017, and to complete the statistical analyses, as well as writing a paper, in June 2017.

Project Progress Reports
Banner Alzheimer's Institute

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Native American Outreach Program. Dawn Batchuluun, CCRP, Anna Burke, MD, Lori Nisson MSW, LCSW, Edward Zamrini, MD, Jan Dougherty, RN, MS, Nicole Lomay, Richard Caselli, MD, Eric Reiman, MD, Pierre N. Tariot, MD. Banner Alzheimer's Institute; Mayo Clinic Scottsdale; Banner Sun Health Research Institute; University of Arizona; Arizona Alzheimer's Consortium.

Specific Aims: 1) To forge a close working relationship with members of our Native American Community in the awareness, care, and scientific understanding of Alzheimer's disease (AD) through educational and service-related outreach activities. 2) To support the participation of interested Native Americans in the Arizona Alzheimer's Disease Core Center (ADCC) Clinical Core and research studies of interest to them without detracting from our other outreach and partnership-development goals. 3) To work with our Native American partners to identify and begin to prepare for one or more research studies that advance the understanding of Alzheimer's disease and/or service to patients and families from this understudied, underserved population.

Background and Significance: Native Americans facing the problem of Alzheimer's disease constitutes the most underserved and understudied population in the United States. Since 2006, we have established an outreach program to help address the educational and clinical needs of patients, families and health care professionals; developed culturally sensitive educational and service programs; and demonstrated to the Native American communities our strong interest in serving these needs whether or not they participate in research studies. We have continued to attract a number of interested participants from the Urban Native American community to participate in the ADCC Clinical Core.

Preliminary Data and Plan: To date, 67 Native Americans have been followed through the ADCC and whose clinical findings are reported in a national database. As of January 2017, there are 43 active participants, 23 have withdrawn, and 1 died. Over the past year, over 3,400 Native Americans have participated in education and outreach efforts. We continue working relationships with numerous Arizona tribes and have had participation in outreach efforts from tribes outside of Arizona including New Mexico, Colorado, California, Utah, Oklahoma, Nevada and Minnesota. We have hosted the 12th Annual Conference on AD in Native Americans in October in Flagstaff, AZ with over 260 community participants. In November, we developed an AD program with the Office for American Indians, Alaskan Natives and Native Hawaiian Programs Administration on Aging and Administration for Community Living and Indian Health Services for the Long Term Services and Supports (LTSS) in Indian Country National Conference in Minneapolis, MN. This national conference drew 175 participants and included 6 speakers representing Banner Alzheimer's Institute. We are currently formulating a plan to host the 13th Annual Conference on AD in Native Americans in October 2017 in the Tucson, AZ region and will anticipate drawing 250 community participants. We have established a consistent and solid working relationship with many urban and tribal communities to continue on with these efforts.

Proposed One-Year and Long-Term Outcomes:

1. Continue outreach efforts to general Native American communities and education of health care providers for American Indians that will decrease the disparity related to diagnosis and treatment of AD in both reservation and urban dwelling Natives.
2. Retain the 43 Native American cohorts in the ADCC trial in the next 12-months with a goal of recruiting 12 new participants.
3. Refine methods to reach more Native Americans from youth to elders to educate using the Native American Brain Health program.

Funds have been used in a way that complements but does not overlap with funding provided by the National Institute on Aging (NIA, which supports some of our outreach and clinical core enrollment activities), the Ottens Foundation (which provides partial support for our Annual Conference), and funds from Tohono O'odham Nation and Salt River Pima-Maricopa Indian Community to support development of culturally sensitive memory screening/brain health programs.

Annual Progress Summary:

1. During the past year, our education and outreach programs have included 1,785 community participants and 806 professionals from the Native American community across Arizona. We held the 12th Annual Conference on AD in Native Americans, drawing over 260 family caregivers and lay participants from Arizona, New Mexico, Utah and California. As with our past conferences, participants highly rated both the content and speakers. Our pre-conference on "Dementia Friendly Communities" drew a record 48 professionals. As a follow up to the 2015 National Conference on AD in Natives, BAI played a leadership role in planning a National conference with the Long Term Services and Support (LTSS), a division of the NIH Administration on Community Living. We assisted Dr. Bruce Finke, CMO, Indian Health Services, to create a full-day tract of topics on dementia that featured 6 staff/faculty from BAI providing the content. The conference was held in Minneapolis, MN and drew 175 professional participants. In addition, we had a resource table with numerous participants requesting family caregiving education collateral created by BAI NA Outreach team.
2. During the past year, we enrolled 11 new participants, are preparing to enroll 12 additional participants, and completed 25 assessments. We have lost 23 participants to follow-up, and will be working with the ADCC Education Core to find ways to optimize retention in our longitudinal research program.
3. BAI Native American Program received significant funding from the Gila River Indian Community and Tohono O'odham Nation to develop outreach efforts with a focus on raising awareness of improved brain health and recognition of warning signs of AD/dementia. As such our team finalized a brain health program that is culturally sensitive to educate and engage the community in culturally relevant lifestyle habits that include mental and physical exercise, nutrition, stress reduction and sleep. We incorporate interactive methods to assist community members to differentiate "Senior Moments vs. Something More" (dementia) and provide action steps when there is "something more." We commissioned a Native artist to create a painting of Native Brain Health that draws upon Native traditions for wellness. This art work is featured in the Brain Health program and in most of our outreach collateral. We have been successful at presenting this information to lay communities and select elder

groups. We will continue to develop relationships with schools and youth groups to also educate about lifestyle factors and brain health unique to Natives.

During the year, the University of Arizona and Banner secured one of the national Precision Medicine Initiative's (PMI's) first Health Provider-led grants. Under this grant, we are preparing to enroll 150,000 members of Banner Health, who agree to a brief evaluate, provide their anonymized medical records, DNA, blood samples, and be reassessed 3 years later. Our organization will aim to include 7,500 Native Americans and 90,000 Latinos. We have engaged Dedra Buchwald from the University of Washington ADCC Native American Satellite Program at Washington State University to forge collaborations in our PMI Cohort ("All of Us") program, as well as an ongoing genetic, MRI and cognitive study of >1,000 Native Americans from the Strong Heart Stroke Study.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Advanced image analysis techniques for the detection, tracking, treatment, and prevention of Alzheimer's disease. Kewei Chen, PhD, Hillary Protas, PhD, Dhruvan Goradia, PhD, Wendy Lee, MS, Eric M. Reiman, MD. Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Specific Aims:

1. To further develop, test and apply several of our voxel-based image analysis techniques for the early detection, tracking, treatment, prevention, study, and differential diagnosis of Alzheimer's disease (AD).
2. To make our data analysis algorithms available to research laboratories inside and outside Arizona, and do so in a format that is relatively easy for others to implement and use.
3. To further develop a platform for sharing anonymized data and biological samples for the Arizona APOE4 Gene Dose Cohort Study in the most user-friendly, appropriate, and HIPAA-compliant way.

Background and Significance: With non-overlapping grant support from the Arizona Alzheimer's Consortium, BAI's Computational Image Analysis Laboratory has continued to develop PET and MRI image analysis tools with improved power to detect, track, diagnosis, and study AD and improved power to evaluate putative AD-modifying and prevention therapies. Examples include our hypometabolic convergence index (HCI) to characterize the magnitude and spatial extent of AD-related reductions in cerebral glucose metabolism in a single measurement; a voxel-based multi-modal partial least squares (MMPLS) method to characterize the covarying pattern of regional changes in different kinds of brain imaging measurements from the same persons in a single summary measurement; our empirically predetermined "statistical region-of-interest (sROI)" approach to track AD-related brain declines in regional glucose metabolism evaluation AD-modifying and prevention therapies; a fully automated "iterative principal component analysis (ICPA) algorithm to compute longitudinal reductions in whole brain volume; use of a white matter reference region-of-interest to track longitudinal changes in florbetapir PET measurements of amyloid plaque deposition, and evaluate anti-amyloid treatment effects.

Plan:

Developing and Testing Our Image Analysis Algorithms: Capitalizing on data from the AD NeuroImaging Initiative (ADNI), Arizona APOE4 Gene Dose Cohort Study in persons at three levels of risk for AD, and API Biomarker Study in autosomal dominant AD mutation carriers and non-carriers from Colombia, we will continue to optimize the white matter reference region used to track florbetapir PET changes and clarify the impact of brain atrophy and partial volume averaging on the power to track those changes; we will further revise our HCI method to track AD-related declines in cerebral glucose metabolism and compare it to our sROI method; we will extend our MMPLS method to permit its application to resting fMRI and florbetapir PET data; and we will establish the impact of these refinements on the statistical power to track AD and detect AD-modifying treatment effects. **Sharing our Image Analysis Algorithms:** We have

already shared several of our methods with researchers from other laboratories; we continue to share these methods with other laboratories whenever requested; and we continue to forge new collaborations whenever appropriate. *Developing an Improved Data Sharing Platform*: During the funding period, we will continue to collaborate with researchers from other Centers (e.g., Washington University and Rush University), develop a user-friendly graphical user interface, and recruit a data manager with the expertise and productivity needed to advance our data sharing goals.

Progress Summary:

1. A) Using non-overlapping funds from the state of Arizona, we modified our method to track cerebral-to-white matter florbetapir SUVRs to enable us to address the impact of brain atrophy and partial-volume averaging on our findings; confirmed that the white matter reference method has superior power to track florbetapir PET measurements of amyloid plaque deposition and evaluate ant-amyloid treatment effects (Yi et al, Hum Amyloid Imaging Abstr 2017); and supported an NIA grant application to further evaluate the impact of demyelination on these findings. B) We have continued to compare the power of our statistical ROI and subsequently developed longitudinal HCI algorithms to track AD-related declines in regional cerebral glucose metabolism, such that we can select the optimal algorithm to use before data from our API trials are unlocked (Chen et al, AAIC Abstr 2017). C) We have begun to compare our voxel-based “tau convergence index (TCI)” algorithm to the Jagust laboratory’s region-of-interest (ROI)-based Braak staging approach and the Johnson laboratory’s inferior temporal cortex ROI image analysis methods for the detection and tracking of PHF tau abnormalities in persons affected by and at risk for AD. D) We have further developed two methods for the characterization alterations in white matter connectivity using MRI-based diffusion sensory imaging (DTI) data. E) In collaboration with Yalin Wang’s laboratory at Arizona State University, we demonstrated limitations in the ability of estimate clinical trial sample sizes using data from two time points if the treatment interval differs from the observed time intervals; we introduced a Monte-Carlo simulation procedure to compare the onset of different AD biomarker changes from the same individuals; and we summarized our findings in two AAIC 2017 abstracts.
2. We have shared our image-analysis expertise and algorithms with researchers inside and outside Arizona. In addition to our ongoing support for researchers in the Arizona Alzheimer’s Consortium, we have worked closely with and assisted our partners in the API ADAD Colombia and API Generation Trials, and the Imaging Clinical Research Organizations (CROs) that will be needed to implement these procedures for our potentially license-enabling trials.
3. In 2016, we showed our collaborators at Stanford (Dr. Natalie Rasgon group), Shanghai Jiaotong Univ. (Dr. Huang’s group), Beijing Medical Univ. (Dr. Yao and Dr. Zhang groups), Taiwan National Cheng-Kung Univ. (Dr. Lee group), and Beijing University of Technology (Dr. Wu’s group) the adequate procedures to process and analyze neuroimaging data. We provided valuable statistical and image processing inputs to many others for their scientific reports and as a training opportunities for graduate students and young faculty members from inside, outside Arizona and internationally (in the forms of summer intern program, visiting scholar, series of statistical seminars). With the state funding and same as last year, we continued to process and analyze new data and provide statistical and scientific report

assistance to researchers from Brown University, Genentech and Novartis among several others.

4. We have shared our data, blood samples, with more than a dozen research groups, helping to generate new methods and findings, supported the analysis of clinical trial data, and provided a further foundation for AD prevention trials. In addition to the XNAT data management platform, we decided to add REDCap database for multiple projects. The combination of these two together with new database team led by Mr. Don Saner and Dr. Kewei Chen, we have made progress to optimize data management, security, and accessibility to qualified researchers.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Alzheimer's Prevention Registry. Jessica B. Langbaum, PhD, Eric M. Reiman, MD, Pierre N. Tariot, MD. Banner Alzheimer's Institute.

Specific Aims:

1. To increase enrollment into the Alzheimer's Prevention Registry through community outreach and other related efforts.
2. To continue to develop collaborations with academic and non-academic partners to increase enrollment into the Registry.
3. To demonstrate that the Registry accelerates recruitment into AD prevention studies, such as the API APOE4 Trial (now known as the API Generation Study), the ADCS "A4" trial, and others.

Background and Significance: Alzheimer's disease (AD) is the most common form of dementia. This devastating illness takes a significant toll on clinically affected persons and family caregivers, and will take an overwhelming financial toll on society. Results from observational studies suggest that the pathophysiological process of AD begins years, if not decades, before the diagnosis of clinical dementia. It is possible that at least some therapeutic interventions, particularly those that target amyloid pathology need to be started before the clinical onset of AD, when there is already extensive neuropathology, in order to exert their maximum effects. A number of prevention trials have begun, and more are being planned.

The BAI-led Alzheimer's Prevention Initiative (API) is a multi-institutional, multi-partner collaborative mechanism to evaluate promising preclinical AD treatments. API includes prevention trials in individuals at high risk for developing dementia as well as registries to help support the enrollment into these and other trials. Recruitment and enrollment into clinical trials is a major obstacle faced by researchers and study sponsors. It has been estimated that fewer than 10% of Americans participate in clinical trials, mostly due to lack of awareness about study opportunities, resulting in approximately 80% of research studies failing to meet their enrollment goals in the stated timeframes. The web-based Alzheimer's Prevention Registry (www.endALZnow.org) ("Registry") was created in 2012 to help studies meet their enrollment goals in an efficient and timely manner. Based on lessons learned from the Arizona Alzheimer's Research Registry and modeled after other web-based research registries, this Registry was purposely designed to have a low threshold of commitment at entry. At enrollment, individuals are asked to provide their email address and basic demographic information. Enrollees receive regular email communication to keep them apprised of the latest news in Alzheimer's prevention research. In addition, enrollees receive email notifications when study opportunities become available in their communities, with information on whom to contact to explore the possibility of their participation. In November 2015, the Registry launched its GeneMatch program, and IRB research program open to adults age 55-75 in the United States who do not have a diagnosis of cognitive impairment to submit a sample of DNA for APOE genotyping, the results from which are used in part to help match to studies. Beginning in the second half of 2016, new features will be added to the Registry including a Researcher/Study Opportunities Portal which will allow reporting of de-identified enrollment metrics to help demonstrate the utility and impact of the

Registry. The Registry, which aims to enroll at least 250,000 individuals, is intended to provide a shared resource to the AD scientific community to facilitate efficient enrollment in preclinical studies and to complement and enhance local recruitment efforts.

Progress Summary: The Alzheimer’s Prevention Registry is an online community of individuals ages 18 and older who agree to receive emails with information about Alzheimer’s prevention related research updates as well as notifications about study opportunities within their communities. As of the end of February 7, 2017, the Registry had over 260,000 enrollees and GeneMatch enrolled over 32,000.

1. During the funding period, considerable effort was undertaken to increase enrollment into the Alzheimer’s Prevention Registry through community outreach and other related efforts. We have activated 16 GeneMatch partner sites across the United States during the funding period, allowing sites to enroll individuals into the Registry and GeneMatch and distribute recruitment materials. As a result of these efforts we have seen an increase in enrollment into the Registry and GeneMatch, as well as an increase in referrals to studies at those sites. In addition, we ran a successful online advertising campaign in Q4 2016 to increase enrollment into the Registry and GeneMatch.
2. As described above in Aim 1, we activated 16 GeneMatch partner sites during the funding period, helping us to achieve the goal of continuing to develop collaborations with academic and non-academic partners to increase enrollment into the Registry. In addition, our team serves on a number of workgroups and advisory committees aimed at developing strategies to help accelerate recruitment and enrollment into programs such as the Registry and GeneMatch.
3. During the funding period, we have undertaken several steps to demonstrate that the Registry accelerates recruitment into AD prevention studies, such as the API APOE4 Trial (now known as the API Generation Study), the ADCS “A4” trial, and others. Indeed, work on this aim served as the basis for an R01 grant application submitted to the NIH in October 2016 to study the “science of recruitment” leveraging the Alzheimer’s Prevention Registry and GeneMatch programs. As mentioned above in November 2015, we launched an IRB-approved genetic testing program of the Registry, GeneMatch, open to adults age 55-75 residing in the US and without a diagnosis of a cognitive impairment. After electronically signing the ICF, individuals are provided a cheek swab kit by mail for at-home collection of DNA. The sample is returned to a CLIA-certified lab for APOE genotyping. The genetic results are used in part to help match people to research studies. The API Generation Study is the first study to use GeneMatch as its primary recruitment tactic in the US. GeneMatch does not disclose genetic results to participants, and all invitations to participate in a study must be done so as to not inadvertently disclose test results. To date, 32,000 people have joined GeneMatch. GeneMatch began referring participants to the Generation Study in Q3 2016. To date, approximately 250 GeneMatch participants have been invited to the Generation Study, about 50% of whom have accepted their invitation and 1 person has declined. We are taking the learnings from the GeneMatch referral program to develop StudyMatch for the Registry program overall. The goal of StudyMatch, which should launch in 2017, is to provide a secure portal allowing Registry members to authorize sharing their contact information and other PHI as required with enrolling studies. This portal will provide the Registry with important metrics tracking its success at helping studies accelerate their enrollment goals.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Alzheimer's Prevention Initiative. Eric M. Reiman, MD, Pierre N. Tariot, MD, Jessica B. Langbaum, PhD. Banner Alzheimer's Institute.

Specific Aims:

1. To support a prevention trial/surrogate marker development program in 30-60 year-old cognitively unimpaired *PSEN1 E280A* mutation carriers and non-carriers from the world's largest autosomal dominant Alzheimer's Disease (ADAD) kindred.
2. To prepare for a prevention trial/surrogate marker development program in 60-75 yo cognitively unimpaired APOE4 homozygotes.
3. To design, plan and prepare for other prevention trials.
4. To further develop registries for these and other prevention trials.
5. To conduct biomarker studies of ADAD mutation carriers that will help to inform other other prevention trials, including in younger adult carriers using biomarker endpoints.

Background and Significance: Alzheimer's disease (AD) is the most common form of dementia. It takes a devastating toll on affected persons and families, and will take an overwhelming financial toll due to the growing number of people living to older ages. Treatments may need to begin before the clinical stages of AD, when the disease is already extensive, to have their most profound benefit. We have argued that it takes too many cognitively unimpaired persons and too many years to evaluate the range of promising but unproven prevention therapies, have introduced strategies to accelerate the evaluation and approval, and helped to launch a new era in AD prevention research. We incorporated elements to provide a better test of the amyloid hypothesis (the leading theory about the molecular processes involved in the development of AD) than failed trials in the clinical stages of the disease. We have capitalized on groundbreaking public private-partnerships to address goals that none of the parties could accomplish on their own, as well as ground-breaking data and biological sample sharing agreements to ensure that the study will help the entire research community find even faster ways to find, approve and support the availability of prevention therapies.

Progress Summary: To date, we have generated data to support the design and size of prevention trials in persons at increased risk; initiated the first prevention trial of the anti-amyloid antibody treatment crenezumab in unimpaired members of the world's largest ADAD kindred in Colombia, who are at virtually certain risk for early-onset AD, and ushered in a new era in AD prevention research; initiated an international prevention trial of an two other anti-amyloid treatments (a vaccine therapy and oral BACE inhibitor) in unimpaired persons with two APOE4 alleles, who are at the highest imminent risk for the more common late-onset AD; generated biomarker, cognitive, and genetic risk findings to inform these trials; established exceptionally large registries to support enrollment in these and other trials; and continue to address the scientific, logistical, ethical, social, and regulatory issues needed to provide the best possible chance to find, approve and support the widespread availability of prevention therapies

by 2025. To date, we have secured nearly \$500M to support this effort, helped the field invest in other prevention trials, and enabled us to prepare for other prevention trials to come.

1. To date, the Colombian API Registry has identified and performed baseline assessments in >5,700 members of the *PSENI E280A* kindred, including nearly 1,200 mutation carriers at virtually certain risk for early-onset AD. The API ADAD Colombia Trial completed enrollment of 252 kindred members on 02-27-17, has been associated with minimal attrition, and is expected to be completed five years from then. We submitted and received a nearly perfect impact score of 11 for our competitive renewal grant, which will help us to incorporate tau PET into the trial (at 26 and 52 months) and provide an infrastructure for the sharing of baseline data and post-study data and samples in accordance with Collaboration for Alzheimer's Prevention guidelines. In collaboration with Yakeel Quiroz, we continue to help characterize the trajectory of age-related biomarker changes in relationship to the kindred's clinical onset, most recently including information about tau PET changes, which begin approximately 6 years before the onset of MCI.
2. To date, the Alzheimer's Prevention Registry has enrolled >260,000 persons in North America and its GeneMatch Program has enrolled >30,000 55-75 persons who provide a cheek swab for APOE testing, and elements of API's Registry, GeneMatch, genetic counseling program, and an emerging portal-based StudyMatch program (to match participants to trials and monitor the impact of our enrollment efforts) continue to progress in ways that will support API and other prevention trials. The API Generation Trial has begun, starting at BAI; site enrollment continues to be slow but methodical; and our clinical research organizations continue to work through the challenges involved in conducting such a large and complex program.
3. Several API co-led prevention trials are in the preparatory and/or planning stages, and at least one of these trials will be announced later this year. In the meantime, API and A4 investigators are working together on a grant application to conduct a prevention trial of the amyloid antibody treatment aducanumab in unimpaired amyloid-positive older adults. If this treatment's biomarker effects are confirmed in ongoing trials to be associated with a clinical benefit (Phase 3 Study readouts are expected in 2019), the proposed trial could set the stage to find and support the approval of a prevention therapy by 2023 and to evaluate a wider range of prevention therapies in with wider at-risk population using reasonably likely "surrogate endpoints." Meantime, we generated a comprehensive, multi-faceted proposal, entitled "Preventing Alzheimer's Together by 2025," to help find and support the approval, affordability and widespread availability of effective prevention therapies by 2025. The proposal included 24 elements, the possibility of up to \$50M in additional philanthropic support, and an opportunity to leverage the effort with >\$2B in NIA and industry funding. While it was not selected for consideration by the MacArthur Foundation's \$100M grant solicitation, it generated interest and support from 84 leaders at 72 international organizations, as well as the interest of several major funders. We will begin to explore ways in which to advance as many of the elements in that proposal as possible.
4. We continue to expand the Alzheimer's Prevention Registry, a web-based registry focused on encouraging enrollment into prevention studies. It is intended to be an online community of individuals who want to stay informed and engaged about Alzheimer's prevention research, including receiving email notifications about study opportunities, providing a shared resource to accelerate enrollment in other prevention trials. We are honored to have

leaders in the field serve on the Registry's executive committee. The Registry has over 260,000 enrollees. In November 2015, the Registry recently launched its GeneMatch program which collects genetic samples from participants age 55-75 for APOE genotyping and uses the genetic results in part to help match people to research studies. This will be one of the primary recruitment sources in the United States for the API Generation Study. To date, over 32,000 people have joined. We exceeded our ambitious goals for the Colombian API Registry, to date having enrolled nearly 5,700 autosomal dominant kindred members, including 1,171 mutation carriers and several new families. Registry expansion will continue during 2017.

5. We continue to analyze two-year longitudinal follow-up MRI, FDG PET, amyloid PET, CSF and plasma biomarker data from a subset of 24 young adult mutation carriers and non-carriers (individuals who would not be eligible for our prevention trial) as well as clinically affected carriers. These findings have already had a major impact on the field's understanding of the earliest biological and cognitive changes associated with the risk of AD. We are partnering with Dr. Yakeel Quiroz to collect tau PET data in a subset of kindred members, and have begun to track the age-related trajectory of tau PET changes. Dr. Quiroz just received an outstanding priority score (4th percentile) to extend this work to the longitudinal studies, including in younger adult mutation carriers and non-carriers, and complementing the longitudinal data we are acquiring in the API ADAD Colombia trial.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Development of a centralized data management program for the Arizona Alzheimer's Disease Core Center (ADCC), Brain and Body Donation Program (BBDP), and Apolipoprotein E4 (APOE4) Gene Dose Program. Don Saner, PhD & Kewei Chen, PhD, Laura Wojtulewicz, Davy Weissenbacher, Travis Johnson, Matthew Huentelman, Bruce Petersen, Thomas Beach, Richard J. Caselli, Eric M. Reiman. Banner Alzheimer's Institute; University of Arizona; Arizona State University; Mayo Clinic Arizona; Banner Sun Health Research Institute; Translational Genomics Research Institute; Barrow Neurological Institute, Arizona Alzheimer's Consortium.

Specific Aims:

1. To assemble a data management team, further assess the existing ADCC Clinical Core, ADCC Neuropathology and Ancillary BBDP Core, and Arizona APOE4 Gene Dose Cohort Data Management Programs, work closely with the Program PIs and other relevant stakeholders, and begin to establish a centralized, standardized, and highly productive data management program for these and other longitudinal research cohorts in the Arizona Alzheimer's Consortium.
2. To develop a common data management platform that a) sets the stage for initiating the data sharing among these programs at the end of this fiscal year and from other longitudinal cohorts as well in the future; b) sets the stage for the team to manage the database using other grant and contract funds; c) establishes a plan to incorporate information about available DNA, other biological samples, and brain images and sets the stage to incorporate genetic, biomarker, and imaging data into the database in the future; d) makes it possible to upload error-free ADCC data to the National Alzheimer's Coordinating Center (NACC) in the most timely and productive way using ADCC funds; e) acquires information about relevant research program metrics and generates real-time metrics reports; f) sets the stage for each of the programs to provide respond to data inquiries and data and sample-sharing requests in the most user-friendly, timely, productive and appropriate way; g) ensures that data are handled in the most appropriate way, including the provision of anonymized data, appropriate confidentiality and privacy protections, and the necessary quality-assurance and control procedures; h) cross-trains team members in a way that maintain the program when individual team members are not available; i) works closely with clinical and neuropathology site investigators, data entry specialists, and ADCC Core and Research Program PIs, and sets the stage to further develop and optimize the data management program in the future; and j) develops the centralized data management program in a manner that doesn't detract from the existing programs ongoing needs.
3. To establish the kind of program that will permit Arizona Alzheimer's Consortium researchers to conduct research studies, share data and samples, and generate research grants and publications in the most productive, impactful, and appropriate way.

Background, Significance, and Preliminary Findings: The Arizona Alzheimer’s Consortium has three longitudinal research programs which are internationally recognized for their productivity, impact, and value to researchers inside and outside of Arizona in the scientific fight against AD, PD, and related disorders, and the study of normal brain aging. These programs include common data elements, are administered through separate data management programs, and could provide even greater value under a common data management program that is optimized to fulfill the programs’ common and complementary research goals. a) With support from the National Institute on Aging (NIA), the Arizona ADCC Clinical Core is the nation’s first NIA-sponsored AD Center with multiple clinical core sites (including those at Mayo Clinic Arizona, BSHRI, UA, BNI, and BAI); it provides annual assessments in ~500 research participants with AD, related disorders, and cognitively unimpaired older adults; it includes individuals who are enrolled in BSHRI’s BBDP, cognitively unimpaired individuals with two, one and no copies of the APOE4 allele, the major genetic risk factor for AD, members from Arizona’s understudied Latino and American Indian communities, and other clinically affected and unaffected research participants; and it provides a shared resource of participants and data for researchers to generate new findings, publications, and grants. b) The BBDP includes >800 annually assessed research participants from the ADCC, the National Institute for Neurological Disorders (NINDS)-supported National Brain and Tissue Resource for PD (NBTR-PD), and other longitudinally assessments from older adults who consent to brain donation after they die, neuropathological data and exceptionally high quality brain and body tissues from >1,500 expired BBDP participants; it has been the world’s leading resource of neuropathology data and brain and other body tissue samples for AD, PD and other neurodegenerative disease researchers around the world, has contributed to hundreds of research publications and grants, and continues to make major contributions to the study of AD, PD, related disorders, and brain aging. c) With support from NIA, the state of Arizona and Mayo Clinic and BAI, the Arizona APOE4 Gene Dose Program provides a longitudinal cohort of research participants and data with two, one and no copies of the APOE4 gene, reflecting three levels of genetic risk for AD, including a sub-set of subjects with extensive brain imaging and other biomarker data. This program has made pioneering contributions to the conceptualization of “preclinical AD,” established a foundation for the Alzheimer’s Prevention Initiative (API) and the accelerated evaluation of prevention therapies, and includes an invaluable resource of data and samples to help researchers detect and track the earliest biomarker and cognitive changes associated with AD, contribute to the understanding of genetic and non-genetic risk factors, develop data analysis techniques with improved power to detect and track AD and evaluate promising but unproven AD prevention therapies. (Consortium researchers lead other valuable longitudinal research programs, which despite fewer common data elements, may benefit from either a shared data management program and/or mechanisms to find other relevant data in the future. In this project, we propose to assemble the team needed to evaluate each of the data management programs, begin to develop a centralized data management program that more fully addresses the needs of researchers inside Arizona and around the world, dramatically increase the number of productive researchers who used our shared data resource, and optimize our impact on the fight against AD, PD, related disorders. While more work will remain to be done to further optimize and demonstrate the added value of the proposed one-year funding period, and much of the work needed to implement and maintain the program will come from existing ADCC, APOE4 Gene Dose Program, and organizational funds and other grants to be secured in the future, we are

extremely excited about the chance to provide the most user-friendly and productive resource for the sharing of data and samples that we can.

Research Plan: We are pleased to note that we have retained Don Saner, to lead our data management program. He has extensive experience in the development and management of highly user-friendly productive and accountable data management programs and the development of the needed data management teams. He will work closely with Kewei Chen (Director of the Arizona ADCC Data Management Core), Richard Caselli (Director of the ADCC Clinical Core and Co-leader of programs in the Arizona APOE4 Gene Dose Cohort), Thomas Beach (Director of the BBDP), leaders and relevant personnel from each of our programs and ADCC Cores, our data management consultants, and Eric Reiman (Director of the Arizona Alzheimer's Consortium). He and/or Chen will also visit data management program directors from NACC and 1-2 of the most productive AD Center Data Management Programs in order to exchange information and clarify how to optimize the program in a way that would have greatest value to the AD research community. During the proposed one-year funding period, he will assemble a data management team, further assess the existing ADCC Clinical Core, ADCC Neuropathology and Ancillary BBDP Core, and Arizona APOE4 Gene Dose Cohort Data Management Programs, and begin to develop the centralized data management program in a manner that incorporates some of the elements below.

On-Site Data Collection: We will standardize the Electronic Data Capture (EDC) taking place at the sites around a single set of technologies, including REDCap (Research Electronic Data Capture) and Teleform™. We have extensive experience with REDCap, an open source tool created by Vanderbilt and used successfully throughout the world in thousands of research projects. REDCap permits the rapid creation of web base electronic collection forms through an intuitive web based interface without the need for custom programming. Additionally, REDCap exposes the data via an Application Programming Interface (API) which permits the integration of data collected through REDCap with a larger infrastructure. In order to deploy an instance of REDCap two servers will need to be provisioned; one to house the application written in PHP and the other to house the MySQL relational database. The uniform way in which data is collected using UDS3 paper forms opens the possibility to use form scanning and Optical Character Recognition products such as Teleform™ which could greatly reduce the amount of time spent on data entry. With this option, the forms would be scanned locally at each site and transmitted to a central site which would then process the scanned images and store the results in a relational database. While Teleform™ cannot perform with 100% accuracy they do have a validation step which displays a portion of the scanned form alongside the computer's interpretation for human validation.

Inventory of Existing Legacy Data: We will perform an inventory across sites and programs to assess common elements that can be consistently extracted. In order to help prioritize this work, we propose to establish a governance committee with both technical and scientific representatives from the sites to ensure feasibility and retention of high priority data. All raw data from each site will be archived and the extraction and harmonization of data will take place in phases as prioritized by the governance committee.

Creation of a Central Data Repository (CDR). Much of the design of the database schema will be informed by the governance committee, and by the inventory of legacy data. We propose to build the CDR using an MSSQL relational database which will require provisioning an additional server. In order to integrate existing data we will perform imports from legacy data stores into a staging area. The data will be mapped and integrated in this area prior to moving to

the CDR. For prospective data, we will author code that regularly Extracts, Transforms and Loads (ETL) from source systems into the staging area and ultimately into the CDR.

Implementation of Open Source, Commercial and In-House Developed Software. In order to facilitate the implementation of these applications we intend to create dimensional star schema data marts within the CDR. We will use the open source JasperSoft for routine metric reporting; the open source i2b2 and commercial Tableau™ for end user queries and custom in house developed software for period data upload to central agency NACC. The architecture of the proposed systems is illustrated in the figure below.

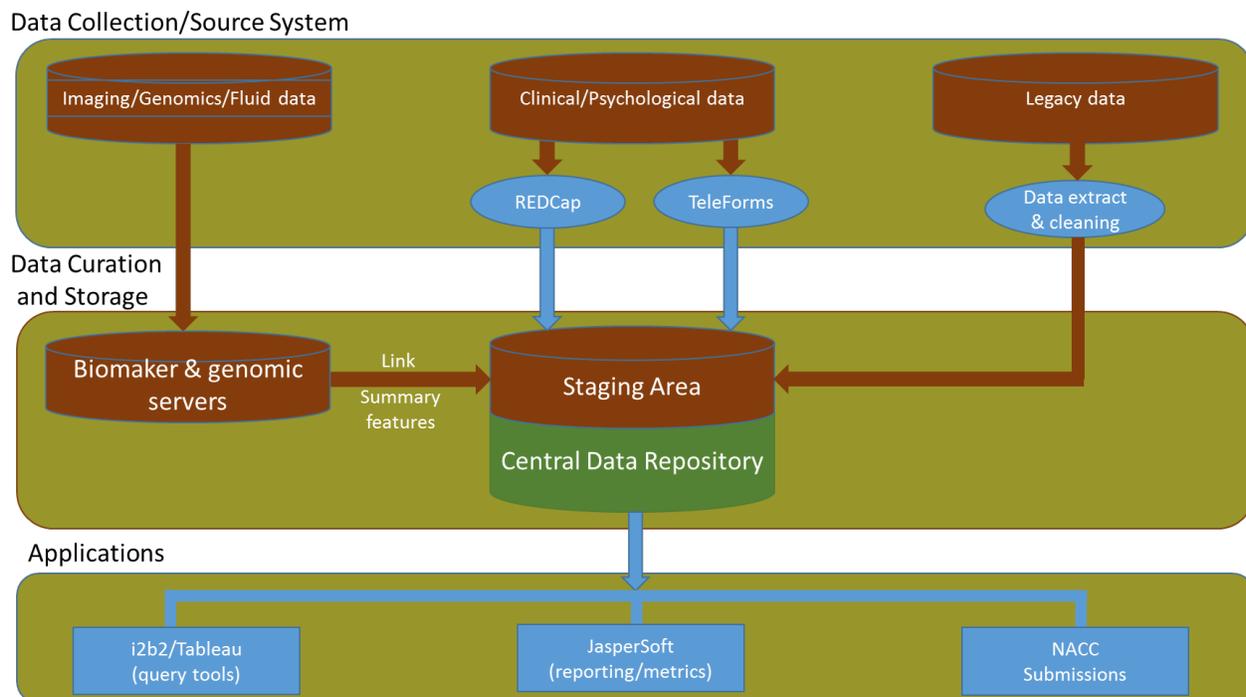


Figure. Illustrations of the overall architecture of the proposed system with Source Systems shown on top, Staging and CDR in the middle and Applications on the bottom

Year End Progress Summary: Significant progress has been made towards implementing the infrastructure proposed above. For each of the two current programs, Arizona Alzheimer’s Disease Core Center (ADCC) and the Apolipoprotein E4 (APOE4) Gene Dose Program, we are finalizing the implementation of REDCap based electronic data collection instruments as we originally proposed. The decision to use REDCap for our data entry/collection tool was welcomed by study coordinators from all ADCC sites and from BAI and Mayo teams for the APOE4 Gene Dose Program. For the Brain and Body Donation Program (BBDP), we are keeping the extensive MS Access database based infrastructure for the near term, and are looking to interface this system with our centralized infrastructure. Long term decision will be collectively made by Dr. Caselli, Dr. Reiman, Mr. Saner, Dr. Chen, and especially, Dr. Beach.

In addition to the REDCap implementation, we have authored a key piece of software, REDCap2Relational, that expedites and automates the export of data from REDCap projects into a relational database. REDCap2Relational can interrogate the meta data of any REDCap project,

create a relational database schema based on the meta data and then extract the data from REDCap into the newly created relational schema. All of this is accomplished through the REDCap Application Programming Interface (API).

In our implementation, data is extracted from REDCap using the REDCap2Relational into the Staging Area. From the Staging Area, the data is then moved into the Central Data Repository using SQL Server Integration Services (SSIS). During the Extract, Transform and Load (ETL) process from the Staging Area to the Central Data Repository, we harmonize the data from the different data sources (in the case of ADCC, this is the data from the three different UDS versions). We have further leveraged SSIS to extract the data from the Central Data Repository into flat files that can be uploaded to NACC. For the ADCC data we have performed end to end testing from entering data in REDCap, extracting it to the Staging Area, performing ETL into the Central Data Repository and finally generating and uploading NACC extracts. We are planning a go-live date for this part of the system for the end of February.

A SQL Server Reporting Services (SSRS) server has also been provisioned which will provide on demand web based reports that can be exported to various formats, including CSV, Excel and PDF. Sample reports have been created based on templates provided by members of the ADCC. The primary purpose of deploying SSRS is to make the data available to core leaders so they can monitor key metrics used to assess the progress and performance of ADCC, APOE and future studies. We also envision reports that could help assist in scientific investigations.

Based on our testing to date we feel confident that the infrastructure established thus far is extensible to additional projects and data types. As we continue to expand our Central Data Repository, we will be establishing a robust web based tool that can be used by internal and external investigators to interrogate the available data and biospecimens available and ultimately make requests for these resources. We plan to have an initial version of this platform completed by the end of June 2017.

Project Progress Reports
Banner Sun Health Research Institute

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Towards single-cell analysis in human brain neurodegenerative disease: a pilot study. Thomas G. Beach, MD, PhD, Geidy Serrano, PhD, David Brafman, PhD, Douglas Walker, PhD, Lih-Fen Lue, PhD, Matthew Huentelman, PhD, colleagues from each of the participating Alzheimer's Consortium sites. Banner Sun Health Research Institute; Banner Alzheimer's Institute; Mayo Clinic Arizona; University of Arizona; Arizona State University; Translational Genomics Research Institute; Arizona Alzheimer's Consortium.

Specific Aim 1: Develop, optimize and standardize a method for producing single-cell suspensions from rapidly-autopsied human brains, allowing the analysis of proteins, RNA and DNA from single cells and phenotypically-specified cell populations.

Specific Aim 2: Biochemically characterize single cells and phenotypically-specified populations of cells from single-cell suspensions created with an optimized standardized method (from Specific Aim 1).

Specific Aim 3: To provide the foundation of a shared resource of separated cells to researchers within and outside Arizona.

Background and Significance: Biochemical analysis of human neurodegenerative brain tissue, especially from Alzheimer's disease (AD) and Parkinson's disease (PD) patients, has produced much of what is known about these conditions, and has led to the major FDA-approved therapies. The typical approach has been to homogenize whole pieces of brain tissue and separately characterize the proteins, RNA, DNA and other macromolecules within. While this has been sufficient to identify large changes, there is very little ability to identify small changes, or changes in small subsets of cells. Furthermore, neurodegenerative disease often leads to massive losses of the targeted and disease-relevant cells, for example the entorhinal cortex layer II stellate neurons or substantia nigra pigmented neurons. Whole-homogenate analysis of such brain regions can give completely misleading results, as any biochemical constituent that is selectively localized to the depleted cells will appear to be "down-regulated", whereas in fact it has most likely been lost only as an "innocent bystander". Also, a relevant loss or increase might be completely missed, if the biochemical entity is found in many cell types, diluting the "lost" signal from the cell of interest, especially if that cell type is uncommon or rare. To effectively investigate the biochemistry of neurodegenerative disease in the brain, with its thousands of different cell types, we must first separate the cells, and study them as phenotypically-defined populations and even as individuals.

Laser-capture microscopy (LCM) is an early method that could pick individual cells off a cryostat section, but is severely limited by the large time and personnel investment as well as the limited ability to phenotypically mark target cells. In recent years, methods have been developed that allow an initial creation of single-cell suspensions from solid tissue followed by analysis of phenotypically-defined cells sorted on the basis of cell-type identifying proteins or RNA expression¹⁻⁶. These methods are much more time and labor-efficient than LCM, and allow sorting by a much more diverse panel of markers. Some groups have already published

intriguing results⁷⁻⁹ from AD brain cells, but as yet there has not been a comprehensive exploitation of these novel technologies. This set of experienced neuroscience investigators, together with a unique rapid-autopsy brain tissue resource, are well-suited to apply these methods on a large scale to AD and other neurodegenerative brain diseases.

Preliminary Data: The PI is Director of the Banner Sun Health Research Institute's Brain and Body Donation Program (BBDP), a clinicopathological study of aging and neurodegenerative disease based in Sun City, AZ since 1987¹⁰. The BBDP has made rapid autopsy a priority, with a 3.0-hour median postmortem interval for the entire collection. Tissue quality is correspondingly high, with a median RNA Integrity Number (RIN) for frozen brain tissue of 8.9. Between 80 and 110 autopsies are done each year, allowing the rapid acquisition of relatively large numbers of new subjects. The PI and Co-Pis have prior experience with LCM single-cell harvesting and population analysis of phenotypically-defined human control and AD neurons and glia derived from the BBDP¹¹⁻¹⁷. Drs. Walker and Lue have focused their entire careers on separation and culture of a variety of cell types from BBDP postmortem human brains¹⁸⁻²¹. Dr. Brafman is experienced in the usage of fluorescence-activated cell sorting (FACS) for antibody-based separation and analysis of defined cell types, including neural progenitor cells²²⁻²⁷. Dr. Huentelman has developed novel methods of genomic and cellular analysis and applied these to several disease conditions, especially Alzheimer's disease²⁸⁻⁴¹.

Experimental Designs and Methods

Specific Aim 1

Creation of single cell suspensions. Multiple methods using various combinations of enzymatic and mechanical dissociation techniques have been used but many investigators⁴²⁻⁴⁶ have found it advantageous, for efficacy and standardization, to use an automated device, the GentleMACS Dissociator (Cat No. 130-093-235, Miltenyi Biotec) and we will therefore also initially base our protocol on this, while also assessing alternatives. Simultaneous enzymatic digestion with different enzymes, e.g. trypsin, papain, will be trialed, as well as removal of excess myelin with Myelin Removal Beads⁴⁶. Subsequent filtration will be followed by suspension in cryopreservative and freezing at - 80C for future usage. The objective will be to maximize recovery of morphologically intact and separated cells.

Estimation of quantitative cell recovery. Companion, adjacent tissue samples (cerebral cortex ~ 600 mg) will be dissected for comparison of cell numbers recovered, using unbiased morphometric cell density estimates, performed on one companion sample, as the gold standard. The density of total cell nuclei, expressed per tissue unit weight, as well as immunocytochemically identified neurons (NeuN), astrocytes (GFAP) and microglia (Iba-1) will be assessed and compared with densities determined through FACS (Brafman), magnetic bead sorting (Walker and Lue) and RNASeq (Huentelman).

Specific Aim 2

Drs. Walker and Lue: Separate cell suspension aliquots will be incubated with antibodies NeuN, GFAP and Iba-1 followed by magnetic beads (Microbeads, Miltenyi Biotec) conjugated with anti-mouse immunoglobulin. The cell suspension is washed by centrifugation and separated through an AutoMACS Pro cell separation device (Miltenyi Biotec). Cells conjugated to beads are positively selected, eluted from the column and recovered by centrifugation. The resulting cells are frozen prior to subsequent Western blots with the same antibodies to determine purity of

cell separation, as well as RNA isolation and quality assessment by RNA integrity number and RNA yield analyses.

Dr. Brafman: Cells will be washed twice with stain buffer (PBS, 1 mM EDTA, and 0.5% FBS) and resuspended at a maximum concentration of 5×10^6 cells per 100 μ l. For staining of intracellular proteins (NeuN, GFAP, Iba-1), cells will be fixed for 10 min on ice with BD Cytotfix Fixation Buffer (BD Biosciences). The cells will then be washed twice with stain buffer and permeabilized with BD Phosflow Perm Buffer II (BD Biosciences) for 30 min on ice. Cells were then washed twice with stain buffer and the primary antibody will be added cell suspension. Cells will be stained overnight at 4^oC, washed twice, and resuspended in stain buffer. Cells will then be stained with the species appropriate secondary antibody for 1 hour on ice, washed twice, and resuspended in stain buffer at a maximum concentration of 1×10^6 cells per 100 μ l. Cells will be analyzed on a ACCURI C6 (BD Biosciences). Results will be analyzed using FlowJo software using appropriate control isotypes to set thresholding gates.

Dr. Huentelman: RNA Sequencing of Single Cells. Next generation RNA sequencing (RNA-Seq) will be performed on cryopreserved single cells using the 10X Genomics droplet-based approach. Briefly, single cell suspensions will be thawed and individual cell containing droplets will be merged with reaction buffer droplets resulting in cell lysis and bar-coded cDNA synthesis. Library preparations will be performed using Illumina-based chemistries and the resulting libraries will be sequencing to a depth of approximately 5 million reads per cell. Analysis will be performed to identify major and minor cell populations that may be present in the single cell suspension sample.

Proposed One-Year and Long-Term Outcomes: The one-year goal will be to have a scientific report accepted for publication that describes the standardized method for creating single-cell suspensions as well as a biochemical characterization of sorted neurons, astrocytes and microglia. This publication will serve as preliminary data for planned NIH grant proposals to compare sorted cells from different neurodegenerative diseases as compared to normal control cells. These grant proposals will be directed at supporting both the cell core and individual investigator-initiated projects.

Progress Summary to April 3, 2017

Specific Aim 1: Develop, optimize and standardize a method for producing dissociated-cell suspensions from rapidly-autopsied human brains, allowing the analysis of proteins, RNA and DNA from single cells and phenotypically-specified cell populations.

Creation of dissociated-cell suspensions

Multiple methods using various combinations of enzymatic and mechanical dissociation techniques, as well as time and temperature differences, have been used in the past but there is insufficient data to allow decisions on the best approach. It is possible that more than one method may be necessary to allow a wide range of experiments and analyses. Also, complementary methods will allow critical cross-validation of results, including validation with the current gold-standard, laser-capture microdissection. We are currently pursuing two strategies for producing dispersed cells that would form a shared resource for a wide range of investigations (Table 1).

Physiological cell dissociation seeks to keep cells alive during processing at 37 C, with cocktails

including varying combinations of serum proteins, growth factors, gentle mechanical and enzymatic manipulation, antibiotics, anti-oxidants and oxygenation. **Hypothermic cell dissociation** seeks to minimize molecular changes caused by processing, by cold processing.

Table 1. Comparison of methods for single-cell isolation and analysis. There is at present insufficient data to determine which approaches are best. It is possible that all three methods are complementary and can serve to cross-validate each other. The cell dissociation methods, however, offer the best prospects for creating a resource that could be shared with multiple projects.

Laser-Capture Microdissection	Advantages 1. Allows selection of cells in microanatomical context, e.g. cortical layer, morphology. 2. All cell types are available for selection and analysis. 3. Is current gold-standard for single-cell isolation and analysis. 4. Open databases exist, allowing cross-comparison of data.
	Disadvantages 1. Very labor intensive, restricting sample analysis numbers. 2. Limited ability to phenotype with antibodies. 3. Includes surrounding neuropil that contaminates data.
Physiological Dissociation	Advantages 1. Living cells can be cryoprotected and used later for in vitro experimentation. 2. Agonal changes in macromolecules may be reversed. 4. May allow higher yield and integrity of macromolecules including RNA. 5. May allow a greater range and accuracy of sorting by phenotype.
	Disadvantages 1. May be associated with significant process-associated molecular changes. 2. May lose cells selectively or non-selectively, during processing. 3. May be more time-consuming and require more expertise. 4. Advantages may be lost with neurons, most of which do survive.
Hypothermic Dissociation	Advantages 1. Low temperature processing may limit molecular changes of processing. 2. May lose cells selectively or non-selectively, during processing. 3. May be less time-consuming and require less expertise.
	Disadvantages 1. May reduce yield and integrity of macromolecules including RNA. 2. May result in greater loss of proteins needed for phenotyping. 3. Agonal changes in macromolecules will not be reversed.

The physiological dissociation approach is being conducted by Drs. Lue and Walker, with procedures adapted from their longstanding postmortem cell culture protocols. The hypothermic approach is being pursued by Drs. Serrano and Beach.

With both approaches, tissue is first minced with a razor blade and then mechanically disrupted by repetitive pipetting. More recently, Drs. Serrano and Beach have used an Omni tissue grinder. Following this, the physiological approach employs digestion with 0.25 mg/ml

papain at 37°C for 45 minutes, while the hypothermic approach uses enzymatic digestion with Accutase for 4 hours at 4°C.

Both methods then remove myelin, neuropil and other cellular debris using percoll centrifugation. Using either approach, the final products are single-cell suspensions of glia and neurons that will be useful for further customized processing and analysis by user researchers.

To date, 42 autopsies have been performed by the BBDP since July 1, 2016. This is a low autopsy rate compared to recent years, limiting the project's progress to some extent. Of these 36 autopsies, tissue from 15 has been used to develop and refine the cell dissociation protocols. A subset of cell pellets are embedded in paraffin, sectioned and stained with H&E. Figure 1 shows representative images of a single-cell suspension derived from physiological dissociation while Figure 2 shows an initial cell pellet using the hypothermic approach. Figure 3 images are from a cell pellet obtained with the most recent modification of the hypothermic app

Figure 1. Dissociated single-cell suspension obtained with the physiological approach, at three successively higher magnifications. The preparations are almost entirely made up of nuclei (blue-purple spherical structures) and perinuclear cytoplasm, with no detectable cell debris.

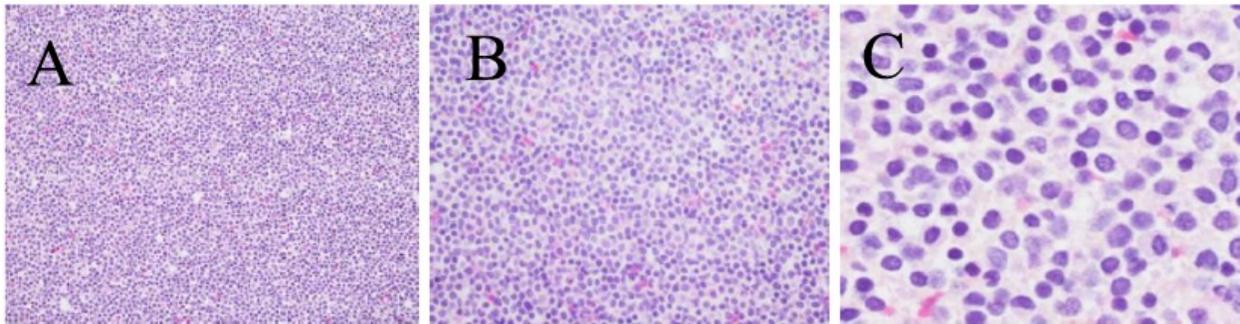


Figure 2. Dissociated single-cell suspension obtained with an initial protocol using the hypothermic approach, at three successively higher magnifications. Many nuclei are present but there is also abundant eosinophilic (pink) cellular debris, composed of disintegrated neural and glial processes and synaptic terminals (neuropil).

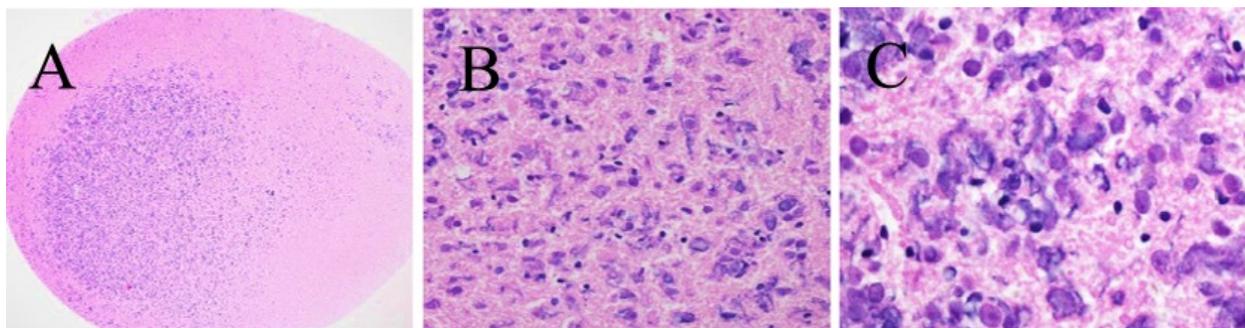
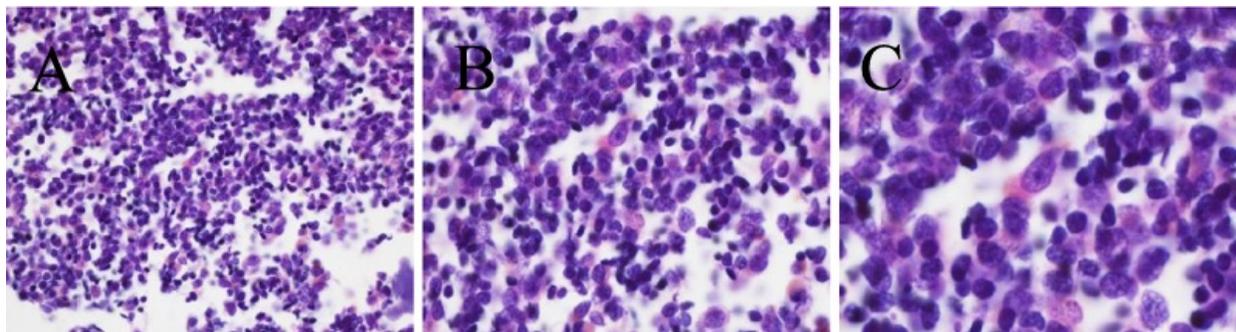


Figure 3. Dissociated single-cell suspension obtained with the most recent protocol utilizing the hypothermic approach, at three successively higher magnifications. The preparations are relatively restricted to nuclei (blue-purple spherical structures) and perinuclear cytoplasm, with very little detectable cell debris.



Specific Aim 2: Biochemically characterize single cells and phenotypically-specified populations of cells from single-cell suspensions created with an optimized standardized method (from Specific Aim 1).

Characterization of RNA obtained from dissociated cell preparations

The objective is to create dissociated-cell suspensions with intact protein and RNA, allowing for comprehensive biochemical characterization. Two criteria were used to assess RNA quality, yield and integrity. Table 1 lists results from the two approaches to date. The data show that relatively intact RNA can be isolated from cells separated through the two procedures. However, there was considerable variability, for reasons that need to be resolved. It is known, from more than two decades of work from the Walker-Lue lab, that much of the case-to-case variability is due to initial brain tissue status, which is variable due to differing agonal conditions, postmortem cadaver temperatures and postmortem interval prior to commencement of autopsy.

Also, the RNA concentration may not be easily comparable due to differing concentrations of cellular perikarya between methods. As shown in Figures 1 and 2, initial protocols for the hypothermic approach did not exclude large amounts of neuropil that were free of cellular perikarya, with a probable proportional impact on RNA concentration. More recent hypothermic protocols have excluded most of the neuropil (Figure 4) and should allow for a more direct comparison of RNA concentration in the two methods.

Due to these factors, at present only RNA integrity may be directly compared in the two methods. Case 16-53 shows that, when original tissue status and processing protocols are optimal, excellent RNA concentrations, yields and integrity may be achieved with both methods. RNA integrity numbers for dissociated cell preparations for this case were 8.0 and 8.4, respectively, for the hypothermic and physiological approaches.

In three other autopsies where both methods were used, the RNA integrity number tended to be higher with the physiological approach. In future cases, the usage of RNAase inhibitors may be employed to protect RNA during processing. To date, this has not been used, as both protocols have been derived from protocols intended to preserve cell viability for eventual cell culturing. As this is not the intended usage of the dissociated cell resource, RNAase inhibitors are not contraindicated and it is likely that adding RNAase inhibitors will preserve RNA, allowing for more reliable gene expression assessment.

Phenotypic characterization of cells from dissociated cell preparations. Another objective is to create single-cell suspensions with representative populations of neurons and glia. To establish whether major cell types are present, the paraffin-embedded cell pellets were immunohistochemically stained with antibodies specific for neurons (neurofilament), astrocytes (glial fibrillary acidic protein, GFAP) and microglia (Iba1).

Preliminary examination of stained cell pellets prepared with the hypothermic approach show cells stained with all three antibodies, in Figure 4. The figure shows that all three major cell types appear to be present, based on the presence of positive staining in select cell populations. However, double-staining needs to be performed to ascertain whether any cells are marked by more than one antibody. As these antibodies are known to be completely selective for one cell type only, there should not be any double-stained cells.

Another way to determine what cell types are present in the cell suspensions is to use fluorescence-activated cell sorting (FACS). This method also provides “pure” populations of cells, depending on the antibodies used. Dr. David Brafman has done preliminary FACS analysis of cell suspensions derived from the hypothermic approach.

Cells were washed twice with stain buffer (BD Biosciences) and resuspended at a maximum concentration of 5×10^6 cells per 100 μ l. For staining of intracellular proteins, cells were fixed for 10 min on ice with BD Cytfix Fixation Buffer (BD Biosciences). The cells were then washed twice with stain buffer and permeabilized with BD Phosflow Perm Buffer II (BD Biosciences) for 30 min on ice. Cells were then washed twice with stain buffer and one test volume of antibody was added for each 100 μ l of cell suspension. Primary antibodies were incubated overnight at 4°C and then washed twice with stain buffer at RT. Secondary antibodies were incubated at RT for 1 hr. Cells were analyzed on an ACCURI C6 (BD Biosciences). Gates were set using secondary only controls. Analysis of four primary cortical samples (16-50, 16-52, 16-53, and 16-57) revealed that GFAP percentages ranged from 8.0-20.5%, Neuronal Nuclear protein (NEUN) percentages ranged from 85.3-95.0%, and IBA1 percentages ranged from 84.8-96.7%.

The next step will be to determine the percentages of cells that are double-labeled. Again, as these antibodies are known to be completely selective for one cell type only, there should not be any double-stained cells.

Discussion and Future Plans:

1. Dissociation protocols may need further refinement to determine whether the observed variability in RNA yield and integrity is protocol-related, and whether this may be improved by adding RNAase inhibitors. If the cell suspensions are to become a reliable research resource, all will need to have RNA quality analysis in order to determine whether they are suitable for further usage.
2. Further characterization of the cell suspensions, in both paraffin-embedded cell pellets and by FACS, is needed to determine whether cell subsets labeled with the cell-type-specific antibodies are mutually exclusive as would be expected, and to determine whether ratios of neurons and glial cell types are what would be expected from brain tissue. This would be done using antibody double-labeling. Also, the dissociation methods may selectively lose some cell types and it would be important to know this and devise protocol changes to address this as researchers will often want to have all cell types present.

3. RNA quality analysis needs to be repeated on cell subpopulations after undergoing FACS, to ensure that RNA quality is maintained and that sorted cells will be useful for further study, e.g. transcriptome analysis.
4. Analysis of cell suspensions using next generation RNA sequencing (RNA-Seq) and the 10X Genomics droplet-based approach, to be initiated once further characterization and quality analysis of the cell suspensions has been performed (as in 2 and 3 above). This transcriptome analysis of sorted cells will also be important to confirm the accuracy of cell-type sorting by FACS.
5. Once data from 1-4 above has been acquired, a detailed comparison will be made of the hypothermic and physiological cell dissociation methods. It is likely that the methods will be complementary, with some projects more suited to one and some projects better served by the other.
6. The optimal methods for long-term cryopreservation of cell suspensions will need to be determined in order for these to become a shared resource.
7. Once steps 1-4 above have been completed to initial satisfaction, a methods paper will be written and published so that user-researchers will have confidence in the methods used to prepare the cell suspensions.
8. Once methods have been optimized, results from specific autopsies should be compared with those obtained using laser-capture, as this has previously been the gold standard for cell-type specific gene expression analysis.
9. After a methods paper has been published, in the next year of funding, one or more high-profile projects should be undertaken, to further establish the importance of the general approach and to further awareness of the resource among the neurodegenerative disease scientific community.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Developing a shared resource of cerebrospinal fluid, plasma, serum, and peripheral blood mononuclear cell (PBMC) samples from Arizona's Longitudinal Brain and Body Donation and Apolipoprotein E4 (APOE4) Gene Dose Programs. Thomas G. Beach, PhD & Edward Zamrini, Geidy Serrano, Kathryn Demarco, David Weidman, Lucia Sue, Richard J. Caselli, Charles H. Adler, Donald Saner, Eric M. Reiman. Banner Sun Health Research Institute; Banner Alzheimer's Institute; Mayo Clinic Arizona; University of Arizona; Arizona State University; Translational Genomics Research Institute; Arizona Alzheimer's Consortium.

Specific Aims:

1. To develop a repository of cerebrospinal fluid (CSF), plasma, serum, and PBMC samples from well characterized, longitudinally assessed, and consenting participants in Arizona's Brain and Body Donation Program.
2. To develop a repository of CSF, plasma, serum and PBMC samples from well characterized, longitudinally assessed, and consenting participants in Arizona's APOE4 Gene Dose Program.
3. To provide a shared resource of CSF, plasma, and serum samples and data to researchers inside Arizona and around the world.

Background, Significance and Preliminary Findings:

The Arizona Brain and Body Donation Program (BBDP) provides an invaluable scientific resource of longitudinal cognitive, motor, clinical, and genetic data from >800 living older adults who have standardized annual assessments, consent to brain (and frequently body) donation, and provide a resource of unusually high-quality brain tissue, postmortem CSF and blood samples (which differ in some respects to samples that are acquired in life) and neuropathological data after they die. The program includes but is not limited to research participants with the clinical features of Alzheimer's disease (AD) or related disorders and cognitively and neurologically unimpaired older adults with support from the National Institute on Aging (NIA)-supported Arizona AD Core Center (ADCC), research participants with the clinical features of Parkinson's disease (PD) and related disorders and cognitively and neurologically unimpaired older adults with support from the National Institute of Neurological Disorders (NINDS)-supported National Brain and Tissue Resource for PD and Related Disorders (NBTR-PD). The BBDP has provided an invaluable resource of data, brain tissue and DNA to researchers around the world. CSF and blood samples would enhance the value of the BBDP in several ways, including a) the chance to clarify whether the participants have CSF evidence of amyloid- β and tau pathology (biomarkers of AD), b) the chance to evaluate, further develop emerging CSF and blood-based biomarkers in terms of the extent to which they predict subsequent clinical decline and the neuropathological diagnosis of AD, PD, and other disorders, and c) the chance to use CSF- and blood-based measurements to further help in the clarification of disease mechanisms and risk factors.

The Arizona APOE4 Gene Dose Program provides an invaluable scientific resource of longitudinal data from initially cognitively unimpaired research participants with two, one and no copies of the APOE4 allele, the major genetic risk factor for AD. The program includes nearly 200 participants who were initially late-middle-aged participants with a first degree family

history of dementia who are followed every two years with a battery of clinical ratings, cognitive tests, FDG, amyloid and now tau PET scans, and MRIs, who have provided plasma, serum and PBMC samples that are stored at Mayo Clinic, and who have begun to provide CSF samples with support from a longstanding NIA grant. It also includes more than 200 other participants, with or without a family history and through youngest to oldest adult ages, who are followed using state and organizational Arizona Alzheimer's Consortium funds, and who have not yet provided CSF, plasma and serum samples. CSF and blood samples in state-supported APOE4 Gene Dose participants would increase the value of the Arizona APOE4 Gene Dose Program in several ways, including a) the chance to detect and track the earliest fluid biomarker changes associated with the predisposition to AD, b) clarify the extent to which they are associated with subsequent cognitive decline and clinical progression, c) help to distinguish the cognitive changes associated with preclinical AD from those associated with aging in the absence of AD pathology, d) help researchers clarify the extent to which emerging AD biomarkers could be detected at earlier ages, and e) provide promising endophenotypes to help in the clarification of AD risk factors.

Research Plan: During the one-year funding period, we propose to further develop the infrastructure to conduct lumbar punctures (LPs), acquire up to 30 ml of CSF and 40 ml of blood and process CSF, plasma, and buffy coat (for PBMCs) samples from BBDP participants at BSHRI and APOE4 Gene Dose Program participants at BAI, to acquire, process, aliquot and store samples using standardized procedures, and to establish a repository of these samples at BSHRI. This year, we propose to acquire CSF samples in 100 returning BBDP participants at BSHRI and in 50 returning state-supported APOE4 Gene Dose Program participants at BAI who consent to LPs; and we propose to acquire blood samples in 400 returning BBDP participants and in the 50 APOE4 Gene Dose Program participants.

CSF Samples. LPs will be acquired by trained and experienced personnel standardized procedures established for other longitudinal cohorts. We propose to acquire up to 20 ml of CSF, as the volume of CSF has not been associated with differences in the risk of post-LP headaches. CSF will be centrifuged at 1,500 rpm for 10 min at 24°C. The supernatant will be collected, placed into 0.25 ml aliquots, and stored at -80°C. One (1) ml of CSF from a randomly selected set of at least 5% of cases to conduct standard analyses on cell count, protein and glucose levels, and hemoglobin levels.

Blood Samples. We propose to acquire up to 40 ml of venous blood in EDTA tubes. Blood will be centrifuged at 1,500 rpm for 15 min at 24°C to separate plasma, buffy coat and red blood cells. The plasma will be collected and placed into 1.7 ml microcentrifuge tubes and then centrifuged again for 5 min, 4°C at 14,000 rpm. The plasma supernatant will be collected and stored at -80°C. From blood samples collected at BSHRI, the buffy coat at the red blood cell/plasma interface will be diluted in Hank's Balanced Salt Solution (HBSS) to a total volume of 50 ml. A total of 25 ml of HBSS-diluted blood will be gently layered on top of 10 ml of Histopaque 1077 and then centrifuged at 400 x rcf for 30 min at 24°C. The opaque interface containing peripheral blood mononuclear cells (PBMC) will be collected and then brought up to 50 ml with HBSS. After centrifugation at 3000 x rcf for 15 min at 24°C, the supernatant will be discarded and each pellet resuspended in 3 ml of HBSS and then transferred to 1.7 ml microcentrifuge tubes. The resuspended cells will then be centrifuged at 14,000 rpm for 5 min at 4°C to pellet the PBMC. The PBMC pelleted cells will be stored at -80°C.

Fluid Repository. All samples from Specific Aims 1 and 2 will be stored at BSHRI in ultra-low temperature freezers protected with redundant temperature-activated alerts, banks of emergency CO2 tanks, redundant air conditioning units and backup diesel alternate power supply. BBPD staff are on constant call to respond to freezer alerts. A biological sample distribution committee involving the BBPD and APOE4 Gene Dose Program PIs will evaluate all research proposals involving the use of shared biological samples.

Proposed One-Year and Long-Term Outcomes: Our one-year goal is to collect, process and store the samples as described. Our long-term goals are to extend this effort to all consenting participants in the BBPD and APOE4 Gene Dose Programs, incorporate relevant information about these samples in a centralized database, secure NIH funding to support the longitudinal acquisition of CSF and blood samples in this invaluable cohort, provide a shared resource of biological samples to researchers inside and outside of Arizona, and use these samples to make significant contributions to the scientific study of AD, related disorders and cognitive aging.

Progress Summary to April 3, 2017

Specific Aim 1. To develop a repository of cerebrospinal fluid (CSF), plasma, serum, and PBMC samples from well characterized, longitudinally assessed, and consenting participants in Arizona's Brain and Body Donation Program.

Blood Samples. These efforts started in the previous, 2015-2016 funding year, when we collected plasma and white blood cells from 222 BBPD participants. To date since June 30, 2016, we have obtained blood samples from 222 additional participants. By clinical diagnosis, the collected blood samples are from 269 non-demented controls, 21 subjects with mild cognitive impairment, 29 subjects with a clinical diagnosis of dementia due to possible or probable Alzheimer's disease, 74 subjects with Parkinson's diseases and 22 with other diagnoses. It is projected that we will meet our 2016-2017 goal of collecting blood samples from 400 BBPD participants.

CSF Samples. For CSF from the BBPD, of 180 participants that had agreed, on their most recent BBPD consent, to contribute CSF samples, 39 were ruled medically ineligible for lumbar puncture, mostly due to history of spinal surgery, and thus were not contacted as this is a medical contraindication. More than 60 others have been contacted by phone and of these, 30 have changed their minds and now decline to donate CSF. Twenty-five were scheduled for lumbar puncture. Nine of these cancelled their clinic appointments in the few days prior to the appointment; of these, four have stated that they are willing to be re-scheduled. Two subjects were found to have scars in the lumbar area consistent with prior surgery (despite having no database history of spinal surgery) and were thus sent home. Thirteen subjects had a lumbar puncture of which 10 were successful but 3 did not obtain CSF. The project is thus behind schedule. Much of this is due to an unexpectedly high rate of refusal from subjects that had previously indicated on their consents a willingness for CSF donation. Additionally, we have had unanticipated difficulty in hiring a suitable nurse practitioner (NP). Only a single staff member (Dr. E. Zamrini) is currently qualified to do lumbar punctures and he has had time available for only 2-4 attempts, at most, per week. An NP was hired (Alisson Gilbert) in January 2017 and will be undergoing lumbar puncture training over the next 2-3 months. A suitable training program is under development in consultation with nurse administrators at the Arizona Mayo Clinic, who have prior experience with this and with a scheduled visit by an external expert, Dr. Elaine Peskind (scheduled for May 8-10th). We anticipate being able to obtain CSF

from 50 subjects by the end of the project but will need an encumbrance until September 30th to accomplish this.

Specific Aim 2. To develop a repository of CSF, plasma, serum and PBMC samples from well characterized, longitudinally assessed, and consenting participants in Arizona's APOE4 Gene Dose Program.

Longitudinal biofluid samples from 18 subjects enrolled in the APOE imaging cohort (CSF, plasma, and serum) have successfully been transferred from BAI to BSHRI. This effort has achieved the longtime need for unification of APOE imaging cohort samples in one laboratory with the required expertise. These are currently stored at BSHRI in ultra-low temperature freezers protected with redundant temperature-activated alerts, banks of emergency CO2 tanks, redundant air conditioning units and backup diesel-powered alternate power supply.

No subjects have yet been referred to BAI for this project from the non-imaging APOE cohort. However it is anticipated that blood and CSF will be obtained from 50 participants by the end of September 2017.

In preparation for the availability of an NP (Alisson Gilbert) who will be performing lumbar puncture procedures and collecting CSF for the APOE study cohorts, the APOE study teams of Mayo Clinic and BAI have together begun the recruitment process of non-imaging APOE study participants currently undergoing participation exclusively at Mayo Clinic. It is our intention to hold an informational session at Mayo Clinic for this group of dedicated participants for their convenience. This informational session will allow program investigators to provide detailed insight regarding the value of study participation directly to these esteemed volunteers. Results from similar informational sessions at BAI and BSHRI show that efforts such as these prove to be tremendously fruitful for recruitment and retention of research participants. Existing APOE imaging cohort participants have been presented information regarding the importance of biofluid collection and analysis at their long-established volunteer appreciation events. This has shown to increase participant enthusiasm and has encouraged participants to independently reach out to study staff to requesting to complete biofluid collection procedures, such as lumbar puncture, without additional promotion made by the research team.

Specific Aim 3. To provide a shared resource of CSF, plasma, and serum samples and data to researchers inside Arizona and around the world.

Ultimately, because the collected samples will be tied to a postmortem neuropathological diagnosis, this will constitute a unique and invaluable "reference resource" for rigorously evaluating potential biofluid biomarkers of AD, PD and other neurodegenerative diseases. Therefore, it will be important not to disburse all of the samples prior to obtaining autopsy diagnoses. For this reason, and because the collected samples do not as yet constitute a sufficiently large sample size, we have not as yet undertaken any promotion of the availability of the new antemortem biofluids resource. At present we are fulfilling our sharing mandate by disbursing from our large collection of postmortem blood serum and CSF. Since July 1, 2016, we transferred 455 serum and/or CSF samples to 9 different researchers located in 7 different states.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Population survey and clinicopathological study initiation for Incidental REM Sleep Behavior Disorder in Sun City, Arizona. David Shprecher, DO, MSci, Thomas G. Beach, MD, PhD, Charles H. Adler, MD, PhD, Eric Reiman, MD, Richard Caselli, MD, Shyamal H. Mehta, MD, PhD, Joseph Hentz, MS, Geidy Serrano, PhD, Brad Boeve, MD, Ron Postuma, MD. Banner Sun Health Research Institute; Mayo Clinic Arizona; Banner Alzheimer Institute; Mayo Clinic Rochester; McGill University.

Specific Aim 1: Conduct a population-based survey of the prevalence of idiopathic REM sleep behavior disorder in Sun City, Arizona

Specific Aim 2: Initiate a longitudinal clinicopathological study of idiopathic REM sleep behavior disorder in the Arizona Study of Aging and Neurodegenerative Disorders and the Banner Sun Health Research Institute Brain and Body Donation Program.

Background and Significance: Idiopathic rapid-eye-movement (REM) sleep behavior disorder (iRBD) is a harbinger of neurodegenerative disease in the elderly. A definite diagnosis of requires presence of dream enactment behavior, absence of a secondary cause (such as medications, brainstem lesions in tracts mediating REM atonia, or neurodegenerative disease) and polysomnogram confirmation (demonstrating REM atonia and the absence of an RBD mimic such as nocturnal frontal lobe epilepsy or arousals related to sleep apnea. Over the last 15 years, evidence from multiple research groups world-wide has indicated that approximately 50% of those with RBD will develop either parkinsonism or dementia within 10 years, with 80% or more converting after 20 years. The mean time interval between RBD onset and cognitive impairment or parkinsonism is 6-7 years. Autopsy studies have shown that the great majority of those dying with RBD have a brain disorder characterized by the accumulation of a protein called alpha-synuclein, and are hence termed "synucleinopathies". The major synucleinopathies are Parkinson's disease (PD) and dementia with Lewy bodies (DLB). There are currently no preventative treatments for PD or DLB, and prevention trials have not been feasible due to the low incidence rates and absence of clear clinical predictors of disease development. RBD subjects, however, with their high rate of progression to a synucleinopathy, are ideally suited for prevention trials. Recruiting for prevention trials from sleep clinics would be convenient but will be limited by the relatively small numbers of definite iRBD subjects that come to medical attention, especially if, as expected, multiple agents and trials will be needed. Probable RBD (pRBD) can be identified using the RBD single item questionnaire ("have you ever been told, or suspected yourself, that you seem to act out your dreams") with a sensitivity of 93.8% and specificity of 87%. Recruiting pRBD directly from the elderly population would be expected to generate the needed subject numbers, as RBD has been estimated to be present in about 0.5%-17% of older adults. However, there has never been a true population survey for the prevalence of RBD in the United States. Existing data comes almost entirely from subjects identified after presentation to a healthcare organization and are thus a selective and possibly distinct subset of RBD. A well-designed population survey is essential for understanding the true prevalence of RBD while neurological observation and ultimate autopsy diagnosis are essential for

understanding the percentage that eventually develop PD, DLB or other neurodegenerative diseases. These studies are a necessary step towards eventual clinical trials aimed at preventing the molecular cascade of synucleinopathy and hence PD and DLB. The study could provide a foundation for the study of cognitively normal older adults at risk for PD and PLB, who are longitudinally assessed using cognitive-behavioral, motoric, sleep, and other measurements to help in the preclinical detection and tracking of AD (informing the design and size of future prevention trials), and who are enrolled in the Brain and Body Donation Program to clarify the extent to which these ante-mortem measurements are associated with subsequent clinical course and post-mortem neuropathology.

Preliminary Data: The principal investigators are recognized experts in the clinical and neuropathological evaluation of synucleinopathies and have published multiple studies of preclinical markers including RBD. Dr. Shprecher has previously undertaken broad-based surveys of neurological illness, including a mail-out survey for the presence of RBD in 7,888 subjects in Salt Lake City, UT, to which 1,344 respondents included 13% that indicated the presence of dream enactment behavior (manuscript in preparation). Co-PI Dr. Adler is Co-Director of the Arizona Study of Aging and Neurodegenerative Disorders (AZSAND) and leads the clinical assessments of all enrolled subjects. Over 800 subjects are examined annually and Dr. Adler has published extensively on the clinical biomarkers seen in PD, AD, and other neurodegenerative disorders. Co-PI Dr. Beach is Co-Director of AZSAND and is Director of the Banner Sun Health Research Institute's Brain and Body Donation Program (BBDP), a clinicopathological study of aging and neurodegenerative disease based in Sun City, AZ since 1987. Between 80 and 110 autopsies are done each year, allowing the rapid acquisition of relatively large numbers of new subject, and autopsies are obtained in more than 90% of those enrolled. Tissue is made available to investigators worldwide, with more than 150 tissue transfers each year. Drs. Reiman and Caselli will also participate in this program, given the relevance of RBD to the preclinical study and prevention of DLB, and the opportunity to leverage resources from the Arizona AD Core Center.

Experimental Methods:

Specific Aim 1: Conduct a population-based survey of the prevalence of REM sleep behavior disorder in Sun City, Arizona.

We will identify the age-adjusted population prevalence of probable RBD subjects within a single zip code (85351) by random telephone interviews with 1,000 subjects in Sun City, AZ (Census Designated Place) approximately 6.6% of the more than 15,000 residential telephone numbers listed for this zip code. A survey with a random sample of 1,000 people is widely regarded by polling organizations as having a margin of sampling error of 3% for the estimated percentage of the whole population. Telephone numbers will be selected using a random number generator in conjunction with a commercial calling list. Telephone interviews are less subject to respondent bias than other screening methods that require more effort from the respondent, such as mail questionnaire. Using available demographic information, we will compare respondents to non-respondents to determine whether there are any systematic differences in age, gender or type of domicile. The call list does not include cellphone numbers and this will be a recognized bias. However, households that exclusively use cellphones tend to be younger and we expect that Sun City, as a retirement community, will have a high proportion of landline servicing. Calls made Monday through Friday have a larger probability of getting a woman than a man, because there

are more housewives than househusbands; however here again as Sun City is a retirement community this bias should be less expected although we do expect more women to answer than men due to the higher proportion of women surviving to older ages.

Probable RBD will be identified using the validated Mayo Clinic Sleep Questionnaire and a minimum definition of RBD will be defined as an affirmative answer to the presence of recurrent dream enactment behavior. An affirmative answer to the presence of recurrent dream enactment behavior has high sensitivity and specificity for polysomnogram-confirmed RBD. The survey will also include confirmation of demographic data as well as questions on existing neurological and other medical conditions. The survey questions will be designed after consultation with RBD experts including Dr. Bradley Boeve (Mayo Clinic Rochester) and Dr. Ronald Postuma (McGill University), as both have consented to assisting with the design of our planned study. Telephone interviews will continue until at least 1000 subjects have fully completed the questionnaire. Using a rough average prevalence of 5%, this will identify 50 RBD subjects.

To supplement those RBD subjects expected to be identified through the community-based telephone survey, we will administer the same questionnaire to the approximately 200 cognitively normal subjects currently on the BBDP enrollment “waiting list”. This may identify 10 or more additional subjects who will be invited to enroll.

Specific Aim 2: Initiate a longitudinal clinicopathological study of probable REM sleep behavior disorder in the Arizona Study of Aging and Neurodegenerative Disorders at the Banner Sun Health Research Institute Brain and Body Donation Program.

All of the identified RBD subjects will be invited to enroll in AZSAND and the Banner Sun Health Research Institute Brain and Body Donation Program (BBDP) a longitudinal clinicopathological study established in Sun City since 1987. As part of the BBDP, subjects will receive annual research-quality cognitive and movement disorders assessment batteries as well as general medical examinations. Detailed medical histories will be obtained through subject questionnaires and standardized abstraction of requisitioned private medical records. Also to be included are smell tests (RBD patients often have hyposmia) as well as autonomic symptom and environmental exposure questionnaires. All of the subjects will also be invited to undergo polysomnography, with the gold-standard diagnostic result being REM sleep without atonia. Polysomnography results will be used to estimate the percentage of questionnaire-positive probable RBD subjects that meet definitive RBD diagnostic criteria. Development of cognitive impairment and/or parkinsonism will be documented by clinical examination and ultimately autopsy, while neuropathological examination will determine the molecular disease process.

Proposed One-Year and Long-Term Outcomes: The one-year outcome is expected to result in a published scientific report that would be the first truly population-based study of RBD prevalence in a US community. Establishment of a cohort of prospectively-assessed RBD subjects will be used as preliminary data to obtain NIH, PCORI, and/or Michael J. Fox Foundation grants to enlarge the cohort, conduct directed studies of clinical progression biomarkers, and potentially begin prevention trials to slow or stop progression to PD or DLB. As the principal investigators have a long-established record of obtaining federal and non-federal out-of-state funding, this project has a high probability of leading to larger, long-term state revenue inflow and increasing local employment.

Year End Progress Summary: We completed IRB approvals through WIRB and Mayo Clinic, training and start up (including creation of a RedCap database) for our survey on prevalence of REM sleep behavior disorder in Sun City, AZ. Survey telephone calls started on January 3, 2017. Our team called an average of sixteen phone numbers per day and until January 25th, (for a total of 204 phone numbers called.) These numbers included calls that resulted in completed surveys, declined surveys, numbers out of service and no answer calls. Surveyor tried each number six times if there was no answer, before the number was considered a non-successful call. From the 204 covered phone numbers, 34% were numbers out of service, 22% declined participation, 38% without answer and 6% (twelve calls) resulted in a fully completed the survey. None of the participants reported recurrent dream enactment behavior, which is not surprising due to the small number of surveys currently collected.

As a consequence of these preliminary results, we have determined that a landline-based telephone survey is not practical in Sun City, AZ. We will therefore revise our survey to be a mail survey sent to home addresses in Sun City zip codes. In order to prevent duplicates, mail surveys will ask patients to indicate if they have already completed a telephone or mail survey. In order to adhere to the current budget, we will reduce staff time and effort supported on the grant proportional to the cost of postage and return response envelopes for the survey. Where indicated, we will enlist qualified research volunteers to complete data entry from the surveys.

In February 2017, we aim to begin enrolling our first participants with REM sleep behavior disorder into our observational cohort study. We have completed several direct to patient outreach talks on Parkinson disease and Lewy body dementia, including a Webinar with a state-wide audience, with specific mention of the need for participants in this study. We will expand recruitment efforts by also arranging for recruitment materials to be available at all Banner sleep clinics in Maricopa County. Once the Sun City survey has been completed, we will also arrange direct advertising in the local newspaper (using a public service announcement) and arrange television or radio interviews on this topic through our public relations department.

Related manuscripts in preparation:

1. Utility of the Mayo Sleep Questionnaire for REM sleep behavior disorder in Predicting alpha-synuclein pathology
2. Prevalence of REM sleep behavior disorder and other risk factors for parkinsonism in Salt Lake City, Utah

Project Progress Reports
Barrow Neurological Institute

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Cognitive and Neural Correlates of Aging in Autism Spectrum Disorder. Leslie C. Baxter, PhD, Blair Braden, PhD, Jiong Shi, PhD, Curtis McKnight, MD, Christopher Smith, PhD, Jieping Ye, PhD. Barrow Neurological Institute, St. Joseph's Hospital and Medical Center; Arizona State University; Southwest Autism Resource and Research Center; University of Michigan; Arizona Alzheimer's Consortium.

Specific Aims: There are very few studies of the effects of aging in Autism Spectrum Disorder (ASD). Young adults with ASD struggle with executive functions, such as working memory, inhibition, and set shifting. Conversely, ASD individuals often have preserved or enhanced visuospatial skills, such as embedded figure recognition and detail processing. Atrophic changes associated with brain aging is more pronounced in the frontal lobe, and the cognitive profile of normal aging reflects these structural brain changes with impairment in some frontal lobe mediated functions, such working memory and set shifting. *Given that ASD individuals struggle with many cognitive functions that are related to frontal lobe integrity in young adulthood, and that the frontal lobe is susceptible to normal age-related changes, there may be an exacerbation of deficits beyond normal aging in ASD.* The present study expands the limited prior research in aging and autism by assessing cognitive functioning in middle-aged ASD individuals using tasks that represent both intact and impaired domains in younger patients. Further, we will correlate cognitive results with measures of functional and structural brain integrity.

Specific Aim 1: Do middle-aged (40-60 y.o.) ASD individuals show cognitive deficits as compared to age-matched controls?

Hypothesis: Middle-aged ASD individuals show selective cognitive deficits, performing worse on executive tasks than age-matched Controls, with preservation of semantic memory and visuospatial tasks of detailed local processing.

Specific Aim 2: Do middle-aged ASD individuals recruit brain networks differently during task-based fMRI than age-matched Controls? Do the differences correlate with cognitive profile?

- Hypothesis 1: On fluency, working memory and inhibition fMRI tasks, middle-aged ASD individuals will exhibit a more diffuse pattern of frontal lobe activation and will recruit additional posterior brain regions to perform these tasks, as compared to age-matched controls.
- Hypothesis 2: Connectivity differences will be observed comparing middle-aged ASD individuals to age matched controls, indicating reduced functional connectivity between areas of the frontal cortex and association cortices (parietal, temporal, and occipital).
- Hypothesis 3: Using multi-task learning techniques, combining cognitive and imaging (connectivity and gray matter/white matter integrity) will show different profiles based on group status, and that weaker connectivity of the frontal lobe with more posterior regions will correlate with greater impairment on executive functioning cognitive tasks.

Research Plan: This project is capitalizing on a multi-institutional group of Arizona researchers who have expertise and interest in aging and ASD. We are partners with Dr. Christopher Smith, research director of the Southwest Autism Research and Resource Center (SARRC), who collaborates with study development and participant recruitment. Blair Braden is co-investigator with all main work, transition to Arizona State University. We are also partnering with Dr. Jieping Ye, at the University of Michigan. Dr. Ye develops statistical packages for combining different sources of data (e.g., imaging, behavioral, cognitive, genetic) for patterns and differences. We also have scientific input from Dr. Rogalsky, with whom Dr. Baxter partners in imaging studies, and share a graduate student through the ASU-BNI Neurosciences program. Our study benefits from the combined clinical and imaging expertise of this group. We also partner with Dr. Woodruff at Mayo Clinic Arizona (MCA), who has also worked with Dr. Smith in a study of cognitive abilities in a group of 50 ASD adults ranging in age from 20 to 58 years; he will participate in the conceptualization and manuscript preparation of this study. Dr. Caselli, also at MCA, has incorporated measures of ASD in his longitudinal APOE cohort.

In our second year, we continue to recruit typically developing (TD) Control males, ages 40-70, who are right-handed, and a group of ASD and TD age-matched young adults (age 18-25) to perform a battery of cognitive testing with a focus on frontal lobe/executive abilities and also undergo structural, functional (resting state and task-based) imaging. Funding from the Department of Defense funding enables us to expand the cohort to a total of 70 ASDs/age-matched controls and obtain two data points, two years apart.

2016-2017 Progress:

Results: Data were collected on the first wave of a longitudinal/cross-sectional cognitive aging study in ASD. We analyzed 16 ASD and 17 typically developing (TD) controls. All participants underwent cognitive testing and MRI scanning including evaluation of white matter (DTI) and gray matter integrity. Participants also performed functional MRI (fMRI) tasks during the scanning session. The cognitive tasks used and the data obtained from the first cohort's baseline time point. Middle-age adults with ASD made more errors on an executive function task (Wisconsin Card Sorting Test; WCST) but performed similarly to NT adults on tests of verbal memory (Rey Auditory Verbal Learning Test) and local visual search (Embedded Figures Task). Independent component analysis of a functional MRI working memory task (n-back) showed decreased engagement of a cortico-striatal-thalamic-cortical neural network in older adults with ASD. Structurally, older adults with ASD group had decreased white matter integrity (fractional anisotropy) bilaterally in the fimbria/fornix of the hippocampus and corpus callosum genu, and reduced bilateral hippocampal volumes, as measured by FreeSurfer. Furthermore, decreased corpus callosum genu integrity predicted worse performance in the executive function task (WCST) in the ASD group, implicating white matter differences as the underlying brain mechanism of this cognitive struggle. Findings expand our understanding of ASD as a lifelong condition with persistent cognitive and functional and structural brain differences evident at middle-age. These findings are currently under review.

We presented our findings on anxiety and depression in ASD at the International Meeting for Autism Research in May 2016. Briefly, the literature indicates that ASD individuals have greater rates of comorbid anxiety and depression. We collected anxiety and depression self-report at the time of cognitive testing and MRI scanning. These data showed that 88% of the middle-age ASD group reported significant levels of anxiety and 44% reported significant depression, as compared to 45% in the young-adult ASD group for both anxiety and depression. Social network measures did not significantly correlate with mood measures in either middle-age

or young-adult ASD, and the report of caregivers was not correlated with the symptom severity reported by the participants (we do not report the TDs in these analyses since they are excluded if they have psychiatric illness or symptoms). Interestingly, anxiety and depression symptoms correlated with several cognitive measures for the young ASD group, but there was no correlation in the older ASD group with cognition. This suggests that the cognitive deficits observed in the older ASD participants are not due to the presence of anxiety and depression but instead anxiety and depression may be independently affected in aging. We also received extramural funding to further investigate emotional status in our older ASD cohorts. We have begun to recruit our cohort of ASD participants for the study during which we obtain a clinical interview by a psychiatrist and will be assessed using the Structured Clinical Interview for DSM Disorders (SCID for DSM5) to better understand how individuals with ASD express/self-report anxiety and depression.

Year End Progress Summary:

- Submitted first manuscript of baseline data comparing older ASD to typically developing (TD) controls showing working memory network dysfunction and decreased performance on a behavioral working memory task. Manuscript finding decreased white matter integrity in motor-related brain regions in the older ASD group compared to TDs, which correlates with fine motor performance is in process.
- Collection of second time point for older adults underway with over 90% retention thus far.
- Collection of comprehensive assessments of anxiety and depression in the younger and older ASD groups begun. Presented preliminary data at the 2016 annual meeting of the International Meeting for Autism Research in May, 2015.
- Joined the Autism Brain Imaging Data Exchange (ABIDE). Contributed our imaging and demographic findings to the consortium.

Proposed One-Year and Long-Term Outcomes:

- Continue acquiring longitudinal data from a cohort of 70 each ASD elderly and age-match controls, along with a smaller subset of younger adults, and their age-matched controls.
- Continue acquiring anxiety and depression data from older and younger adult ASD participants.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Neuroprotective microRNA pathways in non-cognitively impaired elderly. Elliott J. Mufson, PhD, Sylvia Perez, PhD, Michael Malek-Ahmadi, MS. Barrow Neurological Institute; Banner AZADC; Arizona Alzheimer's Consortium.

Project Description: The presence of Alzheimer's disease (AD)-related neuropathology among cognitively normal individuals has been well-documented and it has been proposed that these individuals may represent a pre-clinical AD population. In the last year our group has shown that elderly individuals who died with a premortem clinical diagnosis of **no cognitive impairment** display extensive neurofibrillary tangle and amyloid plaque pathology similar to that seen in AD, which does not correlate with cognitive test scores. The mechanism(s) underlying this preservation of cognition may be related to neuroplastic compensatory events in the face of AD pathology. MicroRNAs (miRNAs) that regulate mRNA stability have been linked to amyloid production and tau phosphorylation in AD. Recently, we found in the frontal but not the temporal cortex that a down-regulation of either miR-212/132 or miR-23a/b is associated with an up-regulation of sirtuin 1 (sirt1) mRNA, which mediates neuroprotective cell stress responses in amnesic MCI (aMCI) suggesting that miRNA-mediated up-regulation of the sirt1 pathway represents a compensatory response to the onset of the disease. Whether alterations in these miRNAs and sirt1 occur in the cortex of individuals that died with a clinical diagnosis of NCI but postmortem display extensive AD pathology remain unknown. Therefore, the following Specific Aims will advance our understanding of the molecular mechanisms underlying an individual's ability to maintain cognitive ability despite extensive AD pathology. These findings may potentially open up new avenues for preclinical AD therapy.

Specific Aims:

Aim 1: We will test the hypothesis that there is a down-regulation of either miR-212/132 or miR-23a/b in the frontal cortex of aged non-cognitively impaired subjects with high Braak scores and amyloid pathology. The findings will be correlated with cognitive test scores and subject demographics.

Aim 2: We will test the hypothesis that sirtuin 1 (sirt1) mRNA is up-regulated in these aged non-cognitively impaired cases using qPCR technology. These findings will be correlated with the miRNA and clinical and pathology data.

Background and Significance: Progress in slowing the course of Alzheimer's disease (AD) has been hindered by a lack of disease modifying therapeutics. Given the vast complexity of this multisystem disorder, therapeutic development will depend on a greater understanding of the intricate molecular mechanisms that regulate the maintenance and survival of selectively vulnerable neuronal populations during disease progression. The presence of small non-coding microRNAs (miRNAs) that negatively regulate mRNA stability presents an under examined mechanism for fine-tuning gene expression within complex cellular networks, which may play a key role in the balance between health and disease. Various miRNAs regulate diverse brain functions including neurogenesis and differentiation, synaptic plasticity, and energy metabolism suggesting that perturbations in miRNA function could be involved in the pathogenesis of

complex neurodegenerative disorders including AD. In this regard, AD brains display altered expression of several miRNAs that regulate the β -secretase BACE1 enzyme involved in the generation of amyloid- β ($A\beta$) plaque pathology. In addition, miRNA dysregulation has been linked to tau phosphorylation and pro-inflammatory activity. The extent to which these changes have physiologic consequences in “preclinical AD” remain unclear. We recently reported that two families of miRNAs, miR-212/132 and miR-23a/b, were down-regulated in frontal cortex in aMCI and AD compared to NCI, yet remained stable in temporal cortex. Down-regulation of either miRNA family was predicted to up-regulate the deacetylase sirtuin 1 (sirt1), which is involved in mediating protective neuronal cell stress responses. Sirt1 mRNA levels were higher in frontal cortex of aMCI subjects but stable in inferior temporal cortex (IFT), suggesting a link between miR-212/132 and miR-23a/b down-regulation and reduced transcriptional repression of sirt1 target mRNA. Experimental down-regulation of miR-212 and miR-23a in cultured neurons up-regulated sirt1 and provided neuroprotection against $A\beta$ toxicity. Given the relatively delayed involvement of frontal cortex in AD pathogenesis and the ability of this region to respond to the onset of dementia by neuronal reorganization, these data suggest that miRNA-mediated up-regulation of sirt1 is a novel neuroprotective pathway activated during prodromal AD. We recently reported aged individuals with a postmortem neuropathologically evaluation of high Braak neurofibrillary tangle scores and amyloid loads received a premortem clinical diagnosis of no cognitive impairment suggesting that neuroplastic events play a role in the maintenance of cognition in the face of AD pathology in these cases. However, whether similar microRNA and mRNA changes occur in the brain of people who died with a premortem clinical diagnosis of no cognitive impairment but were neuropathologically characterized with high Braak scores and amyloid loads suggestive of AD, remains to be determined. These findings generated in the proposed studies may potentially open up new avenues for preclinical AD therapy.

Preliminary Data: We recently demonstrated that mir-132/212 and miR-23a/b are selectively down-regulated in the frontal cortex in aMCI and that these alterations are functionally linked to an up-regulation of sirt-1 and sirt-1 mediated protective responses; see Fig. 1 below). This novel finding adds to a growing literature on miRNA involvement in AD pathophysiology. However, rather than implicating another group of miRNAs in promoting neurodegeneration, our data support the concept that innate neuronal compensatory miRNA-mediated pathways are also activated early in AD progression. A greater understanding of these and other miRNA pathways functioning during the prodromal stages of AD holds the promise that these pathways could be harnessed pharmacologically for drug development.

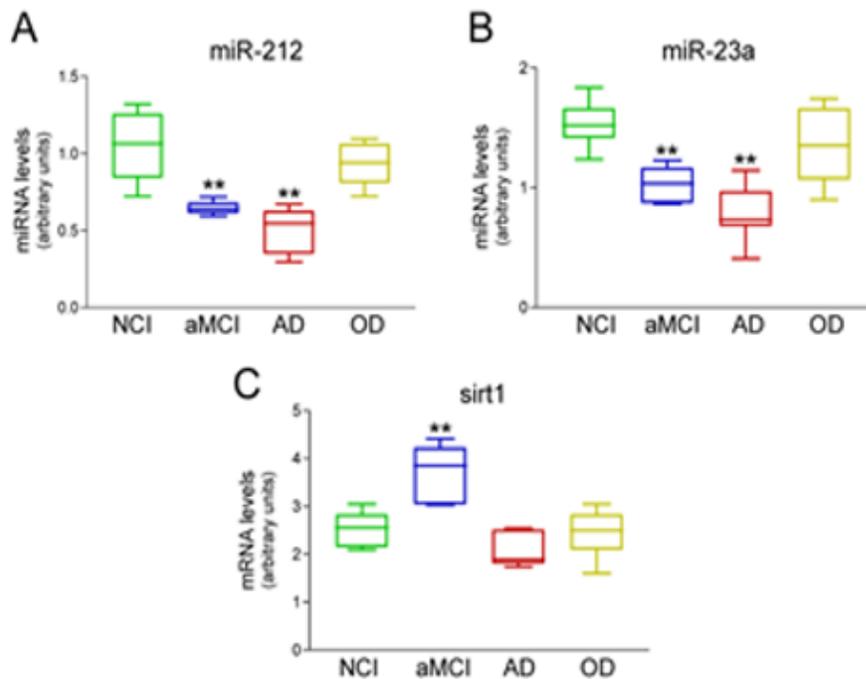


Figure 1. Differential expression of miR-212, miR-23a, and sirt1 transcripts in aMCI frontal cortex. qPCR analysis was performed on frozen frontal cortex tissue samples from NCI ($n = 12$), aMCI ($n = 10$), mild AD ($n = 10$), and other dementia (OD, $n = 5$) neurologic control subjects. Box plots show (A) miR-212 and (B) miR-23a were significantly down-regulated by ~50% in aMCI and by ~60% in AD, whereas their predicted mRNA target sirt1 was up-regulated by ~40% in aMCI. miRNA expression levels were normalized to the human RNU48 control miRNA, whereas sirt1 mRNA was normalized to GAPDH for quantitative analysis. **, $p < 0.01$ via one-way ANOVA with Bonferroni correction for multiple comparisons.

Experimental Designs and Methods:

Subjects: Tissue will be obtained from 123 older deceased and autopsied subjects from the Rush ROS cohort with no cognitive impairment and no coexisting clinical or neurological condition judged to be contributing to cognitive impairment at the last evaluation who agreed to annual clinical evaluations and signed an informed consent and an Anatomic Gift Act donating their brains at time of death that have been used in a large number of clinical pathological investigations supported by our ongoing NIA program project grant (PPG) entitled the “Neurobiology of Mild Cognitive Impairment in the Elderly” (PO1AG14449). Cases were chosen from all RROS brains that came to autopsy, which has a rolling admission. Subjects were selected using a stringent set of exclusion criteria; no large strokes, cerebral vascular disease, Lewy body disease, Parkinson’s disease, frontal temporal dementia, mixed pathologies or any condition that contributed to dementia). The pathological methods employed to determine these conditions have been reported previously. The average interval from last evaluation to brain autopsy was 0.73 ± 0.79 years.

Clinical Evaluation

Participants underwent annual uniform, structured, clinical evaluation and self-report medical history obtained by a team led by a neurologist and annual cognitive function was determined by

a trained neuropsychological test technician as previously reported. All medications used by the participants within the previous two weeks of the examination were reviewed and classified. After review of all clinical data and examination of the participant, a board certified neurologist or geriatrician with expertise in the evaluation of elderly persons with dementia made a final diagnosis. Diagnostic classification of no cognitive impairment was performed as previously described. After death, a neurologist reviewed the medical history, medication use, neurologic examination and results of cognitive performance testing as well as the neuropsychologist's opinion of cognitive impairment and dementia to render a final clinical diagnosis blinded to pathology data.

Cognitive Domain Composite Scores

Composite scores are based on the results of 17 individual cognitive tests divided into five domains of cognition as reported previously. Mini-Mental State Examination (MMSE) was used to describe the cohort but not used in the composite scores. Briefly, episodic memory was evaluated with tests including immediate and delayed recall of story A from Logical Memory and of the East Boston Story, and Word List Memory, Recall, and Recognition from the Consortium to Establish a Registry for AD (CERAD), semantic memory was assessed with three tests including a 15-item version of the Boston Naming Test, Verbal Fluency and a reading test that involves reading single words aloud and a 10-item reading test. Scores on the 3 tests are converted to a standard scale and averaged to get the composite score. Working memory was assessed using Digit Span Forward and Backward and Digit Ordering, perceptual speed tests included Symbol Digit Modalities Test, and Number Comparison and visuospatial ability included a 15-item version of Judgment of Line Orientation and a 9-item version of Standard Progressive Matrices. For each test, raw scores were converted into z-scores based on the mean and standard deviation of the sample. The z-scores from the individual tests were averaged to create individual domain composite scores. The Global Composite Score (GCS) is an average of 17 individual domain z-scores.

Tissue Preparation and Neuropathological Diagnosis

Brains accrument and processing was described previously. Briefly, one hemisphere of the brain was cut into 1 cm thick coronal slabs using a brain slice apparatus and immersion fixed in 4% paraformaldehyde for at least 72 hours and prepared for paraffin embedding. A board-certified neuropathologist or trained technician blinded to all clinical data counted total number of NPs, DPs, and NFTs in one square mm area (100x magnification) per cortical region as reported previously. Tissue from the opposite hemisphere is flash frozen for molecular biological experiments. Since the cohort of RROS NCI subjects we examined did not contain Braak stage VI cases, we operationally divided the present cohort into three groups: Group 1 compared low Braak stages= 0 - II to high=III - V, where the intermediate stage III was included in the high Braak group. Group 2 used the more conventional grouping of low 0-II, medium=III-IV and high=V-VI Group 3 compared low (I-II) and high (IV-V) with the intermediate stage III cases [29]. The inclusion of the intermediate stage III cases with either the low or high groups allowed for a determination of whether stage III subjects would affect the statistical interaction with the cognitive test scores. We will then separate stage III cases from the low and high groups and compare each against the cognitive tests examined. ApoE genotype is available for all cases.

miRNA expression profiling

A miRNA screen will be performed by Exiqon microarray profiling services as previously reported. Approximately 30% of the miRNAs on the array are proprietary and not available for analysis in public databases. qPCR validation for select transcripts using frozen tissue and OD neurologic control cases were performed. Total RNA is extracted (miRvana, Ambion, STAR+TE) and RNA integrity and concentration is verified using Bioanalysis (Agilent). Samples will be assayed on a real-time PCR cycler (7900HT, Applied Biosystems, State) in 96-well optical plates. Target miRNAs of interest as well as the RNU48 artificial normalization control will be amplified using specific Taqman hydrolysis probe sets (Applied Biosystems). In addition, Taqman probe sets specific for *sirt1* and control glyceraldehyde 3-phosphate dehydrogenase are used to quantify *sirt1* transcript levels in the same samples. The ddCT method will be employed to determine relative expression levels of each amplicon. Variance component analyses will be used to reveal relatively low levels of within-case variability, and the average value of the triplicate qPCR products from each case will be used in subsequent analyses.

Dual in situ hybridization/immunohistochemical localization of miR-23a and *sirt1*: To determine cellular localization of probes we will perform *in situ* hybridization to detect miR-23a on 10 μm , cryostat-sectioned samples from the same cases used above using a digoxin (DIG)-labeled hsa-miR-23a probe (Exiqon), adapting the protocol of Doné and Beltcheva. Briefly, tissue sections were fixed in 4% paraformaldehyde overnight at room temperature (RT). The next day, sections are treated with 20 $\mu\text{g}/\text{mL}$ proteinase K for 10 min at 37°C followed by hybridization with 40 nmol hsa-miR-23a probe for one hour at 55°C. The sections are then blocked with 2% sheep serum/1% bovine serum albumin for 15 minutes at RT followed by incubation with alkaline phosphatase-conjugated sheep anti-DIG Fab fragments (1:500, Roche, STATE) for one hour at RT. The sections are then incubated with the alkaline phosphatase substrates NBT (nitro blue tetrazolium)/BCIP (5-bromo-4-chloro-3-indolyl-phosphate; Roche) for two hours at 30°C revealing a dark purple reaction product. Following miR-23a visualization, the sections are incubated overnight at 4°C with a rabbit anti-*sirt1* monoclonal antibody (1:100, Origene) in Tris-buffered saline (TBS, pH 7.4)/0.25% Triton X-100/1% normal goat serum. Following TBS rinses, the sections are incubated with horseradish peroxidase-conjugated goat anti-rabbit secondary antiserum (Vector Laboratories) for 1 hour at RT. *Sirt1* labeling is accomplished by serial incubations in ABC peroxidase reagent (Vector Laboratories, State) and 3, 3'-diaminobenzidine tetrahydrochloride hydrate at RT to reveal a brown reaction product.

Statistical analysis: miRNA levels quantified by qPCR will be compared across Braak stages and OD neurologic controls via one-way ANOVA with Bonferroni *post hoc* testing. The relationship between specific miRNA and mRNA levels will be assessed by Spearman rank correlations. Shapiro-Wilk test will determined normality of cognitive domains/test data, demographics, amyloid load and plaque count variables (Mufson). APOE $\epsilon 4$ status and gender differences among the cognitive domains will be analyzed using a two-sample t-test. Chi-square test will determined differences in gender frequency and APOE $\epsilon 4$ genotype by Braak group and gene expression. If post-mortem interval and MMSE significantly violated the assumption of normality and demonstrated a significantly skewed distribution, we will used the non-parametric Mann-Whitney test. Linear regression models will used to test Braak group differences on the cognitive domains and expression values. Age at death, gender, education, and time between death and last assessment will be included in the models to account for their effects. Cohen's *d* will assess the magnitude of the group differences. Level of agreement between NIA-Reagan and CERAD criteria will be determined using the weighted kappa test. Logistic regression analyses

will be performed for frontal, ITC and hippocampal amyloid loads as predictor variables for Braak group membership, with the Braak 0 to II group as the reference. Linear regression analyses, adjusted for age at death, gender, education, and time between death and last assessment, will be used to assess the associations between amyloid loads with the cognitive domains and expression data. False discovery rate will be used to adjust for multiple comparisons. Statistical analyses will be performed using Systat 13 (Systat; San Jose, CA). Significance level was set at 0.05 (two sided).

Power analysis: Based on previous gene expression studies using postmortem tissue, the proposed study with 10 cases/diagnostic group (3 groups) will have approximately 80% power to detect an effect size of 1.2 standard deviations.

Proposed One-Year and Long-Term Outcomes: Data and findings from this proposed project will be submitted for presentation at scientific meetings and in peer-reviewed manuscripts. In addition, the results will be used to inform future drug targets for AD. The PI will continue to seek additional external, non-state funding from NIH, industry and philanthropic organizations to support these projects.

We anticipate that miRNA levels will be down-regulated in the frontal cortex but stable in the IFT cortex in cases with no cognitive impairment but with high Braak scores. Low Braak cases may remain unchanged but it is possible that there will be no difference independent of Braak stage or amyloid load. We also predict that sort1 expression will be increased in those cases with high Braak and amyloid scores. On the other hand, all cases independent of AD pathological state may remain stable. The down regulation of miRNA and the up regulation of sort1 expression may correlate with cognitive test performance. Based upon our previous findings (Mufson), we posit that ApoE genotype will not correlate with gene expression finding. However, this possibility may be mitigated by the small number of ApoE e4 carriers per group. Such observations would raise the intriguing possibility that miRNA changes play a role in maintaining cognition in the elderly in the face of AD pathology. Since these studies are labor intensive, we anticipate that they will carry over to a second year of funding. During this time we will discuss the possibility of obtaining tissue from Dr. Reiman's ongoing South American AD cohort for comparative gene expression studies.

During the current grant period, we investigated several key question related to our funded project. In one study, our data suggest that genetic molecular pathways regulating protein homeostasis are altered during the evolution of NFT pathology. These changes likely contribute to the disruption of protein turnover and neuronal survival of these vulnerable NBM neurons during the progression of AD. A second study found that the cell cycle regulatory protein RGCC is upregulated in prodromal and frank AD, correlates with global cognitive decline, and may be involved in facilitating aberrant cell cycle reentry induced by neurotrophin loss in neurons, suggesting that RGCC may be a candidate cell cycle target for neuroprotection during the onset of AD. In a related series of paper supported by my AAC grant revealed that aged persons high Braak stages of neurofibrillary pathology do not demonstrate cognitive impairment defined by several tests of memory. Another investigation found age to be a better predictor of cognitive decline than Braak and amyloid pathology in non-cognitively Impaired elders.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Biomarker studies in Alzheimer's disease. Jiong Shi, PhD. Barrow Neurological Institute; Arizona Alzheimer's Consortium.

Specific Aims:

Aim 1: We will test if Sirtuin3 levels differ between normal control (NC), Mild cognitive impairment (MCI) and Alzheimer's disease (AD) subjects in postmortem brains.

Aim 2: We will test if Sirtuin3 levels correlate with AD pathology.

Aim 3: We will test the specificity of Sirtuin3 in other neurodegenerative conditions.

Aim 4: We will examine the cause-effect relationship of Sirtuin3 and AD pathology in transgenic models of AD.

Background and Significance:

Biomarkers in dementia. The growing aging population is facing the challenge of dementia. However, we don't have a reliable biomarker for early diagnosis. Mild cognitive impairment (MCI) describes a syndrome of cognitive impairment beyond age-adjusted norms that is not severe enough to impair daily function or fulfill clinical criteria for dementia (Petersen et al., 1999). Longitudinal studies have shown that 15% of MCI patients progress to AD per year (Ewers et al., 2012; Landau et al., 2010). Amnesic MCI (aMCI) has the highest conversion rate among all subgroups (Fischer et al., 2007). Current therapy provides limited symptomatic benefit in MCI patients, and disease-modifying therapy will likely be most effective when the disease is diagnosed early. Biomarkers that accurately predict disease progression would ameliorate prevailing uncertainties regarding which MCI patients will develop AD and aid in early treatment.

CSF A β and p-tau biomarkers for MCI and AD. CSF A β 42, total tau and phosphorylated tau (p-tau) are most commonly used biomarkers. Many studies have shown that compared to NC, AD patients have lower CSF A β 42 levels and higher total tau and phosphorylated tau (p-tau) levels. As mildly demented AD patients show elevated tau protein levels (Galasko et al., 1997; Riemenschneider et al., 1996) and decreased A β ₁₋₄₂ levels (Andreasen et al., 1999; Galasko et al., 1998; Motter et al., 1995) in CSF compared to NC, altered CSF tau and A β ₁₋₄₂ levels have been proposed as putative early diagnostic markers for MCI subjects at high risk of developing AD. Patients who converted from MCI to AD showed significantly higher tau levels at baseline compared to NC (Arai et al., 1997). Moreover, subjects with MCI who later develop AD can be identified by the combination of decreased CSF concentrations of A β ₁₋₄₂ and increased levels of tau (Andreasen et al., 1999; Riemenschneider et al., 2002), suggesting that CSF tau and A β ₁₋₄₂ may be valuable to detect the preclinical stages of AD. However, the specificity is relatively low since up to 20% of NC subjects may have abnormal CSF AD biomarkers. Therefore, more specific biomarkers are needed. PACAP is such a potential biomarker. We will determine whether changes in PACAP levels in MCI patients correlate with clinical progression and conversion to AD.

Sirtuin3 as a novel biomarker. We have discovered reduced PACAP level in the brains of patients with AD compared to controls (Han et al., 2015; Han et al., 2014). ADCYAP1 (the PACAP gene) expression was significantly reduced in the Middle Temporal Gyrus (MTG),

Superior Frontal Gyrus (SFG), and Primary Visual Cortex (PVC), while its protein levels were reduced in all three regions plus the Entorhinal cortex (ENT). PACAP protein levels were correlated with higher CERAD amyloid plaque score in the ENT and SFG but not in the MTG or PVC. In terms of neurofibrillary tangles, PACAP levels were reduced in Braak stage V-VI (all AD cases) than in stage III-IV. Therefore, PACAP expression was inversely associated with both pathological hallmarks of AD. Furthermore, the PACAP level in CSF was correlated with that of the brain and was reduced in AD as compared with CN. This reduction in PACAP is specific for AD since PACAP levels in Parkinson Disease with Dementia (PDD) and in Frontotemporal Lobe Dementia (FTLD) were comparable to that of CN. Hence, downregulation of PACAP may be an early pathogenic factor in AD. Therefore, early detection of reduced PACAP levels in the CSF may be indicative of underlying AD pathology in patients with MCI and in those with an increased risk of developing AD. Sirtuin3 belongs to the Sirtuin family and is localized in mitochondria to have its deacetylation activity. PACAP modulates Sirtuin3 production via AMPK. We have found that Sirtuin3 expression is reduced in AD brains and this reduction is closely related to Tau pathology. The dual measurement of PACAP and Sirtuin3 provides a more robust assessment of AD progression.

Preliminary Data: We showed that Sirtuin3 expression is reduced in AD brains. Sirtuin3 reduction in different brain areas was associated with Braak stage, but not CERAD amyloid plaque burden. In addition, we showed that lower Sirtuin3 was associated with poorer cognitive performance.

Experimental Designs and Methods:

1) We will examine whether Sirtuin3 levels differ between NC, MCI and AD subjects. We will first compare Sirtuin3 from patients with AD (meeting NINCDS-ADRDA criteria, (1997)) to age-matched NC individuals. Since the diagnosis of MCI is applied to individuals who experience cognitive decline but do not meet the clinical criteria of dementia (Petersen et al., 1999), we will determine whether Sirtuin3 levels in MCI patients are of an intermediate level between that of AD and NC individuals. Furthermore, we will use Tau ¹⁸F PET image as a gold standard to correlate tauopathy with Sirtuin3 levels in the same population.

2) We will examine whether longitudinal changes in Sirtuin3 levels correlate with conversion from MCI to AD. Because of the need for early diagnosis, we will apply several types of analyses to evaluate longitudinally whether Sirtuin3 levels predict risk of cognitive impairment and progressive decline based on cognitive test scores and/or CDR sum of boxes. Specifically, we will follow subjects > 65 years of age with annual evaluations, and we will investigate whether there is a decrease of Sirtuin3 in NC and MCI subjects that precedes the clinical diagnosis of AD.

3) We will examine the specificity of Sirtuin3 in other neurodegenerative conditions. To test its specificity, we will include cases of Parkinson's disease (without dementia), Amyotrophic Lateral Sclerosis, Frontotemporal Lobe Degeneration and related tauopathies. These neurodegenerative pathologies differ from AD, and so will show whether reduction in Sirtuin3 is specific to AD or universal in neurodegeneration.

4) We will examine the cause-effect relationship of Sirtuin3 and AD pathology in a transgenic model of AD. We have identified Sirtuin3 as a marker of AD, but the cause-effect relationship of Sirtuin3 and A β / p-Tau is unknown. We will use a triple transgenic model of AD to exam the Sirtuin3 levels over a time course of 12-month to delineate its relationship with A β / p-Tau.

Proposed One-Year and Long-Term Outcomes: We will complete data collection for aims 1 and 3 by the end of the fund year. We will be able to set up the transgenic animal colony during the one-year fund cycle. This will enable us to continue on aim 2 and 4 which are longitudinal studies. The long-term goal is to understand the PACAP-AMPK-Sirtuin3 pathway in the pathogenesis of Alzheimer's disease and to explore its diagnostic and therapeutic potentials.

Increasing evidence indicates that Sirtuin 3 (Sirt3) has neuroprotective effects in regulating oxidative stress and energy metabolism, both of which are involved in the pathogenesis of Alzheimer's disease (AD). However, it is unclear whether Sirt3 is associated with cognitive performance and pathological changes in AD. We conducted a case-control study of the brains of late-onset AD, mild cognitive impairment and age matched cognitively normal (CN) subjects. We investigated Sirt3 expression, its association with cognitive performance and AD pathology. We have found that Sirt3 levels were reduced in the entorhinal cortex, the middle temporal gyrus and the superior frontal gyrus of AD subjects compared to CN. This reduction was associated with poorer cognitive test scores and the severity of tau pathology. Further study revealed that amyloid- β increased total Tau protein expression via regulating Sirt3.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Natural killer cells in cognitive deficit. Qiang Liu, PhD. Barrow Neurological Institute; Arizona Alzheimer's Consortium.

Specific Aims: AD is the most common dementing illness. Although several scenarios regarding the mechanisms of neurodegeneration have been suggested, including mitochondrial dysfunction, oxidative stress, and the impairment of protein clearance, mounting evidence suggests that innate inflammatory processes activated by A β participate in the progression of neuronal damage and cognitive impairment^{1, 2}. NK cells are innate lymphocytes that can be swiftly mobilized during the earliest phases of immune responses³⁻⁶, but their role in AD remains unknown. We hypothesize that *NK cells favor cognitive deficits via direct killing and augment of local inflammation in AD.* This proposal will study the role of NK cells in neuronal damage and cognitive deficit during AD, and dissect the underlying pathways. Our specific aims are to:

1) Determine the impact of NK cell depletion on cognitive deficits in 3xTg-AD mice.

Using anti-NK1.1 mAb, we will deplete NK cells in 8-month-old wild-type and 3xTg-AD mice. At 4 weeks after NK cell depletion, we will determine neuronal death and long-term potentiation in hippocampus of wild-type and 3xTg-AD mice subjected to weekly i.p. injections of anti-NK 1.1 mAb or IgG controls. Spatial memory in these mice will be assessed with Morris water maze.

2) Determine the mechanisms of how NK cells impact cognitive function in 3xTg-AD mice.

Using flow cytometry, we will determine the expression NK cell receptors (NKG2A) and inhibitory ligands (Qa-1 and CD1d) on neurons in the brain 3xTg-AD mice (8-month-old), which may lead to the disruption of NK cell tolerance. Next, we will test the ability of NK cells to damage primary cultured neurons from 3xTg-AD mice. Using an ELISA array kit, we will examine the expression of inflammatory mediators in the hippocampal tissues of wild-type and 3xTg-AD mice subjected to injections of anti-NK1.1 mAb or IgG at one month after treatment.

Background and Significance: The sustained formation and deposition of A β causes chronic activation of the immune system. Experimental, genetic and epidemiological data has indicated the disruption of BBB and brain infiltration of the innate immune system as a disease-promoting factor for AD pathology^{1, 2, 7}. But the precise role for specific immune cell subsets in the progression of AD is not defined.

NK cells are large granular lymphocytes of the innate immune system, and are among the earliest responders to danger signals. Their perforin-dependent cytolytic activity against target cells without prior sensitization and their early burst of cytokine secretion enable these cells to orchestrate the nature and intensity of both innate and adaptive immunity^{8, 9}. For example, IFN- γ is produced in large quantities by NK cells in very early phase of immune response. IFN- γ activates macrophages and upregulates MHC II expression on antigen-presenting cells. NK cells readily home to the brain during numerous pathological situations where their immune effector functions may potentiate neuronal damage, or regulate the inflammatory milieu which might be critical for neuronal survival^{6, 10-13}. However, these hypothesized roles for NK cells haven't been studied in AD. This project aims to *unveil NK cells as a contributor to cognitive deficit.* Revealing how NK cells execute their multiple immune effector functions will prove to be the

key to understand the related brain damage. *This study will identify NK cells as a therapeutic target.* Identification of NK cells as direct effector cells or as exemplifiers of inflammation and edema will lead to targeted immune therapies to prevent their entry into the brain or reduce their activation to prevent neuronal damage. The lessons learned from NK cells may be applicable to other lymphocytes.

Preliminary Data:

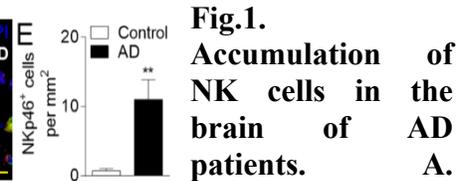
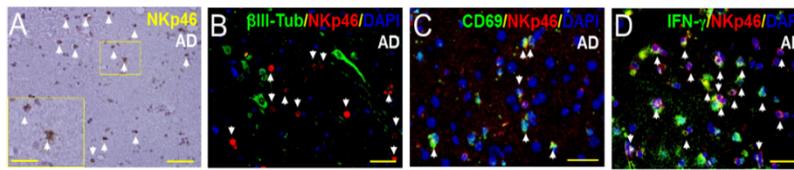


Fig.1. Accumulation of NK cells in the brain of AD patients. **A.** NKp46⁺ (a marker of NK cells) cells in a post-mortem brain section from an AD patient. **B.** Brain-infiltrating NKp46⁺ cells (arrows) in a brain section from an AD patient in close proximity to neurons (Green, β III-Tubulin⁺). **C-D.** CD69⁺ or IFN- γ ⁺ NK cells in an AD patient. **E.** Counts of NKp46⁺ cells in **A**. Controls were from patients without neurological diseases. $n = 5$. Scale: 50 μ m (**A**, right); 20 μ m (**A**, inset); 20 μ m (**B-D**). Means \pm s.e.m.; ** $p < 0.01$.

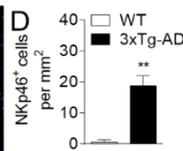
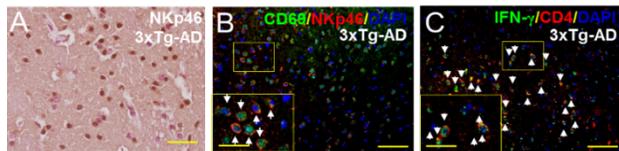


Fig.2. Infiltration of NK cells in the brain of 3xTg-AD mice. **A.** NKp46⁺ cells in a brain section from a 3xTg-AD mouse. **B-C.** CD69⁺ or IFN- γ ⁺

NK cells in a 3xTg-AD mouse. **D.** Quantification of NKp46⁺ cells in **A**. NK cells were not seen in wild type controls. $n = 6$. Scale bars: 30 μ m (**A**); 50 μ m (**B-C**, right); 20 μ m (**B-C**, inset). Means \pm s.e.m.; ** $p < 0.01$.

Experimental Designs and Methods:

1) Determine the impact of NK cell depletion on cognitive deficits in 3xTg-AD mice.

Groups of 8-month-old wild-type and 3xTg-AD mice (12 mice/group) will be subjected to weekly i.p. injection of anti-NK1.1 mAb (100 μ g in PBS) or vehicle PBS for 4 weeks. NK cell depletion (usually >99%, see our publications^{11, 13}) will be monitored by measuring NK cell counts in eye bleeds at day 7 after each injection. At 4 weeks after NK cell depletion, spatial memory in these mice will be assessed with Morris water maze in groups of wild-type and 3xTg-AD mice subjected to weekly i.p. injections of anti-NK1.1 mAb or IgG controls. After behavioral testing, mice will be sacrificed to assess hippocampal synaptic plasticity via long-term potentiation and dendritic/spine morphology by immunostaining PSD-95 and GluR1 as we did¹⁴⁻¹⁶.

2) Determine the mechanisms of how NK cells impact cognitive function in 3xTg-AD mice.

Normal cells express NK cell inhibitory ligands (Qa-1 and CD1d) that are among on the key molecules shielding them from damage by NK cells^{3, 5, 6}. Using flow cytometry, we will determine the expression of Qa-1 and CD1d on neurons and the expression of NK cell receptors (NKG2A) on infiltrating NK cells in the brain of 3xTg-AD mice (8-month-old). Next, we will test the ability of NK cells to damage primary cultured neurons from 3xTg-AD mice by assessing dendritic/ spine morphology via immunostaining PSD-95 and GluR1¹⁴⁻¹⁶. Using an ELISA array kit, we will examine the levels of inflammatory mediators in the cortex and hippocampus of wild-type and 3xTg-AD mice at 4 weeks after receiving weekly injections of anti-NK1.1 mAb or IgG^{11, 13}.

Proposed One-Year and Long-Term Outcomes: The ultimate goal of this application is to understand the precise role of immunity and inflammation in cognitive deficit. This award will enable us to obtain critical data in **Aim 1-2** within the first year. At the second year, we will submit our grant applications to NIH or Alzheimer's Association.

Progress update:

Infiltration of natural killer (NK) cells was seen in the brain sections from patients with Alzheimer's disease (AD) and triple-transgenic mouse model of AD (3xTg-AD). Removal of NK cells via antibody depletion in 3xTg-AD mice at early stage affects A β accumulation and cognitive deficits at late stage. Ongoing studies are to identify the mechanisms of how NK cells impact AD pathology in 3xTg-AD mice.

Reference:

1. Jin WN, Shi SX, Li M, Wood K, Gonzales RJ and **Liu Q**. Depletion of microglia exacerbates postischemic inflammation and brain injury. *J Cereb Blood Flow Metab.* 2017; In press.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Multi-parametric MR Imaging Signatures of Alzheimer's Disease. Ashley Stokes, PhD, Chad Quarles, PhD, Marwan Sabbagh, MD. Barrow Neurological Institute; Arizona Alzheimer's Consortium.

Executive Summary: The goal of this project is to establish advanced MR imaging signatures in persons with no cognitive impairment, mild cognitive impairment, and moderate Alzheimer's disease (AD). This will allow us to non-invasively investigate the underlying neurobiological changes that precede the cognitive impairments characteristic of later stages of AD. The asymptomatic and MCI phases represent a clear potential for early intervention, and the non-invasive methods developed here will permit identification of patients along the clinical trajectory of AD, and thus those most likely to benefit from earlier intervention.

Research Project Details: It has been estimated that a 10-year delay in the onset of AD may essentially eliminate the disease [1], highlighting the importance of early detection. Cerebrospinal fluid (CSF) markers and PET imaging may be sensitive to the early stages of AD [2, 3], but neither is well suited for a population-wide screening tool or longitudinal measurements. On the other hand, MRI is ideal for longitudinal studies, as it is non-invasive and has no ionizing radiation or radionuclides. Structural MRI is widely used in the assessment of AD-induced morphological changes such as brain atrophy [4], which is indicative of neuronal loss. However, morphological changes are minimal during the mild cognitive impairment (MCI) phase [5], with more substantial morphological changes occurring once the patient reaches the clinical threshold for a diagnosis of AD. Functional and molecular changes precede brain atrophy [4, 6] and may be detectable in the earlier MCI phases, when intervention would prove most beneficial. These changes may be detectable using advanced MRI methods.

Specific Aims:

Aim 1: Establish a set of advanced MR imaging signatures that are phenotypical of each stage of AD, from no cognitive impairment to MCI to clinically diagnosed AD.

While structural MRI is known to change with later disease progression, advanced MR imaging of microvascular blood volume (using intravoxel incoherent diffusion), blood flow (using arterial spin labeling (ASL)), molecular species (using chemical exchange saturation transfer (CEST)), and iron deposition (using susceptibility-weighted imaging (SWI)) may provide specific signatures of disease progression. We hypothesize that the corresponding vascular or molecular changes could be an early indicator of incipient MCI or AD, prior to morphological changes.

Background and significance:

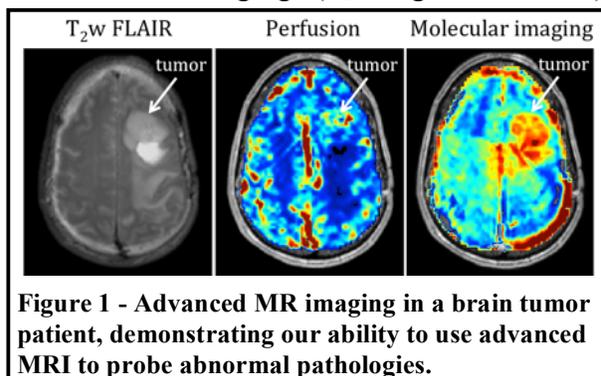
Rationale: Though not directly indicative of tau or amyloid pathology, the proposed set of MRI biomarkers may provide unique and complementary functional information that is indirectly indicative of AD pathology. Perfusion volume fraction and blood flow imaging can identify regional hemodynamic microcirculatory insufficiency that promotes a neurodegenerative state [7]. CEST-MRI can be tuned for sensitivity to myo-inositol [8] and glutamate [9, 10], both of which may be modulated during early stages of AD. SWI is particularly sensitive to local

hemosiderin deposits [11], which result from cerebral microbleeds and are known to occur more frequently in AD patients. Each method was chosen for its sensitivity to a known abnormality related to AD (e.g., perfusion imaging is sensitive to capillary flow patterns that may be a harbinger of altered neuronal function [12]).

Biomedical Significance: The proposed research is significant because it aims to develop multi-parametric imaging signatures that will permit early prognostic identification of patients likely to progress to AD. The data will also shed new insights into the underlying molecular and functional changes that occur in the earliest phases of AD.

Innovation: The proposed studies aim to establish multi-parametric, advanced imaging signatures of neurobiology in patients with MCI and AD. Each metric has been used, individually or in pre-clinical proof of concept studies, to characterize advanced AD status, but, to our knowledge, no study has combined these complementary metrics nor have they been compared in both MCI and AD patients. To date, most of the work using these advanced biomarkers has primarily focused on method development, whereas the studies proposed herein represent the first prospective studies aiming to combine and leverage these functional biomarkers as they correlate to the clinical AD trajectory. Combining these unique biomarkers will allow us to improve our diagnostic accuracy. Thus, the proposed studies represent a significant advancement in AD neuroimaging techniques, providing a more comprehensive, non-invasive strategy with which to characterize neurobiological abnormalities.

Preliminary data: We have extensive experience using the proposed imaging metrics in brain tumor patients (Figure 1), including standard anatomical imaging (T_2 -weighted FLAIR), perfusion imaging, and molecular species-specific imaging. In tumors, both perfusion and molecular-specific (here, amide protons) signatures are elevated in tumor tissue. These methods will be refined for application to AD, but much of the method development is already complete.



Experimental Designs and Methods:

Considering the 1-year timeline, the IRB application is the first action item and will be submitted in July 2016. As this study only requires imaging at one time-point, we do not anticipate issues with IRB approval. In addition, the PI has experience in both clinical trials and the development of advanced MR imaging methods, including those proposed for this study. Patients will be referred to the study by their neurologist (MS, Co-PI), and healthy volunteers will be recruited from the Barrow Neurological Institute. We will recruit a total of 36 people, including 12 patients with MCI and 12 patients with moderate AD. The remaining 12 subjects will be healthy age-matched volunteers with no cognitive impairment. All subjects will undergo cognitive testing using the Mini-Mental State Examination (MMSE) or Montreal Cognitive Assessment (MoCA), functional assessment staging (FAST), and clock draw within 1 week of the MRI.

Data acquisition will be performed at the Keller Center for Imaging Innovation using a dedicated research 3T Philips MRI. Structural MRI using ADNI (Alzheimer's Disease Neuroimaging Initiative) data collection methods [13] will be performed in each study participant, and brain

atrophy reports will be obtained from NeuroQuant (Cortechs Labs). Diffusion-weighted MRI will be acquired using 15 b-values, ranging from 0 to 3000 s/mm², with emphasis on low b-values to reliably measure perfusion fractions. CBF will be measured using a 3D spiral turbo spin echo arterial spin labeling method. A 3D multi-shot gradient-echo method with 48 offsets will be used to obtain CEST spectra. Susceptibility-weighted imaging data will also be acquired. All data will be acquired before May 2017, and data processing will occur as patients are accrued. Groups will be compared using an unpaired t-test. The final months of grant support will be reserved for statistical analysis, preparation of publications, and submission of a larger grant to fund longitudinal studies.

Proposed One-Year and Long-Term Outcomes: Although this study focuses on single time point imaging, the publication of this study will lay the foundation for a longitudinal study that establishes the imaging phenotypes of AD progression. The data obtained through this award will be used as pilot data for a larger grant application through the National Institute on Aging (NIH/NIA), using the K-level funding mechanism for career development. In particular, we will propose a longitudinal study characterizing the timeline of progression of AD, using this data as the first time-point for annual monitoring.

Progress Summary (through Feb 1, 2017): Aim 1: We have completed MRI data acquisition on approximately half of the subjects in each group, along with cognitive testing using MoCA, clock draw, and FAST. The MRI data includes high-resolution structural images, CEST, ASL, DWI, and SWI. All data acquired thus far has been analyzed, including anatomic parcellation of the structural data to assess atrophy and generate regions-of-interest. These regions-of-interest have been applied to the advanced functional biomarkers for blood flow and volume, molecular species, and iron deposition. We will continue to acquire data in the remaining subjects over the next few months, which will provide sufficient time for statistical analysis and manuscript preparation.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Establishing Neuroimaging Phenotypes in Mouse Models of Alzheimer's Disease. Chad Quarles, PhD, Salvatore Oddo, PhD. Barrow Neurological Institute; Arizona State University; Arizona Alzheimer's Consortium.

Project Description: The overall goal of this project is to identify structural, metabolic and molecular neuroimaging signatures of Alzheimer's Disease (AD) in mouse models that recapitulate human pathophysiology. Our multi-parametric approach has the potential to shed new insights into AD progression, inform clinical interpretation of biomarker analysis, and establishes the foundation for its use in pre-clinical drug development. As a platform for future funding opportunities these studies leverage the recently expanded BNI-ASU Center for Preclinical Imaging, and its new microPET system (the first and only system in the state). The data acquired through this proposal will be critical for pre-clinical AD investigators in AZ planning to incorporate MRI and PET neuroimaging into future external grant applications.

Specific Aim: To systematically characterize MRI and PET-based biomarkers of amyloid- β , tau, neuromorphology, and glucose metabolism in wild type and 3xTg-AD, 3xTg-AD/S6K1^{+/-} and S6K1^{+/-} mouse models of AD and correlate with immunohistochemistry.

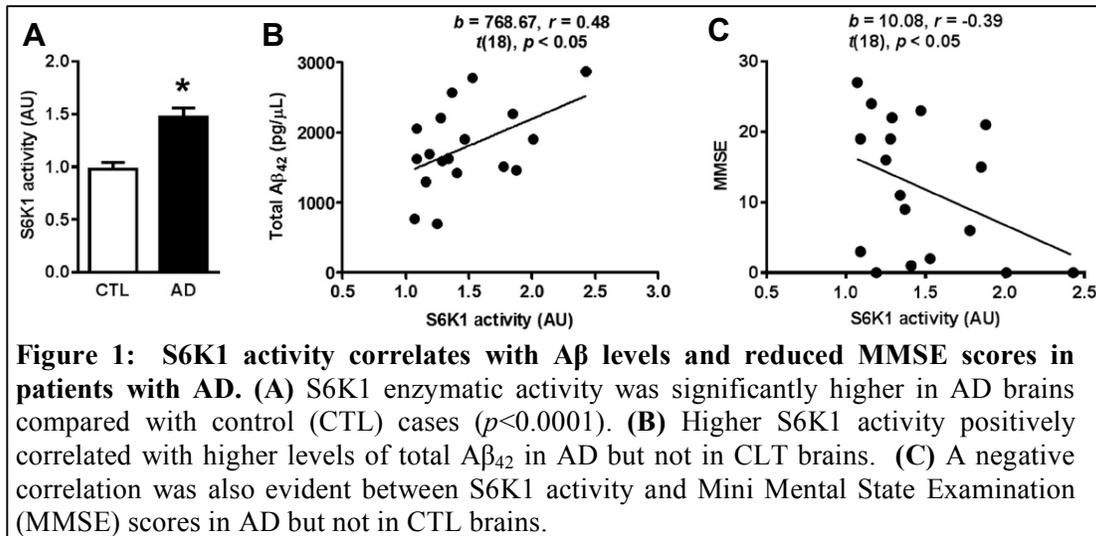
Rationale:

Use of a mouse model that recapitulates human AD pathophysiology: Suppression of the ribosomal protein S6 kinase 1 (S6K1) increases health span and lifespan in several organisms. Dr. Oddo (Co-Investigator, ASU) recently found that S6K1 expression is upregulated in the brains of AD patients. Further, using his well established 3xTg-AD mouse model, genetic reduction of S6K1 improved synaptic plasticity and spatial memory deficits, and reduced the accumulation of amyloid- β and tau, the two neuropathologic hallmarks of AD (Caccamo et al, J Neurosci, Oct 14, 2015; 35(41):14042–14056). These results implicate S6K1 dysregulation as a previously unidentified molecular mechanism underlying synaptic and memory deficits in AD. These findings further suggest that therapeutic manipulation of S6K1 could be a valid approach to mitigate AD pathology. For the purposes of this study, Dr. Oddo's mouse models of AD provide an invaluable system with which to characterize and validate neuroimaging biomarkers. The imaging studies will also add value to his ongoing research. Once characterized, they can be used to *non-invasively* and *serially* assess disease progression and identify critical, transitional time points for conventional histologic analysis.

Use of multi-parametric neuroimaging assays of AD: While most pre-clinical imaging studies of AD evaluate one aspect of the disease (e.g. tau or amyloid- β) we propose to interrogate, non-invasively, multiple and clinically relevant biological features that dynamically and spatially change throughout AD progression. This will enable the characterization of regional and model-specific relationships between the major pathophysiologic characteristics that accompany AD.

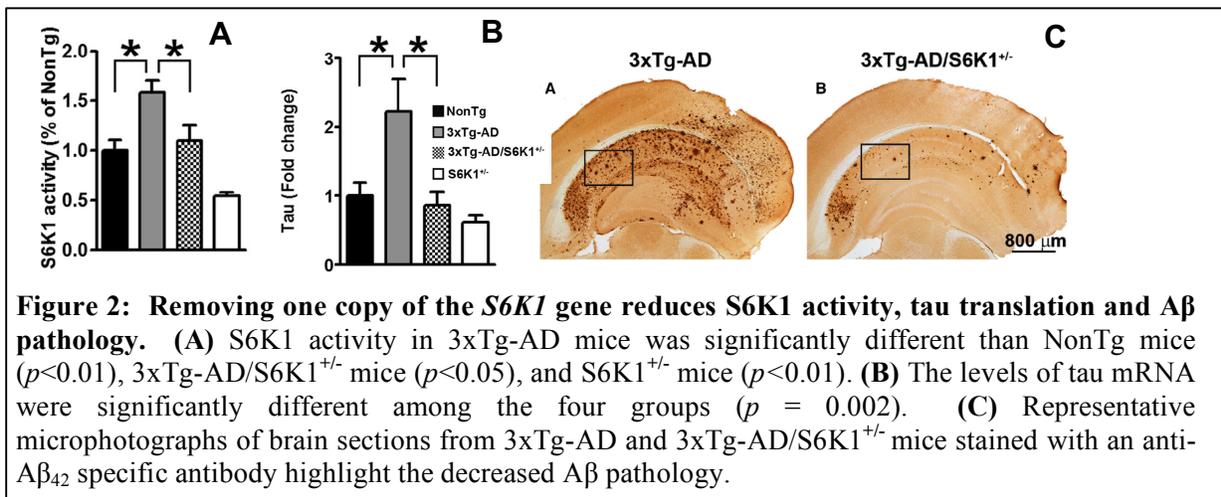
Biomedical Significance: The proposed studies are highly significant as they will i) further establish the role of S6K1 expression as a molecular mechanism underlying AD pathophysiology and potential therapeutic target, ii) establish baseline differences in neuroimaging biomarkers between control and 3xTg-AD mice, and iii) identify imaging signatures of reduced S6K1 activity that could be used as therapy response indicators.

Innovation: This will be the first study to i) establish a multi-parametric MRI and PET based imaging phenotype of AD in mouse models that recapitulate the human condition and ii) characterize this phenotype in mice with reduced S6K1 activity.



Preliminary Results: As described above, Dr. Oddo developed the first AD mouse model (3xTg) that exhibits accumulation of amyloid-β and tau. It has since become one of the most widely used mouse models of AD. He also recently demonstrated, in both humans (Fig. 1) and mice (Fig. 2), the association between S6K1 expression and amyloid-β, tau and cognitive impairment (details of study described in Figures).

Dr. Quarles has extensive experience on the use of MRI and PET in mouse and rat models of disease. For the purposes of this application we have included representative data from a recently study (currently under review in Brain Imaging and Behavior) that describes the use of FDG PET imaging and cognitive tests to assess chemotherapy induced cognitive dysfunction (CICD)



in rats. In rats treated with doxorubicin for 30 days we found decreased [18F]-FDG uptake in the prefrontal cortex and decreased performance on a novel object recognition (NOR) task (Fig. 3). In contrast, FDG uptake and hippocampal-mediated memory was unaffected by treatment.

Reduced FDG uptake in the prefrontal cortex but not the hippocampus represents a neural correlate of the NOR deficits, and likewise suggests a longer-lasting impairment in the prefrontal cortex but not the hippocampus. This study highlights the sensitivity of PET to regional variations in metabolism, such as those being interrogated herein.

Methods and Experimental Design: Four groups (8 mice / group) of mice (wild type, 3xTg-AD, 3xTg-AD/S6K1^{+/-} and S6K1^{+/-}) will be imaged using MRI and microPET. High-resolution MR

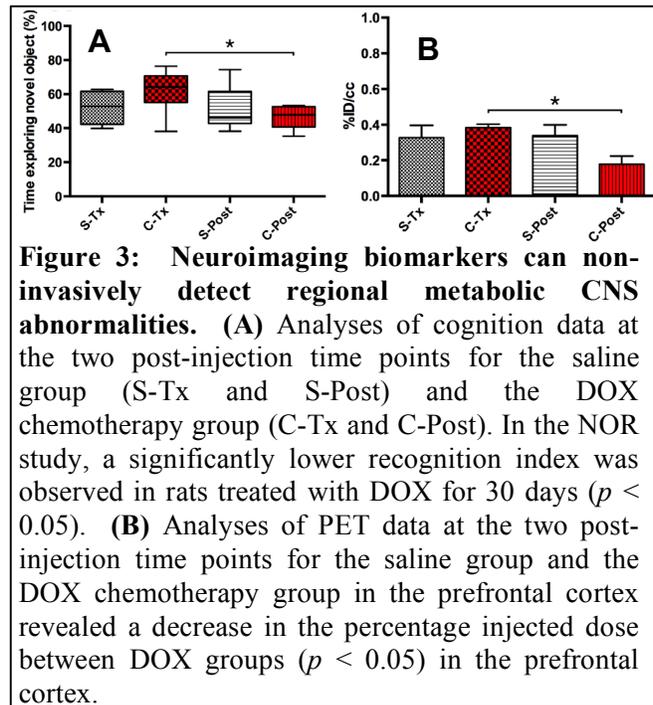


Figure 3: Neuroimaging biomarkers can non-invasively detect regional metabolic CNS abnormalities. (A) Analyses of cognition data at the two post-injection time points for the saline group (S-Tx and S-Post) and the DOX chemotherapy group (C-Tx and C-Post). In the NOR study, a significantly lower recognition index was observed in rats treated with DOX for 30 days ($p < 0.05$). (B) Analyses of PET data at the two post-injection time points for the saline group and the DOX chemotherapy group in the prefrontal cortex revealed a decrease in the percentage injected dose between DOX groups ($p < 0.05$) in the prefrontal cortex.

images will be acquired in order to quantify volumetric changes in the brain, including the whole brain, hippocampus, cortex, total ventricles, and caudate putamen. On three consecutive days (to allow for radiotracer decay from prior injections), PET images will be acquired 1 hour after the injection of [18F]-FDG (glucose metabolism), [18F]-flutemetamol (amyloid) and [18F]-AV1451 (tau). Maps of the percent-injected dose (%ID/cc) will be quantified. Data will be spatially registered to the MRI in order to evaluate regional differences in tracer uptake and correlative analysis with volumetrics. Mice will be sacrificed and brains extracted for histological analysis.

Since our primary goal is to establish imaging biomarkers in AD models we have a straightforward hypothesis that is supported from the *ex vivo* analysis: As

compared to wild type, S6K1^{+/-} and 3xTg-AD/S6K1^{+/-} mice, 3xTg-AD mice will exhibit significantly more uptake of flutemetamol and AV1451, decreased cortical volume, and reduced FDG uptake. Further, the degree of uptake of each tracer will be significantly correlated with ex vivo measures of the same feature.

We acknowledge that the ideal use of these non-invasive MRI and PET biomarkers would be to image mice longitudinally such that their evolution can be tracked during AD progression. However, given the limited funds of the pilot proposal we elected to focus only on a single time point such that our analysis would be sufficiently powered (as compared to, for example, imaging 2 mice per group over time). This approach will enable us to acquire sufficient data for a publication.

Proposed One-Year and Long-Term Outcomes: The publication of this study will lay the foundation needed to apply for an NIH award, which has a long-standing track record of supporting the development and use of image based biomarkers in AD. Dr. Oddo and I have already discussed using the data from this pilot project in order to apply for an R01 grant (multi-PI) focused on imaging disease progression in the models employed herein, with a specific emphasis on using imaging to temporally direct conventional *ex vivo* assays. These studies would also establish BNI's "credentials" in multimodal preclinical imaging of AD, paving the way for other regional investigators to leverage this shared resource through its inclusion in prospective grant applications.

Progress report: The goal of this study is to identify neuroimaging signatures of Alzheimer's Disease in mouse models that recapitulate human pathophysiology. In collaboration with Salvo Oddo (Arizona State University) all mice (n= 24), including 3xTg-AD, 3xTg-AD/S6K+/- and S6K1+/- and wild type, needed for the proposed studies have been transferred to the Center for Preclinical Imaging at Barrow Neurological Institute. Multi-modal imaging data has been collected in all mice and the image analysis and correlative histological evaluation of tissue is currently underway.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Epigenetic dysregulation in cholinergic neurons in PD dementia. Sylvia Perez, PhD. Barrow Neurological Institute; Arizona Alzheimer's Consortium.

Background: Seven to 10 million of people have Parkinson's disease (PD) worldwide and one million in USA. Thirty to 80% of PD patients experience cognitive decline/dementia as the disease progresses. While current PD pharmacotherapy mainly tackles motor symptoms, there is a lack of therapies to treat non-motor cognitive symptoms. Although, the full extent of the neuropathophysiology underlying PD with dementia (PDD) is unclear, degeneration of the cholinergic basal forebrain (CBF) cortical projection system, which is involved in cognition dysfunction and is a major pathologic feature of PD. Notably, CBF neurons within the nucleus basalis of Meynert (Ch4), which innervate the neocortex, exhibit a greater cell loss in PDD than PD and even than Alzheimer's disease (AD). These findings suggest that CBF neuron dysfunction contributes to PD dementia. Since epigenetic mechanisms including histone modification, binding of non-histone proteins, and DNA methylation modulate/coordinate expression of large numbers of genes across many different pathways, they warrant investigation for a potential role in PD pathogenesis including PDD. In fact, decreased deacetylation levels were seen in midbrain neurons in PD, suggesting a major role for epigenetics in the pathophysiology of this disease. Currently, there are no studies of alterations of the epigenoma in CBF neurons and their effect on neuronal gene expression in PD and PDD. To define the cellular and molecular factors underlying Ch4 degeneration in PD and PDD, we will use a novel approach of combining epigenetic immunohistochemical markers and single-cell gene array technology to answer this question. The results will provide novel drug targets to treat cholinergic neurodegeneration in PD and PDD, which may translate to AD and even Down syndrome (DS).

Overview: Studies suggest that dysregulation of epigenetic proteins, such as histone acetylation and deacetylation, which are activated by histone acetyltransferases (HATs) and deacetylases (HDACs), respectively, contribute to neuronal death in PD. We will test the novel hypothesis that a reduction in HDACs in Ch4 neurons alters the expression of cell survival genes in PD and to a greater degree in PDD. **We plan to test the following interrelated Specific aims:**

Aim 1: We will test the hypothesis that there is a reduction HDAC1, HDAC2 and HDAC3 proteins in Ch4 neurons in PD, which will be decreased to a greater degree in PDD, using quantitative immunohistochemistry.

Aim 2: We will test the hypothesis that there is a correlation between the downregulation of HDAC protein levels and classes of cell survival genes in Ch4 neurons in PD and to a greater degree in PDD by combining HDAC immunohistochemistry with single cell gene expression technology.

Significance: Parkinson syndrome is the second most common neurodegenerative disease characterized by the presence of tremor, bradykinesia, rigidity and postural instability and neuropathologically by the presence of alpha-synuclein (α -syn) aggregation and degeneration of nigrostriatal dopaminergic neurons as well as and cholinergic basal forebrain neuron degeneration within Ch4. In PDD, Ch4 neurons are more severely depleted than PD or AD, suggesting that cholinergic neurodegeneration contributes, at least in part, to cognition decline/dementia in PD. Currently, the most effective PD pharmacotherapy for the temporary relief of the motor symptoms is dopamine replacement therapy. Therapies to treat the dementia, associated with PD are lacking. The field of epigenetic provide a unique tool to investigate gene expression and cellular physiology without altering DNA information. In this vein, adding and removing acetyl (A) groups to histone lysine residues by acetyltransferases (HATs) and deacetylases (HDACs) enzymes respectively, is one epigenetic mechanism that regulate gene expression, which in non-pathological conditions are balanced (Fig. 1).

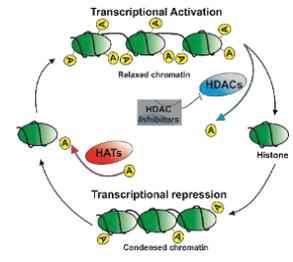


Fig.1

Epigenetic dysregulation has been implicated in neuronal death in PD. Recently, it has shown that acetylation levels in PD are increased in midbrain neurons, whereas levels of several HDACs (HDAC1, HDAC2, HDAC4, HDAC6 and SirT1) were decreased. In addition, it was reported that α -syn could mask histone proteins, preventing their acetylation and promoting neurotoxicity. In several PD models, neurotoxicity was reversed using HDAC or HAT inhibitors, suggesting a role of acetylation/deacetylation enzymes in neuroprotection. These observations suggest that histone acetylation/deacetylation misbalance play a role in neurodegeneration in PD. Although, these studies open the door to a new line of PD treatments using epigenetic approaches that could potentially be used in conjunction with dopamine replacement to treat non-motor symptomatology in PD, a detailed analysis of

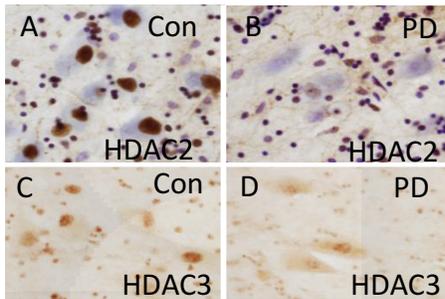


Fig.2. Ch4 immunostained for HADC2 (A, B) and HDAC3 (C, D). Strong HADC2 (A) and HDAC3 (C) positive nuclei in a control (CON), compared to weak HADC2 (B) and HADC3 (D) immunostained nuclei in PD. Tissue in A and B was counterstained with cresyl violet.

alterations in epigenetic markers and its effects on classes of genes are lacking. **Preliminary studies** found a progressive reduction in HDAC2 and HDAC3 protein levels (Fig. 2) suggesting an interaction between deacetylation and cholinergic markers in PD. Whether alterations in HDACs within Ch4 neurons in PD and PDD are associated with a downregulation of HDAC gene expression and other genes remains to be determined. Data generated will provide seminal findings necessary to generate new drug targets for PD and PDD. **Innovation:** Nothing is known about the epigenetic changes and its repercussions in gene expression in Ch4 neurons during PD progression including PDD. The research proposed here is innovative because it focuses on a novel approach using epigenetic markers to evaluate the genetic signature of vulnerable cholinergic neurons, which is an understudied area in PD and will generate new PD drug

targets with possible translation to AD and DS, which also display Ch4 degeneration.

Experimental design and methods: Paraformaldehyde fixed tissue matched for gender, age, PMI, education level and MMSE are examined from clinical diagnostic groups (n=15) including: 1) Pre-mortem clinical diagnosis of no cognitive impairment and without other comorbidities- related to cognitive decline, 2) PD cases clinically defined as Hoehn and Yahr (H-Y) stages 1 to 5 without dementia, and 3) PD with dementia. The Rush ADC Brain Bank approved the tissue request. **Aim1. We hypothesize that HDAC markers in Ch4 neurons are reduced in PD and to a greater degree in PDD.** The relative HDAC2, HDAC3 and HDAC1 protein levels will be quantified using combined immunohistochemistry and densitometry techniques. Briefly, floating sections containing Ch4 will be immunostained using HDAC2, HDAC3 and HDAC1 antibodies (1:500 Abcam). Imaging and quantification of nuclear HDAC immunoreactivity will be performed. **Statistical Analysis:** Data analysis will use one-way ANOVA or Kruskal-Wallis rank sum test depending on value distribution and appropriate post-hoc tests. Correlations will use Pearson or a Spearman rank test. Statistical significance will be set at 0.05 (two-sided).

Aim 2. We hypothesize that a reduction in HDAC markers in Ch4 nuclei will be associated with a decrease in gene expression related to classes of cell survival genes in PD but to a greater degree in PDD. Single population expression profiling using custom-designed microarray analysis will be evaluated using Aim 1 cases. Section will be immunostained using the same HDAC markers as in Aim 1. Labeled Ch4 neurons will be microaspirated using a Zeiss laser capture microscope, mRNA will be extracted as previously reported and custom-designed microarrays will be employed to identify mRNA changes. Approximately 50 individual labeled cells will be micro-dissected via LCM and captured per reaction for custom-designed array analysis. Arrays will be hybridized overnight at 42 °C and washed sequentially at 42°C, exposed to a phosphor screen for 24 hr. and developed on a phosphor imager (GE). Expression of TC amplified RNA bound to each linearized cDNA will be expressed as a ratio of the total hybridization signal intensity of the array to minimize variations due to differences in the specific activity of the probe and the absolute quantity of probe present. Data analysis generates an expression profile of relative changes in mRNA levels among neurons isolated from the three groups. Standard curves and cycle threshold are measured using standards from human postmortem brain RNA expression levels. Experiments are run in triplicate and blinded. Single cell RNA profiling will be validated using qPCR.

Statistical analysis: Relative changes in total hybridization signal intensity and in individual mRNAs will be analyzed by one-way ANOVA with a post-hoc Newmann-Keuls test for individual comparisons. The p-values are adjusted using a false discovery rate to reduce Type I error¹⁸. Data will be correlated with clinical stage and demographic variables.

Expected and Alternative Findings: In Aim 1, we expect a differential reduction in HDAC in PD and PDD with a greater loss of HAD2 in Ch4 neurons related to H-Y score. In Aim 2, we anticipate that gene expression profiles of PD and PDD Ch4 neurons will reveal differential alterations in transcriptional and signaling pathways. Results provide evidence for PD and PDD-specific transcript dysregulation. Alternatively, no difference in gene expression will exist. **Plan for external funding:** Study will provide preliminary data for NIH grants.

Progress Report: The major goal of this study is to define the epigenetic factors underlying cholinergic neuronal degeneration in Parkinson's disease (PD) with dementia (D). Although I had reach to the local/national brain banks to obtain tissue from PD and PDD subjects, they were unable to provide the anatomical characteristics and conditions of the Cholinergic Basal

Forebrain (CBF) tissue necessary to carry out this proposal. Luckily the Parkinson's UK Brain Bank at Imperial College London, run by the Professor Steve Gentleman, provided us with rostral to caudal formalin-fixed paraffin-embedded sections of the CBF as well as frozen CBF tissue from the same PD, PDD and control cases. All these cases have clinical histories that allow us to made correlations between clinical and epigenetic findings. In addition Dr. Mufson has PD, PDD and control cases containing fixed CBF sections, but with very little clinical history, that I would be using in this project too. Attach you can find the date and the amount of tissue that was sent to me from the Parkinson's UK Brain Bank at Imperial College London to complete this project.

Project Progress Reports

Critical Path Institute

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Enabling integration of AD Prevention Study Data by application of CDISC standards: Extension to digital biomarker assessments. Stephen P. Arnerić, PhD and Klaus Romero, MD. Critical Path Institute, Arizona Alzheimer's Consortium.

Specific Aims: **1)** Identify and integrate data from specific prevention trials for Alzheimer disease (AD) to enable the acquisition of additional scientific insights through data modeling and simulation using contemporary biomarker assessments; **2)** Convene a workshop 1Q2017 to advance Clinical Data Interchange Standards Consortium (CDISC) standard development of digital biomarkers; and **3)** Prepare the way for expedited regulatory review of drug development programs by using data standards (e.g., CDISC standards for digital biomarkers, i.e., biosensor measured outcomes) that will create the foundation for qualifying drug development tools that will accelerate innovative preventative treatments. It is proposed to annotate the case report forms from above-mentioned trials with applicable CDISC foundational and AD-specific clinical data standards, and ultimately enable remapping into the CDISC standards format. It is envisioned that these data will be integrated with other AD clinical trial data that reside in the Critical Path Institute (C-Path) Data Collaboration Center (DCC). Once these trials are complete and data are analyzed and released, valuable knowledge can be gained through aggregation and analyses of the integrated data.

Background and Significance:

Data standards: C-Path has, through partnership with CDISC, successfully developed therapeutic-area-specific data standards for AD, Parkinson's disease (PD), polycystic kidney disease (PKD), tuberculosis (TB) and most recently, multiple sclerosis (MS). The Alzheimer's disease CDISC standards represented the first such disease-specific standards and consists of version 1.0 and an update, version 2.0. These therapeutic area standards were developed with funding support from the U.S. Food and Drug Administration (FDA) and represent the preferred format by regulatory agencies for new drug applications for expedited review. Since December 2016, CDISC standards are required by FDA for all new drug applications, strongly suggesting that clinical trials initiating at the present time should adopt these standards. The FDA has estimated that ~40% of the effort to review a new drug submission is expended in preparing the data for analysis. The adoption of CDISC therapeutics-specific data standards in trials will aid in improving the efficiency of regulatory reviews for any novel candidate in development, independent of sponsor or the mechanism of action of the therapeutic candidate.

Data sharing: Additionally, CDISC standards facilitate aggregation of clinical data from diverse sources, enabling the analyses of integrated data. Through analysis of integrated data from the Coalition Against Major Diseases (CAMD) database, the CAMD consortium within C-Path obtained regulatory endorsement for an AD clinical trial simulation tool from both the FDA (June) and the EMA (July) in 2013, and continues to advance the process of qualifying clinical trial enrichment biomarkers as drug development tools with both Agencies. The CAMD-AD clinical trial database is available to qualified researchers and is being analyzed to address specific research questions. In December 2015, the CAMD-AD database was co-linked with

GAAIN (Global Alzheimer's Associate Interactive Network). With the integration of data from interventional trials analyses of the aggregate data sets, additional power to understand the disease-progression continuum, and probe the relationship between drug effects on biomarkers and drug effects on outcome measures will be advanced.

A partnership/collaboration between C-Path and AAC will lay the foundation for additional data standardization and integration of data from prevention trials to facilitate other groundbreaking approaches that will enhance our overall understanding of AD. Such global initiatives currently underway include Global Alzheimer's Association Interactive Network (GAAIN), Innovative Medicines Initiative European medicines information framework, Alzheimer's disease (EMIF-AD), IMI European Platform to facilitate Proof of Concept for prevention in Alzheimer's Disease (EPOC), Consortium for Alzheimer's Prevention (CAP), Alzheimer's and Dementia Initiative (ADDI), and Global Alzheimer's Platform (GAP).

Preliminary Data and Plan: The goal of the v2.0 project is to expand on the concepts for Alzheimer's clinical trials and research, using ADNI as the primary source of input. Substantive additions from v1.0 include ten scales of cognition/function, cerebrospinal fluid biomarkers sample handling and processing, and imaging biomarkers including volumetric MRI, amyloid PET imaging and FDGPET imaging. The AD CDISC standards are ready to be used with current and planned protocols and case report form (CRF) data from ongoing trials. A manuscript detailing these advances for mild cognitive impairment (MCI) is in press (Neville et al., 2017). The team comprised of experts involved in the development of the AD CDISC standards will review preliminary data and new materials provided from relevant prevention trials. These standards will be applied to the data to enable proper classification of this data for the pooling and regulatory submission of prevention studies. **Proposed One-Year and Long-Term**

Outcomes: The one-year outcome of the project will be a report that defines how CDISC SDTM AD standards will be applied to these data, the user guides, annotated CRF's and recommendations for approaches to optimize the processing of the data once this becomes available. Once this information is available, a long-term phase of this project could map the data, which would support long-term outcomes that support Alzheimer's prevention research, such as facilitating FDA review, data pooling, and support other prevention initiatives. The pilot project during year one will lay the groundwork for the future by enabling prevention trials to collect historical and digital biomarker data in a standardized way that will foster pooling and analyses going forward. Several public-private-partnerships are underway globally that CAMD can engage to continue support of this project beyond year one including expanded alliances with Banner AAC.

Year End Progress Summary:

Aim 1: A publication is *in press* that describes v2.0 of the AD CDISC standards. CAMD has grown the existing AD database using CDISC standards (v2.0) that integrate biomarker assessments from prevention trials and the pre-dementia stages of AD.

Aim 2: On March 10, 2017, an international workshop was organized by CAMD in Phoenix, AZ, to initiate the work to create a consensus view on existing gaps and approaches to fill those gaps on CDISC standards for digital biomarkers (aka, biometric monitoring devices, smartphones, remote monitoring devices). In summary: Attendees reiterated the need to focus on

an accelerometer data-based core set of elements (metadata) that can be combined with/compared to other, more traditional measures. That study needs definition and resulting data need to be mapped to SDTM (Study Data Tabulation Model), the standard structure for human clinical trial (study) data tabulations, enabling the creation of standardized datamarts. Concept maps that reflect the user's perspective as well as device capabilities need to be devised; focus on sleep and mobility/frailty appears to be most feasible; a longitudinal dataset (to allow for additional analyses) would be most-effective; data sharing needs to be encouraged. Existing frameworks for integration of such novel measures include existing cohort studies such as PPMI (Parkinson's Progression Markers Initiative) and ADNI (Alzheimer's Disease Neuroimaging Initiative). Correlation with patient-reported outcomes will assure constant alignment with existing measures and allow the potential to assess clinical meaningfulness. For cognition measures, attributes of parameters need to be defined; available devices need to be vetted; the terminology/ontology needs better definition; initially, cognition measures could be included alongside other measures to provide greater perspective. Participants agreed that it would not be useful to develop a new strategy for each new concept - but to be able to reuse a defined standard/standards. Slide presentations and a detailed summary of the workshop is available at: <https://c-path.org/mobile-devices-in-clinical-trials-for-neurological-diseases-cdisc-standards-development/>.

Aim 3: Data from the expanded C-Path database will be used to support the qualification submission in 3Q2017 to FDA of intracranial volume (ICV)-adjusted vMRI images as a prognostic biomarker for use in MCI studies.

Project Progress Reports

Mayo Clinic Arizona

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Normal and Pathological Aging (Preclinical Alzheimer's Disease). Richard J. Caselli, MD, Dona E.C. Locke, PhD. Mayo Clinic Arizona; Arizona Alzheimer's Consortium.

Project Description: Cognitively normal individuals age 21-99 (most age 45-70) undergo 1) APOE genotyping to categorize their relative risk for developing Alzheimer's disease; 2) longitudinal neuropsychological and behavioral assessments; and 3) serve to create a biorepository for DNA, serum, plasma, viable frozen lymphocytes, and immortalized cell lines to determine what factors divert individuals from normal to pathological aging/Alzheimer's disease with the intent of identifying optimal timing of treatment and new potential therapeutic targets for preventing this divergence (prevention of Alzheimer's disease). This "APOE Cohort" also serves as a core resource for multiple collaborative projects within our site and for the consortium.

Specific Aims:

- A. To maintain and grow a unique cohort of human aging in which we characterize the effect of APOE gene dose (a risk factor for Alzheimer's disease) on age-related changes in:
 - 1. Mentation (neuropsychological measures of cognition and behavior; subjective assessments by observers and self; sleep parameters)
 - 2. Brain Imaging (structural brain changes [MRI], functional [FDG-PET], amyloid-PET)
- B. To correlate longitudinal changes on each of these measures with clinical outcomes (mild cognitive impairment, Alzheimer's dementia, non-Alzheimer's dementia)
- C. To characterize the influence of other demographic, genetic, epigenetic, and health factors on cognitive aging trajectories
- D. To create a biobank of serum, plasma, DNA, frozen viable lymphocytes, and immortalized cell lines of this cohort.
- E. To function as a core resource collaboratively supporting other investigators

Background and Significance: Even at the earliest clinical stages of Alzheimer's disease (AD), amyloid pathology has nearly peaked yet neither symptoms nor brain atrophy correlate well with amyloid burden. Failed anti-amyloid therapies have been blamed on being started too late, resulting in new disease modifying strategies that begin during the preclinical, asymptomatic stage. Our work to date has helped to define and characterize the preclinical stage of AD, differentiating normal from pathological aging. Themes of our current research include 1) identification of preclinical disease modifying attributes (genetic, medical, demographic, and others), 2) extension of preclinical testing and precision medicine into the clinical practice domain, and 3) integration of multiple data sources into predictive algorithms.

Preliminary Data: To date we have completed APOE genetic testing on over 2700 participants from which were selected our study population for further testing. We have completed one or more epochs of neuropsychological testing on 751 individuals including 429 APOE e4 noncarriers, 232 e4 heterozygotes, and 90 e4 homozygotes. Of these, 588 have completed two or more epochs of testing, with followup durations of up to 20 years, and an average that is over 9

years providing ample data for longitudinal studies. We have nearly 3000 plasma and serum samples on roughly 400 individuals, and DNA on all. 497 have immortalized cell lines established including all with brain imaging. We established memory aging trajectories for each of 3 APOE genotypes (1), and subsequently on all remaining cognitive domains (2) providing a baseline upon which we are able to distinguish normal aging from preclinical Alzheimer's disease, and the differential impact of modifying factors such as cardiovascular risk factors (3) preclinical amyloid deposition (4) and personality factors (such as proneness to stress) (5) thus generating new hypotheses about amyloid's pathophysiologic role. We have further published TOMM40 related memory trajectories and have found a qualitatively and quantitatively different effect than for APOE (6).

Experimental Designs and Methods: Responders to local media ads undergo APOE genotyping (a blood test); APOE e4 carriers are matched by age, gender, and education to a noncarrier. Screening tests (Folstein MMSE, Hamilton Depression Scale, Neurologic exam, psychiatric interview) confirm reported normality. Blood for the biorepository is obtained at entry for storage of plasma, serum, and DNA. Neuropsychological (and related) testing is performed every 2 years under age 80 and annually over age 80. Individuals developing MCI or AD are rolled over into the NIA-ADCC study.

Proposed One-Year and Long-Term Outcomes: In addition to maintaining the ongoing evaluation of this important cohort, our goals for the next one year include:

1. Complete our analysis of longitudinal FDG-PET, volumetric MRI, and neuropsychological changes that progress to MCI and characterize preclinical AD.
2. Extend our genetic study of unexpectedly young onset dementia patients with whole exome sequencing and bioinformatics analysis of a large gene set encompassing identified risk genes for Alzheimer, disease, frontotemporal lobar degeneration, and Parkinson's disease to examine 4 specific goals:
 - a. do "minor" genetic variants correlate with young onset dementia (implying their generally accepted nonpathogenic status may vary between individuals so that they are more pathogenic in some), and
 - b. how disclosure of such genetic results to patients and families impacts clinical care (e.g., does it lead to CLIA lab confirmation of research results; does the absence of a highly pathogenic variant such as a PS1 mutation offer solace to families concerned about familial transmission)
 - c. explore possible correlations between benign variants in previously reported disease-associated genes with clinical phenotypes (e.g., are MAPT variants more prevalent in focal dementia syndromes)
 - d. extend this analysis to autopsied cases correlating benign variants with neuropathological outcomes (e.g., are variants in Parkinson's disease associated genes more prevalent in patients with Lewy body pathology)
3. continue to evaluate the results of an autism questionnaire with regard to
 - a. the prevalence of a "broad autism phenotype" (BAP) in our cohort members, and whether they "fit" a previously described BAP phenotype, and
 - b. whether a BAP phenotype impacts age-related cognitive decline and the risk for incident MCI and dementia alone or in conjunction with APOE e4
4. Support our collaborative projects

Year End Progress Summary:

Regarding our 2016 goals:

1. Goal: expand our biobanking efforts to include all those with young onset Alzheimer's disease.

Status: We have modified our existing biobanking protocol to include this important subpopulation, and they are now included.

2. Goal: complete a pilot project of whole exome sequencing and bioinformatics analysis of a 55 gene panel in a clinical cohort of patients with biomarker supported young onset Alzheimer's disease (or frontotemporal dementia)

Status: This pilot was originally intended to include 14 young onset dementia patients, as well as 10 controls with Alzheimer's disease over age 80 years and 11 controls with normal cognition over age 80 years, and all have been completed. We have been able to add another 3 young onset dementia patients and these are now in progress. In addition, we received a philanthropic gift to support this work that will allow us to enroll another 20 young onset patients in 2017. We have also applied to receive exome sequence data from the ADSP on patients with autopsy confirmed diagnoses that fit into our targeted disease categories.

3. Goal: publish our analyses of personality factors' influence on age-related cognitive trajectories

Status: completed (reference 5 below). Additionally we have extended this work to examine personality changes that accompany the transition from preclinical AD to MCI (a 2017 goal).

4. Goal: continue data analysis within our large cross sectional study of multiple MRI-based structural, physiological, and vascular measures across the entire adult lifespan (20's-90's), and their correlation with neuropsychological test scores

Status: we are preparing an abstract for the annual Alzheimer's Association Annual Conference that will summarize the results of our longitudinal study of brain imaging (structural MRI and FDG-PET) and neuropsychology in patients who have progressed from preclinical AD to MCI as well as a matched set of controls.

5. Goal: completion of a study evaluating a cognitive "stress test" based upon TOMM40 genotype to further test the proposal that TOMM40 is another genetic risk factor for AD

Status: manuscript has been prepared and is ready for journal submission.

5. Goal: exploration of plasma eosinophil products in patients with vascular contributions to dementia

Status: pilot project completed showing lower eosinophil peroxidase levels in patients with Alzheimer's disease relative to controls and other groups. A larger followup study is underway seeking to test this hypothesis.

6. Goal: collaboration to establish lymphocyte derived iPS cells and differentiated in vitro cortical neurons to explore intraneuronal pathophysiology related to Alzheimer's disease.

Status: lymphocyte derived iPS cells successfully generated and differentiated into cortical neurons that are now under further study by Dr. David Brafman at ASU. An R01 application is additionally being generated to expand this work.

7. Goal: exploration of a partnership with Mountain Park Health Center to address disparities in dementia care and research opportunities in a large Latino population.

Status: space and other challenges have impeded our progress so an alternative strategic partner has been identified (Adelante Health Center in Mesa) and talks are underway.

References:

1. Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, Ahern GL, Baxter LC, Rapcsak SZ, Shi J, Woodruff BK, Locke DE, Snyder CH, Alexander GE, Rademakers R, Reiman EM. Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. *N Engl J Med.* 2009; 361(3):255-63.
2. Caselli RJ, Locke DE, Dueck AC, Knopman DS, Woodruff BK, Hoffman-Snyder C, Rademakers R, Fleisher AS, Reiman EM. The neuropsychology of normal aging and preclinical Alzheimer's disease. *Alzheimers Dement.* 2014; 10(1):84-92.
3. Caselli RJ, Dueck AC, Locke DE, Sabbagh MN, Ahern GL, Rapcsak SZ, Baxter LC, Yaari R, Woodruff BK, Hoffman-Snyder C, Rademakers R, Findley S, Reiman EM. Cerebrovascular risk factors and preclinical memory decline in healthy APOE epsilon4 homozygotes. *Neurology.* 2011; 76(12):1078-84.
4. Caselli RJ, Dueck AC, Locke DE, Hoffman-Snyder CR, Woodruff BK, Rapcsak SZ, Reiman EM. Longitudinal modeling of frontal cognition in APOE epsilon4 homozygotes, heterozygotes, and noncarriers. *Neurology.* 2011; 76(16):1383-8.
5. Caselli RJ, Dueck AC, Locke DE, Henslin BR, Johnson TA, Woodruff BK, Hoffman-Snyder C, Geda YE. The Impact of Personality on Cognitive Aging: a Prospective Cohort Study. *J Internat Neuropsychol Soc* 2016; 22: 765-776.
6. Caselli RJ, Dueck AC, Huentelman MJ, Lutz MW, Saunders AM, Reiman EM, Roses AD. Longitudinal modeling of cognitive aging and the TOMM40 effect. *Alzheimers Dement.* 2012; 8(6):490-5.
7. Caselli RJ, Langbaum J, Marchant GE, Lindor RA, Hunt KS, Henslin BR, Dueck AC, Robert JS. Public perceptions of presymptomatic testing for Alzheimer's disease. *Mayo Clin Proc* 2014 (in press).
8. Caselli RJ, Marchant GE, Hunt KS, Henslin BR, Kosiosek HE, Langbaum J, Robert JS, Dueck AC. Predictive Testing for Alzheimer's Disease: Suicidal Ideation in Healthy Participants. *Alzheimer Dis Assoc Disord.* 2015 Jul-Sep; 29(3):252-4. PMID:25984909. PMCID:4543388. DOI:10.1097/WAD.0000000000000097.
9. Schlosser-Covell G, Caselli RJ. Executive dysfunction in prevalent vs incident amnesic mild cognitive impairment. To be presented at the Alzheimer Association International Conference, July 2014 in Copenhagen, Denmark.
10. Caselli RJ, Dueck AC, Locke DE, Baxter LC, Woodruff BK, Geda YE. Sex-based memory advantages and cognitive aging: a challenge to the cognitive reserve construct? *J Int Neuropsychol Soc.* 2015 Feb; 21(2):95-104. Epub 2015 Feb 09. PMID:25665170. DOI:10.1017/S1355617715000016.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Predicting Cognitive Decline in Cognitively Normal Individuals. Cynthia M. Stonnington, MD. Mayo Clinic Arizona; Arizona Alzheimer's Consortium.

Project Description: Cognitively normal individuals age 21-99 (most age 45-70) undergo 1) APOE genotyping to categorize their relative risk for developing Alzheimer's disease; 2) longitudinal neuropsychological and behavioral assessments; and 3) serve to create a biorepository for DNA, serum, plasma, viable frozen lymphocytes, and immortalized cell lines to determine what factors divert individuals from normal to pathological aging/Alzheimer's disease with the intent of identifying optimal timing of treatment and new potential therapeutic targets for preventing this divergence (prevention of Alzheimer's disease). This project will capitalize on the existing longitudinal data base of imaging, neuropsychological testing, and genetic testing to establish how a clinician might use a combination of such data to identify pre-clinical predictors of disease and to determine the probability of developing disease for any given individual patient.

Specific Aims:

1. To identify participants in our longitudinal study of aging who have baseline imaging and have shown evidence of cognitive decline but are still cognitively normal.
- 2) To identify participants in our longitudinal study of aging who have baseline imaging and have shown evidence of cognitive decline by having developed incident MCI.
- 3) To preprocess MRI scans using cortical thickness, i.e., Freesurfer, and grey matter volume, i.e., SPM, methods. Compare region of interest and whole brain differences between decliners and nondecliners for each of the methods.
- 4) To develop methods to predict decline using FDG PET, MRI, amyloid imaging, genetic, and neuropsychological data by creating training sets of baseline data from participants with decline and from participants who have at least two epochs of data and show no decline.
 - a. Examine the statistical power in distinguishing the two groups using Receiver Operating Curve (ROC).
 - b. Examine prediction accuracy by using machine learning methods.

Background and Significance: Even at the earliest clinical stages of Alzheimer's disease (AD), amyloid pathology has nearly peaked yet neither symptoms nor brain atrophy correlate well with amyloid burden. Anti-amyloid therapies have all fallen well short of expectations to date, for the generally held reason that they are started too late, and that for a disease modifying agent to be effective it must be started during an earlier, preclinical stage, i.e., before patients develop symptomatic memory loss. Preclinical AD is superficially indistinguishable from normal aging. We therefore plan to develop methods to differentiate normal from pathological aging by combining imaging based biomarkers, neuropsychological, and genetic data to better identify those individuals on the cusp of symptoms and therefore most likely to benefit from treatment.

Preliminary Data:

1. From a total of 139 ADNI participants who were diagnosed as MCI and had baseline FDG PET and MRI imaging data, 78 (75.8±7.0 years old) developed incident AD during the subsequent 36 months, and the remaining (75.3±8.0) did not during the same period. FDG PET measured glucose uptake, MRI measured hippocampal volume and ADAS-mod at baseline all distinguished MCI converters from non-converters, but, using ROC, the sensitivity and specificity showed increased statistical power when these modalities were combined (sensitivity=82%, and specificity=80%).
2. From our longitudinal APOE data base of cognitively normal individuals, we have identified 21 individuals with baseline FDG PET and MRI and neuropsychological data who subsequently developed incident MCI, along with 180 in the same age cohort who remain cognitively normal also had FDG PET and MRI and neuropsychological data.
3. From our longitudinal APOE data base of 180 cognitively normal individuals with baseline FDG PET and MRI and neuropsychological data, we have identified 18 who show evidence of cognitive decline but have not yet developed MCI or AD.
4. From our longitudinal APOE data base, we identified 14 individuals with amyloid imaging data who also had evidence of cognitive decline but remained cognitively normal and matched by age, sex, APOE status, and education to 14 individuals who did not show any cognitive decline. At $P < .005$ (uncorrected), decliners had significantly greater evidence of fibrillar A β burden in comparison to nondecliners.

Experimental Designs and Methods: From our ongoing, longitudinal normal and pathological aging study, identify: 1) all participants with baseline imaging exhibiting cognitive decline according to definitions used in our prior studies; and 2) all participants with baseline imaging who developed incident MCI.

Both the FDG PET and PiB PET Distribution Volume Ratio (DVR) baseline images will be coregistered to MRI baseline images, and the MRI Dartel normalization will be used to normalize the MRI and PET data. For PiB PET scan data, the well-known graphical analysis Logan method and an automatically labeled cerebellar region-of-interest will be used to compute parametric brain images of the PiB DVR, a measure of fibrillar A β burden. Together with the effects of age and sex, partial volume effect corrected PET kernel matrices will be created separately for segmented grey matter, cortical thickness, Dartel normalized MRI and PET images, APOE e4 genotype, and cognitive test score data. Regions of interest will be determined from published data that used a data set independent of ours.

Firstly, we will examine the statistical power in distinguishing the two groups using Receiver Operating Curve method. Secondly, we will apply machine learned decision trees to various sets of features from brain imaging, genetic, and neuropsychological data. We will then test diagnostic and prognostic performance using different maximum number of features.

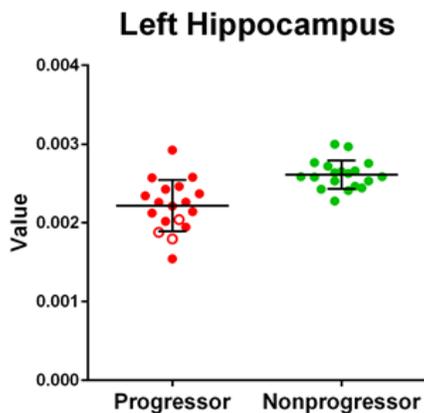
Proposed One-Year and Long-Term Outcomes: Produce computerized systems capable of diagnosis or prognosis for individuals who are cognitively normal based on chains of reasoning that a clinician can evaluate.

Budget Justification: Principal Investigator (6% Salary and Benefits). Cynthia Stonnington, MD Associate professor of Psychiatry will oversee all aspects of this study including procurement of scans, image analysis, coordination of data acquisition and analysis from the APOE cohort with Dr. Caselli, preparation of presentations, manuscripts, and progress reports, and compliance with all institutional and ethical guidelines.

Other budgetary items overlap with the project, Normal and Pathological Aging.

Progress report:

1. We have completed specific aim #1 and #2 as noted above in preliminary data section. We continue to track and update groups regarding diagnosis of MCI.
2. For aim #3, we worked with Yalin Wang at ASU to develop a method that we can then apply to the APOE cohort for the purpose of specific aim #4.
3. For aim #3 and #4: From the APOE cohort, baseline FDG PET and MRI measurements known to be preferentially affected in the preclinical and clinical stages of AD, including hippocampal volumes, entorhinal cortex thickness, posterior cingulate glucose metabolism, and an AD-related hypometabolic convergence index (HCI) were used to compare 18 cognitively unimpaired adults who progressed to the clinical diagnosis of amnesic Mild Cognitive Impairment or dementia due to AD within 1.8 ± 0.8 years to 2035 unimpaired adults who remained cognitively unimpaired for at least 4 years and were matched for their age, sex, education, and APOE4 gene dose. Statistical brain maps were used to further explore between-group differences in baseline differences in regional gray matter and glucose metabolism. In comparison with non-progressors, progressors had significantly reduced MRI and PET measurements in brain regions preferentially affected by AD and significantly increased HCIs at baseline. Among the prespecified MRI and PET measurements, reduced left and right hippocampal volumes were the strongest predictors of subsequent progression to the clinical stages of AD, each with 79% sensitivity and 78% specificity. We have written up manuscript and submitting for publication.



4. Professor Wang's group has also developed a patch-based sparse coding method to classify different stages of AD on hippocampal morphometry.

5. We recently applied patch-based sparse coding and machine learning (Adaboost classification) method to the same group described in #3 above and received the following results:

<i>Hippocampal</i>	<i>RD</i>	<i>RD_left</i>	<i>RD_right</i>	<i>mTBM</i>	<i>mTBM_left</i>	<i>mTBM_right</i>	<i>MMS</i>	<i>MMS_left</i>	<i>MMS_right</i>
Accuracy	0.80	0.64	0.63	0.92	0.69	0.62	1.00	0.77	0.66
Sensitivity	0.75	0.43	0.38	0.75	0.50	0.33	1.00	1.00	0.47
Specificity	0.83	0.86	0.86	1.00	0.82	0.86	1.00	0.75	0.86
ppv	0.75	0.75	0.71	1.00	0.67	0.67	1.00	0.33	0.78
npv	0.83	0.60	0.60	0.90	0.70	0.60	1.00	1.00	0.60

Table 1. Experiment Result of Hippocampal

<i>Ventricle</i>	<i>RD</i>	<i>RD_left</i>	<i>RD_right</i>	<i>mTBM</i>	<i>mTBM_left</i>	<i>mTBM_right</i>	<i>MMS</i>	<i>MMS_left</i>	<i>MMS_right</i>
Accuracy	0.77	0.69	0.54	0.74	0.54	0.50	0.85	0.77	0.60
Sensitivity	1.00	0.67	0.33	0.88	0.33	0.56	1.00	0.91	0.83
Specificity	0.70	0.70	0.71	0.64	0.71	0.88	0.83	0.64	0.50
ppv	0.50	0.40	0.50	0.64	0.50	0.38	0.33	0.71	0.42
npv	1.00	0.88	0.56	0.88	0.56	0.47	1.00	0.88	0.88

Table 2. Experiment Result of Ventricle

For 2017, we plan to write up the above results and submit for publication. We then plan to apply these methods to both the ADNI data set and the APOE cohort, using one for training and the other for testing in order to examine the generalizability of results and method, which is essential for developing a clinical tool to predict dementia.

Project Progress Reports

Midwestern University

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Analysis of the presence and potential role of chronic infection in Alzheimer's disease. G. Jentarra PhD; T.B. Jones PhD; J. Kaufman PhD; D. Jones PhD; P. Potter PhD; J. Vallejo PhD; Tony Tulloh MD / Midwestern University and the Arizona Alzheimer's Consortium

Specific Aims:

Aim 1: Establish the presence or absence of microbial DNA in brain tissue from AD or mild cognitive impairment (MCI) patients, matched non-demented normal controls, and high pathology non-demented controls (HPCs).

Aim 2: Specifically analyze AD or MCI patient and matched control brain tissue for the presence of microbes previously reported in the published literature as being present in AD brain tissue.

Aim 3: Confirm the ability of *Candida albicans* to provoke or increase the neuropathological characteristics of AD in AD triple-transgenic mice. Assess the susceptibility of APOE4 mice to *Candida albicans* infection and determine the nature/magnitude of the inflammatory response generated in the brain.

Background and Significance: Many reports have identified the presence of a wide range of microorganisms in brain tissue in association with AD^{1,3}. Reports include spirochete type bacteria⁴, herpes simplex virus 1 (HSV-1)^{5,6}, and fungi/yeast, including various species of *Candida*^{7,8}. The chronic presence of any of these microorganisms in the brain may be sufficient to produce the inflammation common in AD patients. The described presence of many different microorganisms in association with AD has led us to hypothesize that the specific microorganism present isn't as important as its chronic presence, which may drive a long-term inflammatory response.

The well-established amyloid and tau pathology of AD remain to be explained and there is reason to believe that those aspects of AD could be exacerbated or provoked by the presence of a chronic microbial infection. A β is induced by the presence of an infecting microbe^{2,4} and appears to have strong antimicrobial activity^{9,10}. Hyperphosphorylation of tau, which drives its aggregation, may also be induced by the presence of microbes^{11,12}.

The first two aims of this project are intended to determine if reports of the presence of specific microbes in AD patient brain tissue are reproducible in a well-controlled study, using two different approaches. The third aim is intended to establish if one of the microbes that has been repeatedly reported to be present, *Candida albicans*, is capable of enhancing induction of AD-type neuropathology in the triple transgenic mouse model which carries gene variants associated with early-onset AD. To add to this data, we will also use mice to determine if the APOE4 allele, the largest known genetic risk factor for sporadic AD, leads to greater susceptibility to infection with *Candida* (decreased clearance or increased microbial numbers) or if infection produces an altered inflammatory response, which might help to explain the role of the APOE4 allele in AD susceptibility.

Verification of the presence of specific microbes in the brains of AD patients in a well-controlled study and demonstration that at least one of those microbes can increase AD type pathology could ultimately lead to a substantial shift in how AD is treated. Currently there are no curative treatments for AD or even any treatments that are more than marginally effective.

Linking infection to the development of AD would open up many new, possibly far more effective, treatment options.

References:

1. Krstic D, Knuesel I. *Nature reviews. Neurology*. 2013;9(1):25-34.
2. McCaulley ME, Grush KA. *Int J Alzheimers Dis*. 2015;2015:515248.
3. Goldeck D, Witkowski JM, Fulop T, Pawelec G. *Current Alzheimer research*. 2016.
4. Lim SL, Rodriguez-Ortiz CJ, Kitazawa M. *Microbes and infection / Institut Pasteur*. 2015;17(8):549-556.
5. Heppner FL, Ransohoff RM, Becher B. *Nature reviews. Neuroscience*. 2015;16(6):358-372.
6. Bagyinszky E, Youn YC, An SS, Kim S. *Clinical interventions in aging*. 2014;9:535-551.
7. Perkins M, Wolf AB, Chavira B, et al. *Journal of Alzheimer's disease : JAD*. 2016.
8. Burgos JS, Ramirez C, Sastre I, Valdivieso F. *J Virol*. 2006;80(11):5383-5387.
9. Gale SC, Gao L, Mikacenic C, et al. *J Allergy Clin Immunol*. 2014;134(1):127-134.
10. Bu XL, Yao XQ, Jiao SS, et al. *European journal of neurology*. 2015;22(12):1519-1525.
11. Miklossy J, Kis A, Radenovic A, et al. *Neurobiology of aging*. 2006;27(2):228-236.
12. Harris SA, Harris EA. *Journal of Alzheimer's disease: JAD*. 2015;48(2):319-353.

Preliminary Data and Plan:

Aim 1:

Preliminary Data: Preliminary testing of HSV-1, pan-bacterial, and pan-fungal primers for microbial DNA analysis was performed to verify their function and the expected limits of detection within brain tissue.

Extract DNA from the superior frontal gyrus of AD/MCI patients and controls and use PCR followed by sequencing to survey the microorganisms present. Groups to be analyzed will include (1) patients with AD dementia, i.e. late stage AD patients (2) patients with MCI, i.e. early stage AD patients (3) matched normal control individuals for groups 1 and 2, i.e. non-demented, non-MCI matched controls (4) HPCs, i.e. individuals with substantial amyloid plaque and tau burdens but no cognitive impairment.

Aim 2: Stain brain tissue slices of superior frontal gyrus with antibodies/chemicals targeting fungi (including *Candida* species), herpes simplex virus type 1 (HSV-1), and bacteria (general bacterial markers and spirochete specific stains).

Aim 3: Conduct a longitudinal study of infected triple-transgenic mice to determine if fungal infection increases the production of amyloid plaques or neurofibrillary tangles. Determine if possession of the human APOE4 allele alters the susceptibility of mice to infection and the nature of the immune response in the brain.

Proposed One-Year and Long-Term Outcomes:

1. Compile data from each of the three aims to determine if they suggest a role for infection in the development of AD. Draft and submit 1-2 publications for peer review based on this data. The data will be publishable regardless of whether it supports or fails to support the idea that infection is involved in AD. The information will be important to the field in either case.
2. Use the data to construct an R15 or R21 proposal to the NIH by the end of 2017 (October or November submission deadlines).

3. Utilize the outcomes of this study to design additional studies to further explore the mechanisms which initiate and drive amyloid plaque and neurofibrillary tangle pathology. During the course of the current studies, new potential mechanisms have been identified and will be examined further.
4. Continue the analysis of the potential role of viral infection in AD by expanding the DNA analysis to include additional common viruses such as HBV, CMV and EBV.

Year End Progress Summary:

Aim 1: This aim proved more challenging than initially anticipated due to the unexpected finding that commercially available DNA extraction kits are contaminated with low levels of microbial DNA (confirmed by the companies themselves). A kit from MoBio was ultimately settled on as being the least contaminated (this company supplies the kits for the Human Microbiome Project). In collaboration with MoBio, we ultimately developed a modified method of DNA extraction from brain tissue that would be expected to lyse the cell walls, capsids, or envelopes of nearly any type of microbe that might be present within the tissue. Analysis for the presence of HSV-1 DNA has been completed and was largely negative. Due to the inherent microbial DNA contamination in extraction kits, particularly in regards to bacterial DNA, we then worked with the Arizona State University Microbiome Laboratory to develop an alternate analysis method for bacterial DNA based on creating libraries of 16s rRNA genes present in the extracted brain tissue samples. These are currently undergoing final sequencing at ASU, so the results are pending. The analysis for fungal DNA is currently being performed at Midwestern with the ASU DNA Lab to complete the sequencing of PCR products for the identification of specific fungi.

Aim 2: Our effort with regard to Aim 2 of the project has focused on identifying suitable stains and antibodies against candidate pathogens, optimizing protocols for stains and immunohistochemistry using positive control tissue and cultures, and applying these histological methods to our four groups of human brain tissue samples.

After trying a variety of antibodies from various suppliers, we have identified well-performing antibodies against several pathogens that are candidates for infectious agents associated with AD. These include antibodies against *Candida* (cross-reactive with other fungi), *Borrelia* (cross-reactive with other spirochete bacteria), *Mycobacterium* (cross-reactive with a variety of bacteria), and HSV-1. We have optimized the protocols for these antibodies, and have tested them successfully on positive controls. In coordination with our core histology facility, we are also processing human samples with a variety of traditional pathology stains. These stains include Acid Fast Bacteria, Brown & Brenn Modified bacterial stain, Periodic acid-Schiff stain for fungi, Grocott's methenamine silver stain for fungi, and the Warthin-Starry stain for spirochete bacteria.

Portions of each human sample have been fixed, paraffin-embedded, sectioned, and mounted on charged slides. We are currently running immunohistochemistry using antibodies against *Candida*, *Borrelia*, *Mycobacterium*, and HSV-1. Our core histology facility is continuing to perform the pathology stains. Additionally, we will be using portions of each human sample for optical clearing combined with immunofluorescence, which will allow us to examine larger samples and therefore improve the probability of detecting a pathogen if one is present.

Aim 3: The goal of this aim is to assess the ability of 3xTg-AD mice to clear *C. albicans* infection from the brain and various peripheral tissues. We hypothesized that 3xTg mice would exhibit impaired clearance of the candida and as a consequence, would exhibit a greater fungal load and advanced AD pathology compared to age-matched controls. Since this project began we have made significant progress towards completing the experiments proposed in Aim 3. We proposed to examine the effects of *Candida* inoculation in both young adult and aged mice derived from a colony maintained at MWU. We have completed the inoculation and tissue collection phase for the young adult cohorts (6 mos of age; n = 100) and plan to inoculate the aged mice beginning in June once they reach their target age (12 mos of age). This factor represents a time limitation within this study. We have begun the initial tissue processing and preparation for both the determination of fungal burden and the effects of candida infection on AD pathology (amyloid and tau disposition). Initial observations and results appear promising and are detailed below.

Results: The mortality rate for inoculated 3xTg mice sacrificed 14 dpi was 25% (3/12) compared to 0% for C57 control mice. At both 7 and 14 days post-inoculation, there was a significant increase in the mean splenic weight in 3xTg mice compared to control mice at the same time post-inoculation ($p < 0.0001$). Interestingly, spleen weights in male 3xTg mice were 1.5-2-fold greater than observed in female 3xTg mice while spleen weight was comparable between male and female control mice. The effects of *C. albicans* infection on the liver were similar to those observed in the spleen, although were generally less pronounced. For the liver, there was a significant effect of group, indicating that the livers of 3xTg mice weighed significantly more than those of control mice at 7 and 14 days dpi ($p = 0.009$). There was also a significant effect of time post-inoculation which indicated that the liver weights at 14 days dpi were significantly greater in the 3xTg mice than at 7 days dpi. This was not observed in control mice. There was no difference in the weight of kidney or brain across groups, or across time post-inoculation.

Project Progress Reports
Translational Genomics Research Institute

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Use of extracellular vesicle isolation to increase sensitivity for detection of circulating RNA signatures of neurodegenerative disease. Kendall Van Keuren-Jensen, PhD. Translational Genomics Research Institute; Arizona Alzheimer's Consortium.

Project description: The goal of this proposal is to develop pre-analytical methods to increase the sensitivity of extracellular RNA (exRNA) measurements and reduce variability across collection sites and samples. We will do this by examining pre-analytical protocols for enriching extracellular vesicles, prior to exRNA and protein measurement, that could increase detection of protein differences between patients with neurodegenerative disease and healthy controls. Extracellular vesicles are believed to contribute to disease progression by spreading of toxic seed proteins to neighboring cells. Extracellular vesicles are postulated to be a more consistent source for biomarker signals than cell-free biofluids. The mechanisms by which extracellular molecular cargos make their way from the central nervous system (CNS) into the periphery is still unknown, however there is evidence that disease-related information is transferred from the CNS to the periphery. The biofluids that we will consider for this proposal are both cerebrospinal fluid (CSF) and plasma

There have been no systematic comparisons of vesicle isolation in CSF, and none with the goal to see which fraction and isolation method enriches for specific RNA cargo. We will examine several different methods for the enrichment of extracellular vesicles, exRNA and proteins from CSF and plasma. The results of the proposed experiments will allow us to evaluate the pre-analytical processing steps, and the impact on downstream measurements, to advance the development of exRNAs as sensitive and specific biomarkers of neurodegenerative disease.

Budget Justifications:

Salary support: We require time from a FTE to complete the project goals. (\$10,000)

Bioinformatician to do data analysis. (\$5,000).

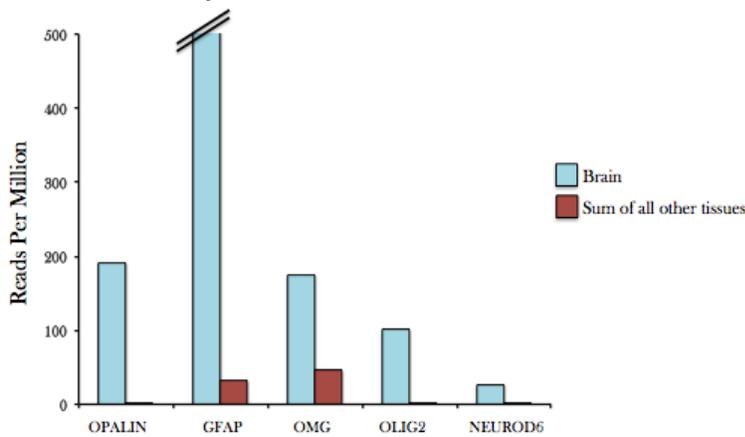
Dr. Van Keuren-Jensen will oversee the work of the FTE and help trouble-shoot any problems associated with the projects (\$5,000).

Supplies: We request money for supplies for isolation techniques, protein detection, RNA isolation, and sample preparation for sequencing. We also request sequencing supplies and bioinformatics analysis of the sequenced data. (\$45,000).

Year end progress summary: Using the Human Protein Atlas, we calculated the top five genes having the highest brain-tissue specific expression: OPALIN, GFAP, OMG, OLIG2, and NEUROD6. Figure 1 displays the relative expression of each gene in the brain compared with all other tissues in the body combined. We want to investigate the presence of these 'brain-enriched' RNAs in biofluids. We can use these RNAs as tools to assess the success of methods that enrich for brain-related information – in the biofluids. We examined the expression of these transcripts in four different sample types; CSF, plasma, urine and saliva.

Table 1 displays our findings from RNASeq. CSF has, as you might guess, the highest expression of these brain-specific transcripts. Surprisingly, for several transcripts, plasma and urine samples have just as many reads per million as CSF (OLIG2).

Figure 1. Data for the top five genes that have the highest level of enriched expression in the brain compared with all other tissues in the body combined.



RNA in CSF samples. We took each cerebrospinal fluid sample and split it in two. From one sample we isolated total cell-free RNA, from the second sample, we isolated RNA from extracellular vesicles. As you can see, there is a difference in the small RNA profiles based on isolation. We will continue to assess different isolation methods and the resulting RNA profiles. Our goal is to identify isolation protocols that have reduced variability and high enrichment for brain-related RNAs.

We examined several vesicle isolation protocols using pools of plasma, CSF and urine and comparing different methods. One method that we found to have low variability and good separation of vesicles from the biofluid, is the Qiagen exoRNeasy kit. This kit isolates vesicles on a lipophilic membrane and lets proteins and other small particles fall through the filter to be discarded.

As an example, in Figure 2, we examined the extracellular

Table 1. The expression of each gene is displayed as reads per million (RPM). The RPM for each gene in brain tissue, all other tissues, CSF, plasma, urine, and saliva are displayed.

Gene	brain	all tissues	Pediatric CSF	SAH CSF	SAH plasma	NHC plasma	NHC urine	NHC saliva
OPALIN	192	0	13	17	7	14	22	9
GFAP	4444	32	1206	509	22	31	34	17
OMG	174	46	11	10	4	5	10	5
OLIG2	101	2	19	10	6	15	18	6
NEUROD6	26	0	9	9	5	5	9	3

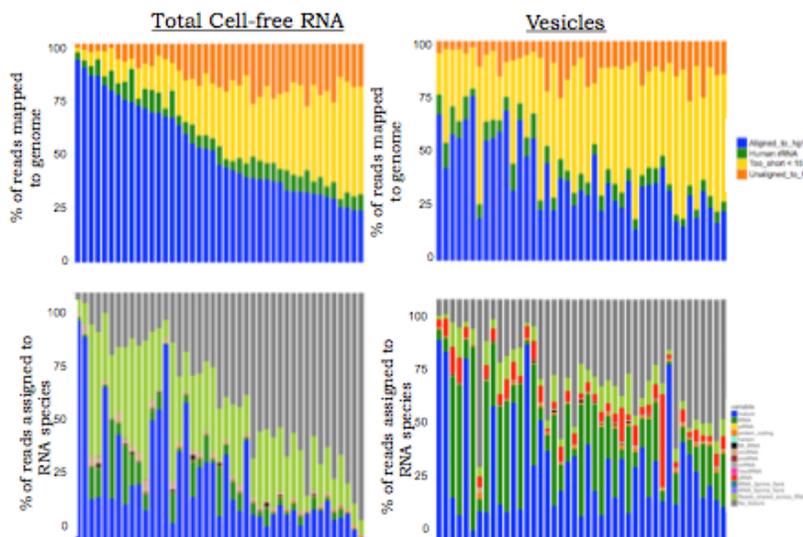


Figure 2. RNA was isolated from CSF samples using one of two kits. miRVana PARIS isolates total RNA (left) or exoRNeasy, which isolates RNA from extracellular vesicles (right). The top two plots display the distribution of reads mapping to the genome for CSF samples. The bottom two graphs represent different RNA biotypes present in each sample.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Single cell transcriptome sequencing to identify age-related changes. Matthew J. Huentelman, PhD, Ryan Richholt, BS, Chris Balak, BS, Ashley L. Siniard, BS, Matt D. DeBoth, BS. Translational Genomics Research Institute; Arizona Alzheimer's Consortium.

Project description: Emerging technology permits a cell-by-cell investigation of the transcriptome using next generation sequencing technology. This ability allows the user to essentially perform an RNA molecular "census" of any selected tissue using an unbiased approach. The end result of such an experimental design will be the detailed identification and quantitation of the cell identities in a given biospecimen and how they change across different experimental states.

Historically the ability to conduct single cell unbiased transcriptional profiling was too expensive and labor intensive to be performed on more than a few dozen cells at most therefore our molecular-based understanding of the census for various brain regions and how those may change in response to health and disease is extremely limited. Of particular interest is the state of normal aging and its influence on how the brain functions and what underlying cellular changes may be present. In lack of large amounts of cell death and morphological changes, we propose that the ages associated with normal aging are significant at the transcriptional level and thereby essentially "change" the cellular census of a given brain region in response to aging thereby manifesting in age-related changes at the network and behavior level. In this proposal we aim to take the first steps to investigate potential age-related changes on the cellular census of the hippocampus – a particularly vulnerable region to normal aging changes.

We propose to first optimize sample preparation from brain tissue for use in single cell transcriptome sequencing on the 10X Genomics infrastructure. Optimization of this will include the dissociation of brain tissue into a single cell suspension and subsequent single cell sequencing. After optimization of this step we will compare the hippocampus from Fisher 344 rats at nine and twenty months of age. This comparison will allow us to look for normal age related changes. Additionally by examining the entire hippocampal formation we are powered to detect changes throughout the structure which we can then localize to the individual sub-region that may be associated with each respective change.

Progress Summary: We decided to start with an ideal sample type – peripheral blood mononuclear cells (PBMCs) – to optimize the single cell sequencing approach from sample prep through analysis. In order to examine our ability to utilize the approach to detect a "condition" we decided to utilize aerobic exercise as our stimulus. We were able to isolate whole blood before and after exercise, purify the PBMCs, and successfully sequence them using the 10X Genomics chemistry. We sequenced approximately 1000 cells from each sample and within each cell we detected and counted approximately 750 genes. This number of genes – while not representative of the entire expressed transcriptome – is still demonstrably more than enough required to classify cells into their various molecular categories. To demonstrate this is true we utilize the sequencing data to classify cells in both a supervised and unsupervised manner. Using

this approach we can identify all of the known sub-classes of PBMCs. We can also identify several potential sub-classes as well as contaminating cell types (like platelets). Finally, we show that aerobic exercise can shift the composition of blood – increasing some cell types and decreasing others. Taken together this demonstrates that the single cell sequencing approach is viable on a well characterized single cell suspension, it can be used to classify cells in the sample, and can detect a change in a sample following a physiological exposure (in our case, a single aerobic exercise bout).

Next we moved on to brain tissue and attempted to create usable single cell suspensions from freshly collected and frozen brain tissue. We attempted multiple types of tissue dissociation approaches including enzymatic-based, mechanical, and ultrasonic. Unfortunately none of the approaches yielded a repeatable, well characterized single cell suspension.

Discussion and Future Plans: We are motivated by the results from the PBMC experiments. It is clear that the main stumbling block for this single cell sequencing approach will be the ability to reproducibly create a single cell suspension from brain tissue. We plan to vigorously explore this during the coming months. Additionally, it is likely much easier to make and utilize a nuclear suspension for sequencing. While the sequencing of the nucleus is possible, it will not yield the entire transcriptional contents of the cell. However, this approach may still yield enough data for cell type classification. We plan to explore nuclear-based sequencing as well using the 10X Genomics platform as an alternative to single cell sequencing – since the creation of single nuclear suspensions will likely be easier and more reproducible.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Characterization of astrocytic circular RNAs in late-onset Alzheimer's disease brains. Winnie S. Liang, PhD, Diego Mastroeni, PhD, Shobana Sekar, MS, Lori Cuyugan, MS, Philip Geiger, MS, Jonathan Adkins, BS, Jacquelyn McDonald, BS. Translational Genomics Research Institute, Arizona State University, Arizona Alzheimer's Consortium.

Specific Aim: To characterize circular RNAs (circRNAs) in astrocytes from different brain regions of late-onset Alzheimer's disease (LOAD) subjects and healthy elderly controls.

Background and significance: In previous work, we investigated the transcriptomes of astrocytes from the posterior cingulate (PC) of LOAD patients in order to understand if processes may be dysregulated in these cells in the context of AD pathogenesis. To continue these analyses, in this study we sought to characterize a non-coding member of the whole transcriptome, circRNAs. CircRNAs are a class of endogenous, non-coding RNAs that form covalently closed continuous loops and are pervasively expressed in the eukaryotic transcriptome. Although circRNAs have been found to possess potential microRNA regulatory roles, they have not been widely characterized in the context of diseases and their relevance in pathological processes is not well understood. Recent studies have reported the existence of preferential back-splicing in neural genes and an abundance of circRNAs in the mammalian brain. Given these findings, the goal of our study is to characterize circRNAs in 3 different regions of Alzheimer's disease brains and healthy control brains.

Year-end progress summary: Postmortem brain samples were collected at the Banner Sun Health Research Institute's Brain and Body Donation Program from 16 clinically classified late-onset AD subjects (2 APOE ϵ 3/3 subjects, 5 APOE ϵ 4/4 subjects and 9 APOE ϵ 3/4 subjects) and 16 no disease (ND) healthy elderly controls (1 APOE ϵ 2/3 subject, 4 APOE ϵ 3/3 subjects, 5 APOE ϵ 4/4 subjects and 6 APOE ϵ 3/4 subjects). To identify and characterize circRNAs, we used

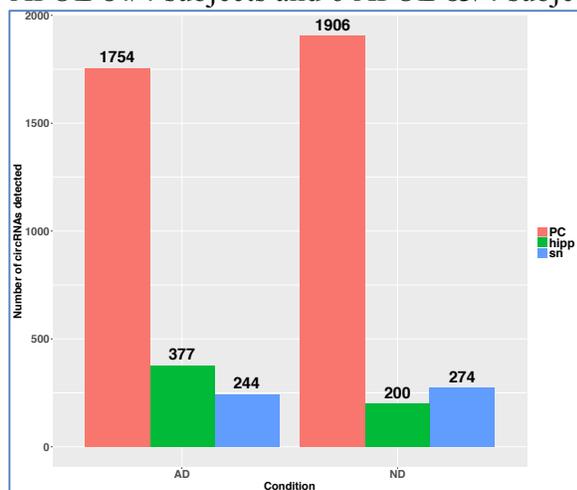


Figure 1: CircRNAs detected in PC, HIPP, and SN regions

data from RNA sequencing (RNAseq) of astrocyte total RNA extracted from the PC, hippocampus (HIPP) and substantia nigra (SN) of these subjects. Raw sequencing data in the form of basecall files (BCLs) were converted to FASTQ format and down-sampled to the exact same number of reads (40,279,128). One LOAD HIPP sample that did not have enough reads was thus dropped from further analysis. For the remaining down-sampled FASTQs, we ran 6 different circRNA prediction algorithms - CIRCexplorer, CIRI, DCC, find_circ Mapslice and KNIFE, using optimized parameter settings as recommended by the tools' authors. Predicted circRNAs from each sample with at least 2 supporting reads were used for further

downstream processing and analyses.

In total, 2,375 and 2,380 circRNAs were predicted across all tools in the AD and ND astrocyte samples respectively (union across all tools; Figure 1). One caveat associated with these analyses is that different markers were used for laser capture microdissection of astrocytes in the PC (ALDH1L1) and in the HIPP and SN (GFAP). As GFAP preferentially mark reactive astrocytes and ALDH1L1 marks both non-reactive and reactive astrocytes, this difference may be a key driver in the dramatic difference in circRNAs detected.

In the PC, 62 and 73 circRNAs were predicted by all 6 tools in AD and ND samples respectively. The most highly expressed circRNAs in the PC are derived from genes including mannosidase alpha class 1A member 2 (*MANIA2*), bromodomain adjacent to zinc finger domain 1B (*BAZ1B*) and insulin like growth factor 2 receptor (*IGF2R*). *IGF2R* encodes the receptor for insulin like growth factor 2, and overexpression of this receptor can lead to an increase in the levels of amyloid precursor protein. The circular form of gene cerebellar degeneration-related protein 1 (*CDRI*), a widely reported circRNA, was detected in both AD and ND PC samples with an average of 48 supporting reads by 3 out of the 6 tools. *CDRI* has been found to be overexpressed in the peripheral blood leukocytes of AD subjects, and the protein was identified in patients with paraneoplastic cerebellar degeneration (OMIM302650).

In the HIPP, 35 and 15 circRNAs were predicted by all 6 tools in AD and ND samples respectively. Genes/transcripts from which some of the most highly expressed circRNAs in this group are derived from include long intergenic non-protein coding RNA 1322 (*LINC01322*), Fas apoptotic inhibitory molecule 2 (*FAIM2*) and *CDRI*. *FAIM2* protects the neurons from death receptor induced apoptosis and is involved in neurite outgrowth and neuronal plasticity. This gene confers neuronal protection in mouse models of ischemia and is regulated during the course of bacterial meningitis.

In the SN, 15 and 20 circRNAs were predicted by all 6 tools in AD and ND samples respectively. The most highly expressed circRNAs in this region arise from genes including *MANIA2*, bromodomain PHD finger transcription factor (*BPTF*) and *CDRI*. In the brains of Alzheimer disease patients, the BPTF protein is localized in a subset of amyloid-containing plaques and its expression was found to be higher in neurodegenerative diseases. Though the relevance of circRNAs in the context of AD is not well understood, we observe that certain key genes give rise to circRNAs to suggest that they could have a potential role in various cellular processes in AD.

As we continue data analyses, we are also optimizing our wet bench and informatics workflows for circRNA detection and characterization. For our next set of analyses, we have procured brain tissue from 5 different brain regions of 6 healthy ND subjects, as well as total RNA from 4 different organs of 6 healthy subjects. For these samples, we will perform a ribosomal RNA depletion step, as well as selective enrichment of circRNAs using Ribonuclease R, an exoribonuclease that digests the majority of linear RNAs but leaves behind lariat or circRNA structures. These 2 additional steps have been used by several recent studies to successfully enrich and identify circRNAs.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Characterization of mitochondrial DNA in late-onset Alzheimer's disease. Winnie S. Liang, PhD, Shobana Sekar, MS, Jonathan Adkins, BS. Translational Genomics Research Institute, Arizona Alzheimer's Consortium.

Specific Aim: To characterize sequence changes in mitochondrial DNA in the posterior cingulate (PC) in late-onset Alzheimer's disease (AD) subjects and healthy elderly controls.

Background and significance: Along with well-established histological and pathological markers, AD is also characterized by energy metabolism deficits in the brain. In particular, the PC is a region that displays early characteristic cerebral metabolic rate for glucose (CMRgl) deficits in AD patients as well as in healthy individuals who carry the APOE ϵ 4 allele, the most well-established susceptibility gene for late-onset AD. However, the molecular basis for these changes remains a mystery. Shedding light into this is key to identifying efficacious therapeutic approaches to support earlier interventions for AD. Given the primary role of mitochondria in energy generation and metabolism, our goal is to characterize sequence changes in the mitochondrial (mt) genome in the PC of AD subjects.

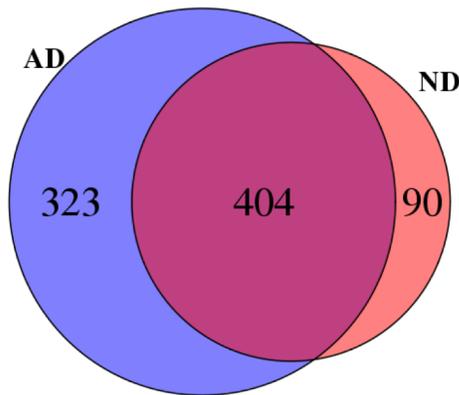
Preliminary Data: In previous transcriptomic studies, we identified dysregulated expression of mt genes in the PC. In non-tangle bearing PC neurons, we first identified widespread decreased expression of 80 nuclear-encoded electron transport and mt translocase genes using expression profiling [1]. We further evaluated PC astrocytes using RNAseq (RNA sequencing) and identified dysregulated expression of immune pathway genes, along with mitochondria-relevant genes including *MT-COI* (mitochondrially encoded cytochrome c oxidase I), *MICU1* (mitochondrial calcium uptake 1), *SLC25A37* (solute carrier family 25, mitochondrial iron transporter, member 37), *TIMM50* (translocase of the inner mitochondrial membrane 50 homolog), *MUL1* (mitochondrial E3 ubiquitin protein ligase 1), *TOMM70A* (translocase of the outer mitochondrial membrane 70), and *MTRF1L* (mitochondrial fission regulator 1-like) [2]. Our previous characterization studies thus provide evidence of a potential role of neuronal and astrocytic mitochondria in characteristic energy metabolism deficits in the PC of AD subjects.

Year-end progress summary: To identify and characterize changes in mtDNA, we performed mtDNA sequencing of the PC in subjects for whom we previously performed astrocyte RNAseq. These subjects include 16 late-onset Alzheimer's disease subjects (eight APOE ϵ 3/ ϵ 3 and eight APOE ϵ 3/ ϵ 4) and 8 healthy elderly controls (ND; four APOE ϵ 3/ ϵ 3 and four APOE ϵ 3/ ϵ 4). This approach entails performing DNA extractions from tissue samples using Qiagen's QIAamp DNA Mini Kit and performing mt enrichment during library construction using Nugen's Targeted Enrichment Mitochondrial Kit, which contains 192 probes that targets the entire mt genome.

From our mtDNA analysis of the PC from 16 AD subjects and 8 ND subjects we generated libraries, performed sequencing on the Illumina MiSeq using 140x14bp reads, and outlined an analytical workflow for analysis and annotation of results by integrating multiple tools. Overall,

we generated 11,906,834 reads with a median mapped coverage of 602.6X following removal of PCR duplicates. Following analysis of sequencing metrics, two subjects were dropped from further analysis (one AD [APOE e3/e4] and one ND [APOE e3/e4]) due to low coverage (<10X). Analysis of the remaining twenty two samples led to the identification of 817 heteroplasmic sites across all samples with 323 sites occurring uniquely in at least one AD PC sample, and 90 sites occurring uniquely in at least one ND sample (Figure 1). Overall, in AD-only heteroplasmic sites, a total of 514 events were detected, and in only ND-only heteroplasmic sites, 102 total events were detected.

Figure 1: Number of heteroplasmic sites identified in PC mtDNA



Data analysis and review is currently ongoing. Notably, AD PC samples have a higher number of heteroplasmic sites compared to ND PC samples. In heteroplasmic sites identified in at least one AD sample, and no ND samples, mt genes *CYTB* (cytochrome B) and *ND5* (NADH dehydrogenase 4) demonstrated the highest level of heteroplasmy with 329 and 513 sites, respectively. *CYTB* encodes a component of complex III in the electron transport chain and *ND5* encodes a component of complex I. While the functional impact of base changes in these changes is unclear, due to the genes' central roles in mitochondria function, these heteroplasmic events

may potentially impact ATP production. In sites identified in at least one ND sample, and no AD samples, the region encoding tRNA-Pro, *CYTB*, and tRNA-Thr demonstrated the highest level of heteroplasmy with 15 sites, a much lower total compared to AD samples. Furthermore, heteroplasmic sites in the AD samples also impacted a higher number of non-coding tRNA regions in the mt genome compared to ND samples. These regions that were only impacted in AD sample(s) encode tRNA-Arg, tRNA-Asp, tRNA-Ile, tRNA-Phe, and tRNA-Pro, and tRNA-Trp. tRNAs are known to be more susceptible to base substitutions and such alterations have been described in mitochondrial dysfunction.

We, however, did not detect any heteroplasmic sites occurring across all AD only samples, or all ND only samples. For heteroplasmic sites that occur only in at least one AD sample, and no ND samples, we observed two sites that were detected by both Mitocall and Platypus. These sites occur at bp position 14766 in mtDNA (occurring in three AD samples) and position 1811 (occurring in four AD samples). At position 14766, which falls within the *MT-CYTB* gene, a non-synonymous C>T alteration was observed in the three AD samples (two of which are APOE e3/e4 and the third being APOE e3/e3). At position 1811, which falls within the *MT-RNR2* gene which encodes 16S RNA, an A>G alteration was observed in the four AD samples, all of which were APOE e3/e4.

While we continue analyses and data review, we will also expand our analyses. In order to perform a more comprehensive analysis of mitochondrial-relevant changes, we worked with Nugen to construct a custom enrichment mitochondrial enrichment bait set that contains all 192 probes that target the mitochondrial DNA (mtDNA), along with probes that target the 360

nuclear-encoded mitochondrial genes that encode proteins that localize to the mitochondria. This bait set includes 11,961 total probes and we received this custom kit in mid-January 2017. We will be analyzing the same twenty four PC samples using this kit to both validate our primary mtDNA analyses, as well as to determine if any sequence changes in nuclear-encoded mitochondrial genes segregate with disease status or APOE genotype.

Lastly, from our previous ADCC pilot study, we performed mtDNA sequencing from serum samples collected from AD subjects and healthy elderly controls. Because sample collection was not controlled for, and resulted in the collection of low quality samples demonstrating elevated levels of hemolysis, we are currently identifying additional AD and control samples that we can use for circulating mtDNA analyses.

References:

- [1] Liang WS, Reiman EM, Valla J, Dunckley T, Beach TG, Grover A, Niedzielko TL, Schneider LE, Mastroeni D, Caselli R, Kukull W, Morris JC, Hulette CM, Schmechel D, Roger J, Stephan DA (2008). Alzheimer's Disease is associated with reduced expression of energy metabolism genes in posterior cingulate neurons. *Proc Natl Acad Sci U S A* 105(11): 4441-6.
- [2] Sekar S, McDonald J, Cuyugan L, Aldrich J, Kurdoglu A, Adkins J, Serrano G, Beach TG, Craig DW, Valla J, Reiman EM, Liang WS (2015) Alzheimer's disease is associated with altered expression of genes involved in immune response and mitochondrial processes in astrocytes. *Neurobiology of Aging* 36(2):583-91.

Project Progress Reports

University of Arizona

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Patient recruitment and outreach for Alzheimer's Disease and related-disorders. Geoffrey Ahern, MD, PhD, Steven Rapcsak, MD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims: This proposal requests complementary support to enhance ongoing efforts for participant recruitment and outreach efforts as part of the UA site of the Arizona Alzheimer's Disease Center (ADC). The Arizona ADC is part of a multi-institutional state-wide consortium that links together the major research institutions in Arizona to advance efforts in the early detection, tracking of progression, and evaluation of treatments and prevention therapies for Alzheimer's disease (AD) and related disorders. As part of the Clinical Core of the Arizona ADC, Drs. Ahern and Rapcsak lead efforts in the participant recruitment for patients with AD, mild cognitive impairment (MCI), and healthy elderly controls in the Tucson-metro area. In addition, they have been actively involved in the recruitment and clinical assessment of patients with other less common forms of dementia afflicting the elderly, including frontotemporal lobar dementia spectrum disorders and the occurrence of AD dementia with an early age-at-onset.

This proposal will support the following primary specific aims:

AIM 1) to recruit, enroll, and evaluate patients with dementia, cognitive impairment, and healthy controls for inclusion in the Arizona ADC;

AIM 2) to support Arizona ADC outreach efforts, providing the Tucson-metro area community with educational information on AD and related disorders and the opportunity to participate in related research, including clinical trials.

Background and Significance: The older adult population is expected to grow rapidly over the next two decades. In the United States, the number of elderly persons will reach over 70 million (US Census Bureau, 16), and public health programs will increasingly need to respond to this escalating growth. Associated with the dramatic increase in the elderly will be an increase in the occurrence of AD and associated cognitive decline. It will be essential to identify new effective treatments and prevention therapies to address the increasing needs of elderly adults with increased risk for dementia. The Arizona Alzheimer's Consortium is a state-wide, multi-institutional research center focused on advancing research to enhance early detection, tracking of disease progression, and evaluating potential treatments for AD. As investigators in the Clinical Core of the Arizona ADC since its inception, Drs. Ahern and Rapcsak have been actively engaged in research to advance understanding of the clinical effects of AD and other age-related neurodegenerative diseases as part of the Arizona Alzheimer's Consortium [see Literature Cited for selected recent publications (1-15,17)]. Geoffrey Ahern, M.D., Ph.D., holds the Bruce and Lorraine Cumming Endowed Chair in Alzheimer's Research and is Professor of Neurology, Psychology, and Psychiatry at the University of Arizona. Steven Rapcsak, M.D. is Professor in the Departments of Neurology, Psychology, and Speech, Hearing, and Language Pathology at the University of Arizona.

Proposed One-Year and Long-Term Outcomes: The primary one year outcomes for this project include increasing the number of new participants enrolled in the Clinical Core of the Arizona ADC as well as to continue to follow currently enrolled participants on a yearly basis to characterize and track changes in cognitive functions and behavior. In addition, we plan to continue and expand our participation in outreach efforts to support our ongoing patient recruitment goals and to provide information to the Tucson-metro area community concerning current research efforts on AD, dementia, and age-related cognitive decline. For example, Dr. Ahern provided a presentation on new directions in the treatment and prevention of AD at the 2nd Annual Conference on Successful Aging (ACoSA), a conference developed and organized by Drs. Alexander and Ryan, collaborating Arizona AAC investigators at the University of Arizona, to provide the most up to date information on aging and the risk for AD to community members in the Tucson-metro area. The focus of this past year's ACoSA meeting was Successful Aging: Reducing your Risk for Alzheimer's disease, and planning for the next conference is underway. Similarly, Dr. Rapcsak has given several lectures on Alzheimer's disease and related dementias at various community centers in the Tucson area.

Year End Progress Report: We have met our recruitment goals by enrolling new study participants from the following diagnostic categories: AD, MCI, frontotemporal dementia (FTD), Parkinson's disease (PD) and normal controls with family history of AD. We have several individuals on the waiting list to join the study. We have continued with our outreach efforts to neurological colleagues, including the Neuromuscular Division at the University of Arizona in order to recruit individuals with FTD/ALS and the Movement Disorders Group to recruit individuals with PD/Lewy Body Dementia. Dr. Rapcsak has given several lectures on the topic of AD and dementia, including presentations at community events, as well as at conferences attended by medical professionals. Dr. Ahern worked with Stephanie Innes of the Arizona Daily Star on her multipart series, "The Hardest Test" (February 2016), which documented the challenges presented to a particular family in Tucson, in whom all three children in one generation suffer from Presenilin 1 Early Onset Alzheimer's Disease. (Of note, all three siblings participate(d) in the ADCC).

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Risk factors for brain aging & preclinical Alzheimer's Disease. Gene Alexander, PhD, G. Alex Hishaw, MD, Matthew Huentelman, PhD, Yann Klimentidis, PhD, David Raichlen, PhD, Judith Su, PhD, Ted Trouard, PhD. University of Arizona, Translational Genomics Research Institute, Arizona Alzheimer's Consortium.

Specific Aims: This proposal requests support to conduct a multi-disciplinary research project with the goal of advancing our understanding of how common health-related factors in the elderly impact brain aging and the preclinical risk for Alzheimer's disease (AD). To accomplish this goal, we have a multi-disciplinary collaborative team of Arizona Alzheimer's Consortium (AAC) investigators, including researchers in the fields of neuropsychology, neurology, neuroimaging, neuroscience, chemistry and optical sciences, genetics, statistics and public health, biomedical engineering, and biological anthropology. This hypothesis-driven, research proposal will use "state-of-the-art" methods for testing human cognition, imaging of brain structure, function, and connectivity, genetics, and behavioral measures of lifestyle, physical activity, and sleep quality. This integrative approach will support efforts to investigate health-related factors, including hypertension and cerebrovascular risk, exercise/physical activity, and sleep quality on the neural systems supporting cognitive function during aging and their impact on the preclinical risk for AD. In addition, this work will explore the development of new methods for early detection of blood-based biomarkers for AD and cognitive decline. Our overall hypothesis is that the common health risk factors of hypertension and genetic risk for AD, as well as the beneficial effects of exercise/physical activity and sleep influence brain aging and the preclinical risk for AD by altering the structure and function of brain networks important for cognitive processes that depend on frontal and temporal brain regions and the integrity of connecting white matter.

In our proposed study, we plan to address the following primary specific aims: 1) to investigate how the health factors of hypertension, genetic risk for AD, exercise/physical activity, and sleep quality affect brain structure, function, and connectivity in the elderly and 2) to determine how hypertension, genetic risk for AD, exercise/physical activity, and sleep quality influence cognitive performance on measures sensitive to the early effects of cognitive aging and preclinical AD (i.e., memory, executive function, and processing speed). Additional Goals: This study will provide substantial added value by 1) acquiring a battery of cognitive and neuroimaging measures to advance new multi-modal analysis methods to detect the earliest effects of preclinical AD, 2) exploring how genetic variation related to risk for AD and cognitive decline influence brain aging and cognitive performance in the elderly, 3) developing and submitting new external collaborative grant proposals on brain aging and preclinical AD, and 4) supporting community outreach and recruitment with our Annual Conference on Successful Aging (ACoSA) and Southern Arizona Healthy Aging Registry (SAHAR).

Background and Significance: The population of older adults is expected to grow rapidly over the next two decades and public health programs will increasingly need to respond to this escalating growth. Associated with this increase in the elderly will be an increase in Alzheimer's dementia and associated cognitive decline. One important and highly prevalent health risk factor

for the development of cognitive decline in the elderly is hypertension. Hypertension is estimated to occur in almost two-thirds of those over the age of 60 and increases the risk for cerebrovascular disease and AD. It is well established that the risk for developing AD is greatly influenced by genetic factors that can increase the probability of developing dementia and cognitive decline over the lifespan. The most common susceptibility gene for late onset AD is apolipoprotein E with $\epsilon 4$ homozygosity conferring the greatest risk. There is, however, great need to evaluate the influence of less common genetic risk factors for AD and the ability to test and replicate findings in very large datasets is needed to address this critically important question. In contrast to these health risks, exercise may help mitigate or improve cognition and brain function during the lifespan. Studies have shown that aerobic exercise can improve cognition during aging and may reduce the risk of AD. In older individuals, high levels of physical activity are correlated with increased brain volume and functional connectivity needed for cognitive processing. Studies investigating cognitive functions and brain imaging in older adults are critically needed to determine the potential for exercise in supporting healthy brain aging. In addition, the importance of sleep quality is an emerging area that may reflect an important factor influencing healthy aging and the risk for AD. In addition, there is tremendous need to develop and test highly reliable and relatively non-invasive methods to aid early detection for age-related cognitive decline and AD. Current methods rely on the use of cerebrospinal fluid (CSF) markers to identify pathological indicators of risk for AD and there is great interest in the development of methods to identify biomarkers in blood for less invasive routine assessments. A new approach with the potential to robustly identify single molecules in fluid samples has been developed and has the potential for applications in identifying AD pathological biomarkers in both CSF and blood.

Preliminary Data: We previously reported patterns of MRI gray matter volume associated with healthy aging [Alexander et al., 2006, 2008; Bergfield et al., 2010] using a multivariate model of regional covariance, the scaled subprofile model (SSM) [Alexander and Moeller, 1994]. We found a pattern of gray matter related to APOE $\epsilon 4$ in young to early middle aged adults, suggesting longstanding brain morphological differences related to this genetic risk for AD [Alexander et al., 2012]. Preliminary results from a new automated white matter hyperintensity (WMH) lesion volume method implemented in Dr. Alexander's lab has shown that using multi-spectral processing for WMH probability maps provides an accurate and reliable approach for evaluating the effects of vascular risk factors, like hypertension, on the brain. Using Freesurfer to measure cortical volume, thickness, and area, we found a pattern of gray matter reduction associated with increasing age (Monte Carlo corrected, $p < 0.05$). Further, we recently found that, after controlling for age, greater regional gray matter volume and area, but not thickness was related to higher levels of aerobic fitness in healthy older adults (Monte Carlo corrected, $p < 0.05$). Together, these findings support the use of MRI to evaluate health and genetic risk factors for preclinical AD. In addition, we proposed a new hypothesis that was featured on the cover of Trends in Neurosciences, suggesting demands for physical activity supported the evolution of the human lifespan and healthy brain aging [Raichlen and Alexander, 2014], helping to show how exercise may prevent AD and cognitive aging.

Proposed One-Year and Long-Term Outcomes: The one-year outcomes for this project include the opportunity to identify new findings on the effects of health factors like hypertension and exercise/physical activity on cognition and predicting cognitive decline, as well as on brain

structure, function, and connectivity. In addition, this work will be leveraged to support complementary projects investigating genetic risk for AD and physical activity; as well as the effects of advanced aging on cognition and brain structure and function. Together, these studies reflect collaborations focused on developing externally funded grant proposals to investigate how cerebrovascular risk factors, differing levels of aerobic fitness, and advanced successful aging impact brain aging and the preclinical risk for AD. The proposed research will provide novel and rich datasets with which to publish findings that will advance our understanding of the brain changes associated with multiple health-related factors that may either enhance or diminish the risk for dementia and age-related cognitive decline. It is expected that this dataset will provide essential pilot data to support new applications for external funding to NIH, NSF, and other external funding sources planned for submission next year. Specifically, this project will provide key data and methodological developments to support pending and planned grant applications by the project investigators, including two NIH applications to investigate the effects of differences in exercise/physical activity and cognitive training on brain aging and cognitive function, as well as an NIH application on the use of novel optical science methods to detect AD biomarkers. This work will also lead to external grant proposals addressing how hypertension and other cerebrovascular risk factors interact with genetic risk for preclinical AD to affect brain aging and cognitive decline, to evaluate the interactive effects of genetic risk and physical activity on AD, and to evaluate the risk for AD in advanced elderly and what factors support successful aging. In addition, we plan to continue our ACoSA and SAHAR to provide for enhanced community outreach, education, and subject recruitment in support of our ongoing studies of brain aging and the preclinical risk for AD, as well as outreach efforts of the Arizona ADC.

Year End Progress Summary: In the past year, we have made significant progress in our studies on individual differences in risk factors and lifestyle characteristics for brain aging and preclinical AD. Analysis from our healthy aging cohort investigated the effects of hypertension status and white matter hyperintensity (WMH) volume on white matter integrity in older adults, 70 to 89 years of age. In this study, we found that WMH volume was related to decreased white matter tract integrity measures assessed by diffusion tensor magnetic resonance imaging (MRI), whereas hypertension status was not associated with tract integrity differences. Together, these findings suggest that WMH volume may be an important vascular risk factor that is separable from hypertension status in the context of healthy aging. Results from this work were presented at the Society for Neuroscience meeting (Nguyen et al., 2016). In addition, we investigated differences in regional white matter integrity in healthy aging by studying 81 healthy participants 50 to 89 years of age. For this study, we excluded participants with a history of hypertension or diabetes and found that mean diffusivity was a more sensitive measure of white matter integrity from MR diffusion imaging in aging than other commonly used markers, with the differences most prominent in the those 80 to 89 years of age. The preliminary results from this work were presented at the Society for Neuroscience meeting (Bharadwaj et al., 2016). The importance of investigating white matter in the context of aging and the risk for AD was emphasized in an invited editorial highlighting new findings that have shown a relation between white matter integrity and amyloid markers of AD pathology in healthy aging, which was published this year (Alexander, *JAMA Neurology*, 2017). We also tested the relation between self-reported physical activity and cognitive performance in a cohort of healthy older adults. This work showed that better performance on measures of executive function and to a lesser extent memory and

processing speed were associated with greater engagement in sport activities and this effect was not influenced by hypertension status. These findings were presented at the Society for Neuroscience meeting (Franchetti et al., 2016) and a manuscript of this work is in preparation. We have also investigated differences in functional connectivity with resting state MRI in a group of young adult endurance athletes compared to age-matched non-athlete controls. We found regional differences in connectivity between the groups that were related to both differences in estimates of physical activity in the groups, with greater connectivity in frontal cortex for the athletes. These findings were published (Raichlen et al., *Frontiers in Aging Neuroscience*, 2016) and received kudos in the press, with articles highlighting the findings in the New York Times and over 60 other news outlets. As a follow up to this work, we are conducting a study of older adult athletes and controls to evaluate differences in cognition, as well as brain function, structure, and connectivity, which is currently underway. The human hypertension studies have been extended to a translational research study with a transgenic rodent model of hypertension in a funded NIH R01 grant (Multiple PIs: Alexander, Barnes, Coleman). This five-year study with AAC collaborators uses “state of the art” epigenetics, cognitive measures, and neuroimaging methods to evaluate the effects of hypertension on the molecular status of brain regions affected by brain aging. A manuscript showing the first regional network gray matter covariance pattern in rodent brain MRI at 7.0T is about to be submitted (Alexander et al., in preparation), supporting human neuroimaging methods in translational studies with small animal models of aging and neurodegenerative disease.

Work from the current AAC study has also supported the ongoing development of a multi-site collaborative project funded by the McKnight Brain Research Foundation (Multiple PIs: Alexander, Cohen, Visscher, Wright) to study the effects of cognitive and brain function in generally healthy advanced older adults, ages 85 to 100+. This study is currently underway and reflects ongoing collaborations between the University of Arizona, the University of Florida, the University of Alabama, and the University of Miami. This work includes an expanded multi-site effort with an additional funded proposal to enhance the development and implementation of novel cognitive measures to assess cognitive decline in this advanced elderly cohort (Multiple PIs: Alexander, Cohen, Levin, Wadley). In addition, this multi-site collaborative work has led to a new five year \$5.7M NIA R01 grant that was funded this year to be conducted by the University of Florida, University of Miami, and the University of Arizona to evaluate ways to enhance the benefits of cognitive training in older adults (UA Field Center PI: Alexander; UA Co-Investigators: Allen, Hishaw, Trouard). This multi-center project will be the largest study to evaluate the use of transcranial direct current stimulation to enhance neuroplasticity during cognitive training in older adults and in relation to risk for AD.

During this past year, Drs. Alexander and Raichlen further developed and refined a novel exercise training method for applications in cognitive aging and the risk for AD. With complementary support from a Tech Launch Arizona Wheelhouse Proof of Concept grant (Multiple PIs: Alexander, Raichlen), we have launched an initial intervention trial, which is currently underway. Drs. Raichlen, Alexander, and Klimentidis have launched an effort to implement the acquisition of actigraphy to provide data on physical activity and sleep characteristics in our AAC studies. To support this effort, we have also initiated an analysis of a large actigraphy dataset to evaluate how individual differences in activity relate to age and mortality. We have plans to submit new NIH and NSF grant proposals to expand on our very encouraging findings with exercise and brain aging during the coming months.

A new collaboration with Dr. Judith Su in the Departments of Optical Sciences and Chemistry and Biochemistry at the University of Arizona is applying a novel technology to evaluate AD-related blood and cerebrospinal fluid biomarkers. This work is in collaboration with Dr. Tom Beach from the Sun Health Research Institute and the Arizona ADC Pathology Core, with a NIH R03 grant on this work currently pending (PI: Su; Co-Is: Alexander, Beach). Drs. Alexander and Ryan continued to lead the implementation of our Annual Conference on Successful Aging (ACoSA), providing members of the Tucson-metro area community with up to date information and new research findings on ways to enhance and support cognitive functions as we age. Our fifth annual conference occurred in February, 2017 with the topic focusing on how decision-making ability is altered as we age. The conference for this year was very successful and plans for our next year's

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Exploring the microbiome-gut-brain axis: impact of microbial communities and their genes on cognition in aging. Carol A. Barnes, PhD, John Konhilas, PhD, Lee Ryan, PhD, Betty Glisky, PhD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims: There are two Specific Aims of the current proposal. Aim 1 is to assess cecum bacterial diversity in young (6mo), middle-aged (15 mo) and old (23 mo) male, F344 rats who have been individually characterized on a large cognitive test battery, and have been assigned to one of three groups: "high functioning", "average functioning" and "low functioning" for each of the 3 ages examined. The results of this experiment will guide our hypotheses in the second aim. Aim 2 is to assess fecal samples from healthy older individuals in a cohort of cognitively-characterized individuals that Glisky and Ryan have followed longitudinally, and who have been determined to be either: a) high-functioning in both the temporal lobe and frontal lobe test batteries, 2) or low-functioning functioning in both the temporal lobe and frontal lobe test batteries.

Background and Significance: There is substantial variation in the impact of aging on cognition in healthy older adults, even those who are unlikely to go on to develop Alzheimer's or vascular dementias. In fact, there is substantial variation in the impact of aging on cognition even in genetically 'inbred' strains of rats whose environmental experience in terms of exposure to pathogens, nutritional and social environments are apparently very similar. This suggests that animal models will contribute important insights into the mechanisms underlying individual differences that result in successful rather than unsuccessful aging phenotypes across species.

Barnes is the PI of a funded RO1 that supports the conduct of an integrated set of experiments designed to assess mechanisms underlying differential cognitive trajectories observed over the lifespan of the F344 rat. These variables include cognitive test batteries over different domains, high resolution MRI imaging, behavior-driven single cell gene expression imaging and brain region- and cell-specific whole transcriptome analysis of brain regions relevant to the behaviors tested. The cognitive test battery used in the large rodent study is conducted to identify rats on the basis of possessing behavioral performance scores that are "high", "average" or "low" with respect to performance distributions from young adult, middle-aged and older adult rats. The behaviors include assessment of hippocampus-dependent spatial memory, motor behaviors, spatial search strategies, visual, general motivational factors, and simple and compound discrimination tests. Other tasks assess ventral visual stream/perirhinal cortical function, frontal cortex-dependent working memory, interference and temporal order tasks. The range of memory tested in these behavioral tasks are analogous to a number of the cognitive tests given in humans in Aim 2. Although not a 'funded' component of this grant, at the time we sacrifice our young, middle-aged and old rats (3 levels of cognitive aptitude in each age group), we also collect gut samples. Konhilas has trained us to dissect the gut into 7 distinct components, and to collect contents and epithelial scrapings from each of these levels for rapid freezing. We can extract the DNA, and Watts can perform the bacterial DNA isolation and 16S gene amplicon generation and

rRNA gene sequencing. Following this, Lussier will apply computational biology approaches that he has developed to these datasets.

Detailed analyses of the gastrointestinal (GI) tracts of these rats will allow us to determine whether different distributions of microbiota in the GI system are related to the way in which individual rats segregate along a cognitive competence continuum throughout life. The answers we obtain from these rats, using technologies that cannot be used in humans at present, will allow direct predictions to be made for our human studies. To begin to explore human cognition we propose to obtain stool samples from cognitively-characterized older individuals from the Glisky/Ryan cohort to begin to explore what characteristics of the microbiome may be advantageous in human populations, and to develop hypotheses about critical brain-immunological interactions for optimizing cognitive health throughout the lifespan.

Preliminary Data: We have obtained cognitive data in both the temporal lobe and frontal lobe domains in both the rats and human participants that will contribute to these studies.

Proposed One-Year and Long-Term Outcomes: The data collected in these two aims will give us preliminary data that we will use to request a supplement for funds to analyze the remaining data in the rodent study, and as preliminary data for an RO1 for a larger human experiment.

Year End Progress Summary: Over the past 2 years we have cognitively characterized and selected animals for inclusion in the high, average, and low cognitive performance groups (n= 29 young, n=28 middle-aged, and n= 25 old). We have also dissected 7 levels of the GI tract in each of these animals, and at each level we have taken a sample of content and a sample of epithelial scraping.

Over the past year we began our pilot analysis of these data, as outlined in **Table 1**. To maximize our ability to detect differences between age and cognitive aptitude (**Aim 1**), we selected rats from only the young and old categories, and only the high and low cognition categories. The pilot funds supported sequencing of a total of 24 animals with two tissue samples from each animal (cecum content and epithelial scraping), 12 in each age category, and within age, an n of 6 at high and low levels of cognitive performance.

COGNITIVE CATEGORY	6 MONTHS	23 MONTHS
HIGH	6	6
LOW	7	6
TOTAL	13	12

Table 1. Number of animals with DNA prepared and sequenced from cecum contents and epithelial scrapings, by age and cognitive category.

We sequenced half of the animals initially, to optimize our pipeline, determine thresholds, and overall approach to the analysis. The second half of the animals were sequenced in January, and our bioinformatician collaborators are analyzing these data now with respect to consistency of results between the first and second batches, and with respect to age and cognitive category. We hope to be able to have the first review of the entire pilot data set next month.

For **Aim 2** we have a target population of 20 older individuals who have been categorized as “high performing” (**H**) on a composite battery of frontal lobe-dependent executive function tasks (first letter) or on a battery of temporal lobe memory tasks (second letter), or as low functioning (**L**), as shown in **Table 2** below. Our original hope was to focus on **HH** (high frontal and temporal lobe function), and **LL** (low frontal and temporal lobe function); however, these individuals are somewhat rare. Thus, we have included people in mixed categories as well as indicated in **Table 2**. These individuals are all participants in a longitudinal study of the impact of age on frontal and medial temporal lobe function as assessed by a neuropsychological test battery that Glisky has developed. We received IRB approval for collecting stool samples and a questionnaire that each individual fills out that includes questions on overall health, well-being and diet (e.g., food type, frequency and serving sizes) using the University of Arizona Diet, Behavior and Quality of Life assessment tool. We are continuing to collect these samples.

COGNITIVE CATEGORY	COLLECTED	TARGET
HH/LH	7	10
LL/HL	6	10
TOTAL	13	20

Table 2. Number of study participants who have been cognitively categorized and have filled out the health and diet questionnaire and have had tissue collected. Target population for each cognitive group is also included.

Once the sequencing data are analyzed from the rat study, and all the samples are collected from our human pilot participants, we will make specific hypotheses about how best to target our sequencing approach with respect to bacterial species that may be related to cognition in this human sample.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

ApoE4: Accelerator of bioenergetic aging in female brain and risk of Alzheimer's Disease.
Roberta Diaz Brinton, PhD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims:

Aim 1: Mechanistic Discovery: Determine ApoE4 genotype regulation of the perimenopausal bioenergetic, inflammatory and lipid metabolism transitions in brain and their association with Alzheimer's pathology. Determine modulatory factors that reduce or exacerbate the impact of ApoE4 genotype.

Hypothesis: APOE4 allele accelerates and exacerbates bioenergetic transition in female brain to increase development of Alzheimer's pathology.

Aim 2: Clinical Translation: Determine impact of perimenopause and the APOE4 genotype on brain metabolism, burden of Alzheimer's pathology, structural integrity and cognitive function in perimenopausal and postmenopausal women. Extend analyses of ELITE cohort (600 postmenopausal women) to determine impact of ApoE4 genotype on metabolic cluster membership and longitudinal cognitive function.

Hypothesis: APOE4 genotype accelerates and magnifies perimenopausal bioenergetic brain aging evidenced by hypometabolism, beta amyloid deposition and loss in hippocampal volume. Further, these brain imaging markers will be predictive of cognitive impairment.

Aim 3: Global Population Translation: Determine the impact of the APOE4 genotype and menopause on the lifespan trajectories of 5000 women derived from 10,000+ MRI derived brain volume metrics, genetics and clinical data available through the ENIGMA network. 33,195 subjects (men and women) with MRIs and genomes will also be investigated using a meta-regression model for the effect of age, ApoE4 and other genotypes on significant brain atrophy.

Hypothesis: APOE4 genotype shifts the postmenopausal endophenotype of brain volume and lifespan trajectory to the left (i.e. interaction between ApoE4 and menopause will be evident earlier as evidenced by glucose hypometabolism in brain accompanied by hippocampal atrophy).

Background and Significance: The greatest risk factors for Alzheimer's disease are age, the ApoE4 allele and female sex. Postmenopausal women constitute >60% of the affected Alzheimer population and are those who will bear the greatest burden of the disease. Twenty years, 2 decades, ago Farrer and colleagues reported a sex difference in the lifetime risk of AD in women. In his risk associated with two copies of the ApoE4 gene in men. This seminal report, women with a single copy of the ApoE4 allele was sufficient to increase disease finding was confirmed a year later in a subsequent report by Payami, Schellenberg and colleagues who found that ApoE4 heterozygote men had lower risk than ApoE4 homozygotes; there was not significant difference between epsilon4 heterozygote males and those without epsilon4. In contrast, epsilon4 heterozygote women had the same significant twofold increased risk as homozygote men. Barnes, Bennett and colleagues found an even greater sex difference in the impact of pathology and risk of AD. Each additional unit of AD pathology was associated with a nearly 3-fold increase in the odds of clinical AD in men compared with a more than 22-fold increase in the odds of clinical AD in women. Increasing evidence links ApoE4 genotype and female sex with

increased risk and severity of AD^{6,14-21} although not all studies find the female sex ApoE4 interaction. The earliest etiological factors that ultimately lead to late-onset Alzheimer's disease, when prevention is still possible, remains unresolved for the primary casualties of the disease, postmenopausal women,

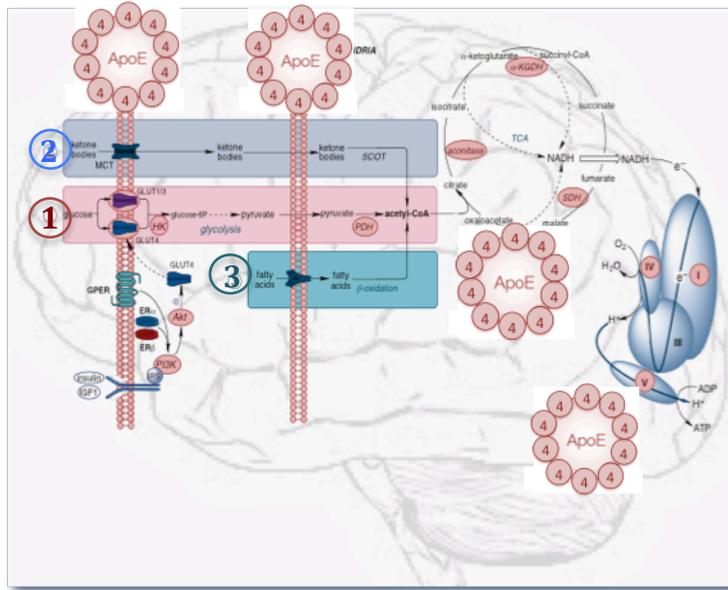


Fig 1. ApoE4 Increases Bioenergetic Crisis in the Female Aging Brain to Accelerate Alzheimer's.

The brain is primarily dependent upon the glucose (pathway 1) as the major metabolic fuel to generate the metabolite, acetyl-CoA, which is at the cross-roads of primary and secondary metabolic pathways to generate ATP (pathway 2, ketolysis and pathway 3, β -oxidation). During the endocrine transition of the perimenopause when estrogen in brain plummets, the systems required for estrogen activation of glucose metabolism and suppression of the ketogenic pathway are disassembled. Following perimenopause, the brain utilizes peripheral stores of ketone bodies as a fuel to generate acetyl-CoA (pathway 2). Reliance on ketone bodies to generate acetyl-CoA ultimately leads to the utilization of brain-derived lipids from

myelin to generate ketone bodies (pathway 3).

We propose that the ApoE4 allele accelerates and magnifies the bioenergetic crisis in perimenopausal brain to both accelerate and amplify the risk of Alzheimer's disease.

finding was confirmed a year later in a subsequent report by Payami, Schellenberg and colleagues who found that ApoE4 heterozygote men had lower risk than ApoE4 homozygotes; there was not significant difference between epsilon4 heterozygote males and those without epsilon4. In contrast, epsilon4 heterozygote women had the same significant twofold increased risk as homozygote men. Barnes, Bennett and colleagues found an even greater sex difference in the impact of pathology and risk of AD. Each additional unit of AD pathology was associated with a nearly 3-fold increase in the odds of clinical AD in men compared with a more than 22-fold increase in the odds of clinical AD in women. Increasing evidence links ApoE4 genotype and female sex with increased risk and severity of AD^{6,14-21} although not all studies find the female sex ApoE4 interaction. The earliest etiological factors that ultimately lead to late-onset Alzheimer's disease, when prevention is still possible, remains unresolved for the primary casualties of the disease, postmenopausal women.

Preliminary Data: Collectively, our mechanistic discovery findings, replicated in mouse, rat and translated to human, indicate that the perimenopause transition can lead to a bioenergetic crisis in the brain that activates a cascade of adaptive responses that ultimately lead to multiple features consistent with AD risk, including glucose hypometabolism in brain, development of a ketone body dependent brain, neuroinflammation, white matter degeneration and deficits in synaptic transmission. Further, dysregulation of metabolism in the human female is associated with significant cognitive decline. In brief, those findings are:

1. A naturalist aging rodent model of human female endocrine aging reveals two distinct aging

programs, one chronological and the other endocrine. The aging program precedes endocrine aging and appears to initiate the endocrine aging program.

2. The glucose dependent bioenergetic system of the brain – including glucose transport, associated gene and protein expression, enzyme activity and mitochondrial respiration – all decline during the perimenopausal transition and remain decreased thereafter.
3. Mitochondrial gene expression declines during the perimenopause and remains decreased thereafter.
4. Surprising to us, the ketogenic system also decreased during the perimenopausal transition and subsequently recovered at the menopause. However, this adaptive bioenergetic pathway was time limited as it dissipated with age.
5. The perimenopausal decline in glucose metabolism is associated with a significant decline in synaptic transmission evidenced by decreased long-term potentiation.
6. Rise in inflammatory signaling pathways also occurred during the perimenopausal transition and were sustained thereafter.
7. Decline in genes required for β -amyloid degradation occurred subsequent to endocrine aging.
8. Astrocytes undergo a transcriptional shift in expression of estrogen receptors with an increased ratio of the nuclear receptors ER α : ER β . DNA methylation of the ESR2 (ER β) UTR correlates with differences in ER β expression suggesting that epigenetic factors may be related to perimenopause-related change in ER expression and loss of transcriptional response to E2.
9. Efficacy of hormone therapy in reducing A β accumulation and improving behavioral outcomes in female 3xTg-AD mice is (i) linked to its ability to suppress microglial activation, (ii) absent in middle-aged mice that have completed the ‘perimenopause’ transition, (iii) diminished in obese mice.
10. A widespread shift in neural estrogen responsiveness following perimenopause transition that likely contributes to the observed loss of hormone therapy efficacy in postmenopausal women.
11. Clinical translational validity of our bioenergetic hypothesis of perimenopause and its concomitant risk for cognitive decline was supported. Postmenopausal women with a clinical profile consistent with a risk of metabolic dysfunction (clinical profile at the limits of normal) exhibited significant decline in cognitive function relative to women of the same age whose metabolic function was well controlled. A rapidly deployable and economically feasible biomarker panel was developed to identify women at risk for developing late onset of Alzheimer’s.
12. Lastly, glucose bioenergetic crisis initiated during the perimenopause activates a genomic and biochemical cascade in brain required to catabolize white matter lipids necessary to generate ketone bodies to fuel generation of acetyl-CoA.

Proposed One-Year and Long-Term Outcomes: Outcomes of our mechanistic to clinical to global population program of research will provide insights into the molecular and genetic mechanisms underlying the increased burden of the ApoE4 gene and risk of AD in the female aging brain. Data derived from the proposed AAC analyses will support federal and philanthropic funding opportunities. We anticipate that findings from the proposed analyses will integrate with and expand upon those of the analyses of *APOE* ϵ 4 carriers conducted by Reiman and colleagues. While our analyses focus on the aging *APOE* ϵ 4 female brain, we anticipate that mechanistic insights derived from this accelerated aging model will inform our understanding of

the increased risk of AD in the *APOE* ε4 male.

Year End Progress Summary:

1. Female bioenergetic transition in perimenopause. Our RNA-sequencing analysis of the hippocampal RNA from perimenopausal rats detected 32,461 poly-A enriched transcripts and identified 266 differentially expressed transcripts in regular- versus irregular cycling rats at 9-10month (Fig. 2A, FDR<0.05). 119 of these differentially expressed genes are from four gene categories: metabolism, inflammation, neuronal function and Alzheimer's (Fig. 2B). Pathway analysis of these genes suggested downregulation in mitochondrial genes controlling both the mitochondrial bioenergetics and redox homeostasis (Fig. 2C).

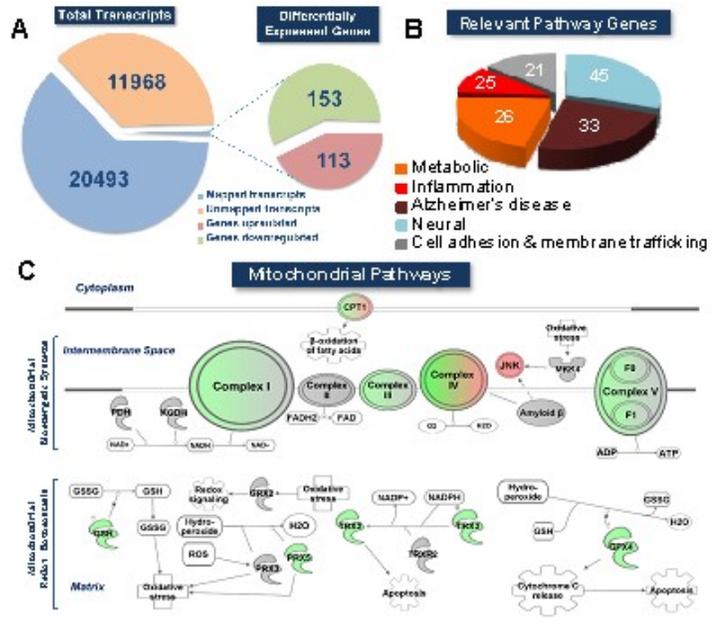


Fig 2. RNA sequencing data from perimenopausal rat hippocampi. A. Transcripts identified in regular- versus irregular cycling rats at 9-10month. B. Genes that have a summary function in NCBI database are from five gene categories. C. The irregular cycling rats have a decline in the expression of mitochondrial genes controlling bioenergetics and redox homeostasis.

Consistently, the endocrine transition from regular to irregular cycling is also characterized by a decline in brain glucose metabolism (Fig. 3), mitochondrial respiration, and long-term potentiation. Onset of acyclicity is accompanied by a rise in genes required for fatty acid metabolism, inflammation, and mitochondrial function. Similarly, in the mouse, both non-Tg and 3xTg-AD brains undergo significant decline in glucose metabolism and mitochondrial function prior to the transition into reproductive senescence.

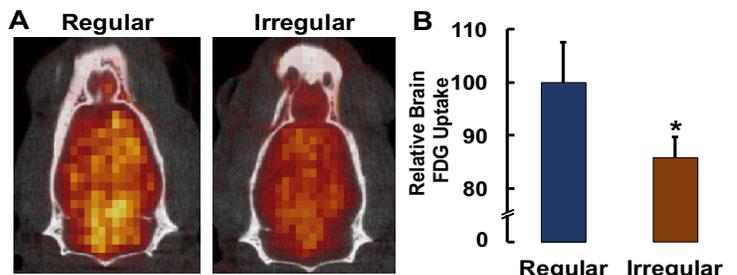


Fig 3. Brain glucose uptake (FDG-microPET/CT) (A) images. (B) measure of 9m regular and irregular cycling rats.

2. Sex difference in normal aging and 3xTG AD mice. Our study in female and male 129/C57BL/6 mice at 6-, 9-, 12- and 15-month-of-age focusing on both

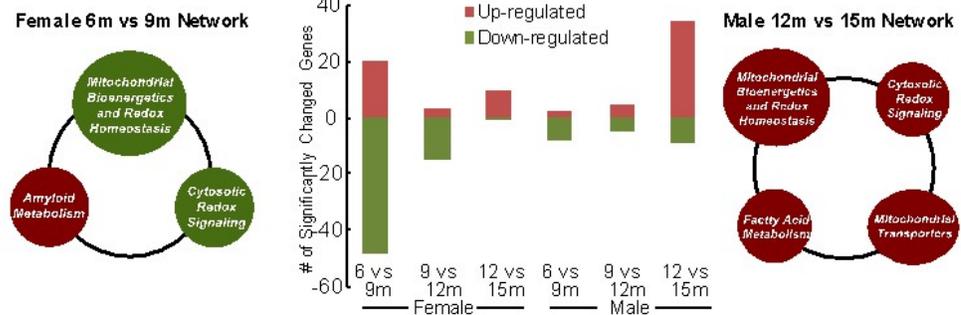


Fig 2. Differentially expressed genes in male and female during each aging transition. Top-ranked gene groups during female 6-9m (left) and male 12-15m transitions (up- or down-regulated).

energy and amyloid metabolism revealed substantial sex disparities in the trajectory of aging changes between their brains (**Fig. 4**): in female brains, most of the changes occurred from 6 months to 9 months with two-thirds of them being downregulated (bioenergetic genes mostly downregulated and amyloid-related genes upregulated); while in male brains, most changes occurred from 12 months to 15 months with the majority being upregulated. Consistently, in a separate study using 3xTG AD mice, significant differences in brain bioenergetic function and amyloid metabolism were observed between sexes: at 12 month-of-age, females exhibited a significantly lower mitochondrial respiratory capacity (**Fig 5A**) and mitochondrial complex IV activity (**Fig 5B**), but higher levels of A β load (**Fig 5C**). Together, results from these studies indicate that female brains undergo age-related changes much earlier than male brains, which signals the onset of a hypometabolic phenotype at risk for AD.

Sex-differentiated impact of APOE4 on bioenergetics. Our preliminary data indicated that APOE^{4/4} mice have significantly lower levels of glucose (in both female and male) but higher levels of ketone bodies (in female only) compared to age-matched APOE^{3/3} mice (**Fig. 6**). In addition, in both APOE^{4/4} and APOE^{4/3} mice, females tend to have a lower peripheral glucose level but a higher ketone body levels, indicating a potentially earlier shift in bioenergetic fuels in female brains. Importantly, a longitudinal FDG-PET study (**Fig. 7**) in rats revealed a significant interaction among age, APOE genotype and sex ($p < 0.01$): APOE^{4/4} females exhibited a lower glucose metabolism at both perimenopause (9-10m, **Fig. 7A**) and post-menopause (12-13m, **Fig. 7B**) compared to males; the APOE4 effect on glucose metabolism was only seen in females at post-menopause stage. Collectively, these data suggest that APOE4 can exacerbate the deficits in glucose metabolism and shift to ketone body metabolism, particularly upon the female rodents during the transition from peri- to post-menopause.

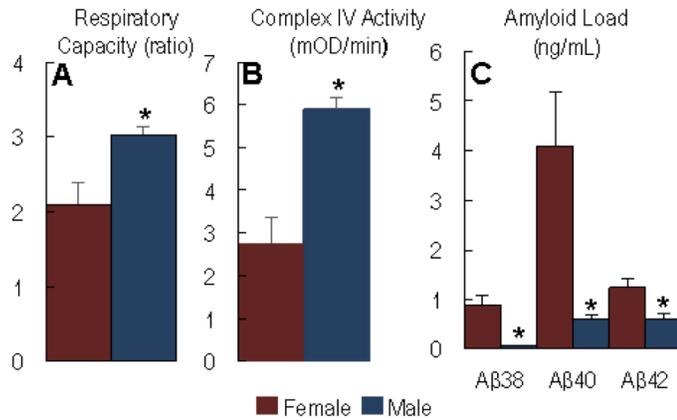


Fig. 5. Sex differences in brain bioenergetic function and A β metabolism. A. mito respiration; B. Complex IV activity; C. A β metabolism.

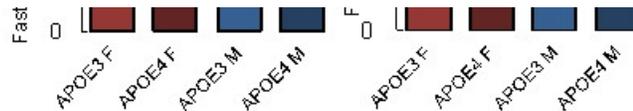


Fig 4. Fasting glucose and α -hydroxybutyrate levels in plasma from APOE4 and APOE3, female and male mice.

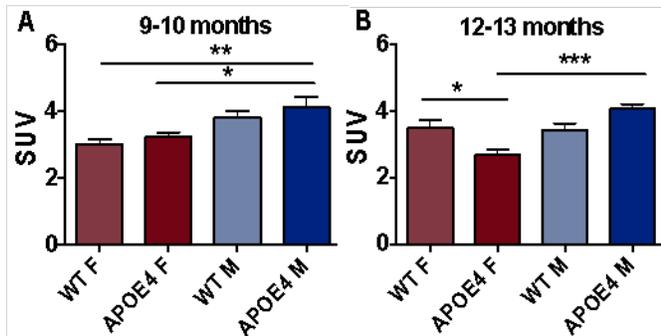


Fig 3. Brain glucose uptake (FDG-microPET) in APOE4 and WT, female and male SD rats at A. 9-10m and B. 12-13m age

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Sleep and circadian function as biomarkers of Alzheimer's Disease risk in Down syndrome.
Jamie Edgin, PhD, Fabian Fernandez, PhD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims:

Aim 1. Extend data collection with a current sample of >100 children with DS to study sleep and circadian function across the lifespan, including young (18-25 years) and middle-age (40-50 years) adults with DS (n = 15 per group).

Aim 2. Publish baseline data regarding intact circadian function, but impaired sleep, in infants and young children with DS (Fernandez, Nyhuis, and Edgin, in press, *Sleep Medicine* 2017).

Aim 3. Determine the relationship between circadian health and memory function in adults with DS.

Background and Significance: It is increasingly clear that the progression of AD will be best understood by examining the course of the illness as early as possible. DS is a population with heightened risk for AD-related neuropathology and decline in adulthood, ultimately resulting in up to 75% prevalence after age 60 years. Partly due to the triplication of the *APP* gene on chromosome 21, those with DS are prone to early and persistent A β accumulation. Deposition of plaques and neurofibrillary pathology is universally found by 35 years of age. A number of risk factors are likely to accelerate the progression of AD in DS, including poor health (high BMI, sleep apnea). Given the numbers of individuals with DS transitioning prematurely to AD in adulthood, DS could serve as a model to develop early prophylactic treatments applicable to the broader population. It is not yet clear when in development individuals with DS begin to show the phenotypic effects associated with AD progression. Finding this transition point could help to determine time windows for effective treatment of AD in DS. This progression may occur before adulthood, but few studies have examined risk factors for AD in young cohorts of individuals with DS and no longitudinal investigations exist with baseline assessments before age 20 years. In the context of studies funded by the Bill and Melinda Gates Foundation, the National Institutes of Health, and the LuMind Research Down Syndrome Foundation, the Edgin lab has collected data on a cohort of >100 individuals with DS including assessments of sleep quality, daily activity rhythms, and cognitive status. Using this cohort, and the expertise on circadian analysis from co-I Fernandez, Edgin and Fernandez will expand the sleep and circadian assessment of this cohort to track the status of individuals with DS as they age. This study will then help determine if those with DS show any early signs of circadian dysfunction that could serve as premorbid markers of decline.

Preliminary Data: Fernandez, Nyhuis, & Edgin (2017) examined the circadian profile of infants and children with DS (n = 78 ; now numbering >100) and compared their rhythms to typically developing (TD) same-age controls (n = 53). Across several metrics, circadian rhythms in our DS sample were robust, showing no differences from TD children. Wake-up, sleep and peaks of daytime activity times were also similar between the groups, but a subset of individuals with DS demonstrated a phase advance of behavioral activity. In contrast to their preserved

circadian function, sleep efficiency and duration in the sample with DS was significantly reduced relative to controls. These results indicate that sleep deficits are likely a greater correlate of developmental intellectual disability in DS than rhythm dysfunction; relations between sleep and measures of cognitive development are not confounded by circadian rhythm disorder. The current proposal will expand on this dataset to study the daily rhythms of young and middle-aged adults with DS. Because circadian rhythms are unaltered in early life, we will explore the possibility that impaired circadian function emerges in later adulthood and may serve as a marker for individuals with DS at greater risk of cognitive decline due to AD.

Proposed One-Year and Long-Term Outcomes: All data from the current project will be submitted for publication in peer reviewed journals, including a first paper submission. Attempts to obtain external funding will be made to the NIH via the R03 mechanism. While several groups have noted the importance of sleep and circadian function as possible biomarkers of cognitive decline in the typical and DS populations, these measures have not been systematically studied in large cohorts of people with DS, allowing us to uniquely contribute to the field's understanding of their diagnostic utility.

Year End Progress Summary: This year, we have continued to collect actigraphy data across the lifespan in DS, having now sampled sleep movement data from 119 individuals with DS from infancy to late adulthood (age-range 6 months- 61 years). The first paper examining circadian/sleep rhythms in this cohort is in press at *Sleep Medicine*. In Fernandez et al. (2017), we evaluated sleep consolidation and circadian activity rhythms in 66 infants and young children with DS, aged 5-67 months, and 43 typically developing age-matched controls. Sleep and measures of circadian robustness, or "timing", were quantified using continuous in-home actigraphy recordings performed over seven days. Circadian robustness was quantified via time series analysis of rest-activity patterns. Phase markers of circadian timing were calculated alongside these values. Sleep efficiency was estimated based on the actigraphy recordings. In this study, we demonstrated further evidence that general sleep quality is poor in infants and toddlers with DS, a population which has a sleep apnea prevalence as high as 50% during the preschool years. Despite poor sleep quality, circadian rhythm and phase were preserved in young children with DS and displayed similar developmental trajectories in cross-sectional comparisons to a typically developing (TD) cohort. Three circadian phase markers showed that 35% of our recruitment sample with DS was phase-advanced to an earlier morning schedule, suggesting significant within-group variability in the timing of their daily activity rhythms. In a sample of older individuals with DS collected this year through AAC funds (n = 10; mean age = 45.47 years), we found evidence for reduction in the robustness of daily rhythms across all measures of circadian amplitude when compared to a younger sample with DS (p <0.01). Sleep efficiency and total time were not statistically different across these age-groups. Given the robust nature of circadian rhythms in young children with DS, early break-down of these rhythms could be an early sign of cognitive decline. Future work will expand on these findings to collect more data in younger and older adults with DS, to compare the trajectory of sleep and circadian rhythm in DS and Fragile X syndrome across age, and to correlate these changes to novel neuropsychological assessments designed in the Edgin laboratory.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Establishing circadian biomarkers for age-related working memory impairment. David Negelspach, BSc, Sevag Kaladchibachi, PhD, Fabian Fernandez, PhD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims:

Aim 1. Use time series analyses and other circadian assessments to quantify robustness of diurnal rhythms in *Drosophila ananassae*.

Aim 2. Quantify phase-resetting and other responses to acute light treatment at night.

Aim 3. Track longitudinal trajectories of circadian health during aging.

Aim 4. Establish a working memory test that can be given to flies repeatedly across the lifespan to detect age-dependent changes in memory performance.

Aim 5. **Long-term Deliverable 1**—Use regression models to identify individual differences in circadian profile that presage cognitive decline.

Aim 6. **Long-term Deliverable 2**—Design phototherapy interventions that can strengthen circadian health starting in middle adulthood so as to mitigate late-life cognitive decline.

Background and Significance: The deleterious effects of advanced age on 1.) circadian rhythms and 2.) working memory have often been siloed areas of research. The aging process unquestionably takes a toll on both, but the possibility that weakening circadian regulation can “seed” age-related memory impairment decades in advance has not been empirically studied. A few largescale epidemiological surveys or prospective studies of older community-dwelling individuals have reported short-term correlations between measures of circadian robustness (i.e., circadian activity amplitude) and performance on tests of executive function/working-memory such as the *Stroop Color-Word Test*, the *Letter Digit Substitution Task* (LDST), and the *Trail Making Test*. However, no work has gone beyond these group inferences to ask whether finer associations can be drawn between particular measures of a younger individual's circadian profile and their tolerance to age-related memory problems. Animal models are indispensable for such work, because they allow investigation of large numbers of individuals across the lifespan and provide control of life history factors that can confound associations between rhythms and memory.

Preliminary Data and Plan: The Fernandez group looks for biomarkers derived from one's circadian rhythms that can accurately identify 'midlife' individuals at risk for cognitive decline associated with normal aging. For this research, they use a cosmopolitan species of fruit fly, *Drosophila ananassae*, that co-evolved with human society. *Ananassae* form highly structured populations around humans, rarely establishing colonies in more natural habitats. Because of this background, the flies show a consolidated pattern of locomotor activity during the day that mimics the diurnal sleep/wake patterns of people and are realistic models of human circadian behavior. *Ananassae* offer the opportunity to screen several dozen properties of the brain's circadian timekeeping clock—and their relationship to memory performance across the lifespan—in just over two months, which is the flies' natural life expectancy. In the current proposal, we will monitor *ananassae* under tightly controlled lighting conditions, measure their

phase responses to light, and determine how their sleep and circadian function worsen with age. From there, we will identify factors within their sleep-circadian profile that correlate with greater than expected memory loss as the animals get older.

To measure *ananassae* working memory, we have devised an apparatus that video-tracks the movement of flies as they explore a miniature Y-shaped maze. When experimental animals are placed in a Y-maze, they usually alternate their movements from one arm or compartment of the apparatus to the next to maximize the territory that they can explore. Decades of experiments in rodents suggest that this alternation behavior is dependent on the animals keeping a running “map” in parts of their brain important for both spatial navigation and online processing of novel stimuli. These maps continually update where the animals have been most recently from those areas they have visited less recently. The Y-maze task is a standard test to evaluate spatial working memory in rats and mice and we have just finished establishing its use in insects (see **Year End Progress Summary** below).

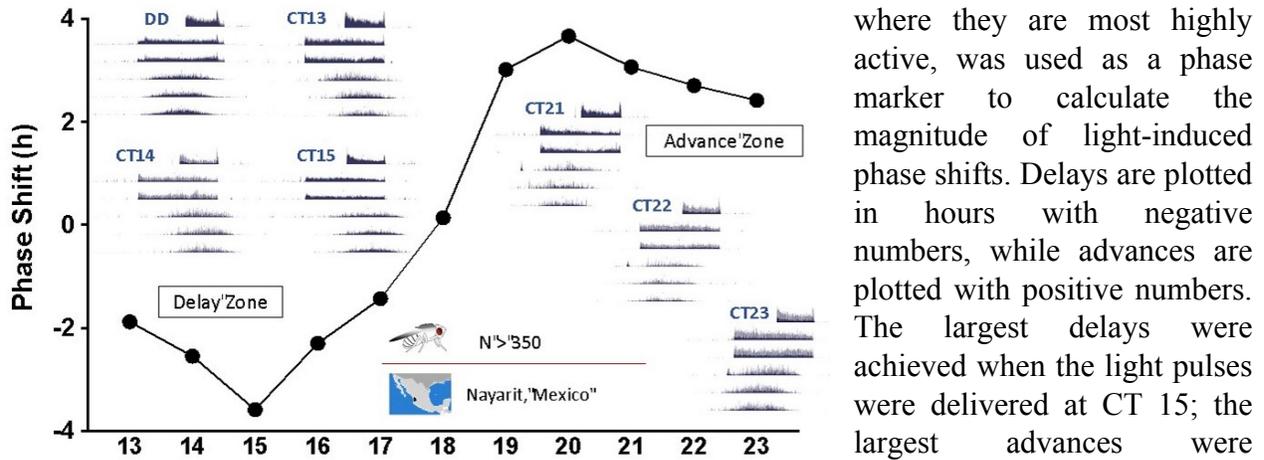
Proposed One-Year and Long-Term Outcomes: All data from the current project will be maintained on secure lab servers and will be submitted for publication in peer reviewed journals. Attempts at outside external funding will be made to the NIH, as well as a number of private foundations dealing with aging and Alzheimer’s disease such as the Alzheimer’s Association. Dr. Fernandez is currently seeking funds from DARPA for aspects of the proposed work. One important long-term outcome of the current project will be to isolate characteristics of the internal clock that might inform efforts focused on rehabilitating circadian function and memory with LED light. For instance, the internal clock does not “tick” at the same exact rate from one person to the next. Though this rate approximates 24h, some individuals exhibit frequencies further removed from 24h than others. Our data may indicate that possessing an internal clock with larger deviations from the 24h solar cycle incurs biological “penalties” as the person ages because the brain is forced to shift its physiology, significantly, every day to match external time. If true, strategies to lessen this burden with phototherapy might yield demonstrable improvements in cognition during aging.

Year End Progress Summary: We have completed the first four specific aims described in the original AAC proposal. Our extensive data suggest that *Drosophila ananassae* exhibit canonical phase response curves to 15-min light pulses delivered at each hour of the subjective night (CT, circadian hour 13-23; **Figure 1**; **re: Aim 2**) and show age-related breakdown of several measures of sleep and circadian robustness (**Figure 2**; **re: Aims 1 and 3**). We have also shown that the flies exhibit a form of spatial search and working memory previously thought to occur only in higher organisms (**Figure 3**; **re: Aim 4**). Our data suggest that both *ananassae* and *melanogaster*, the most common lab strain of *Drosophila*, exhibit significant alternation behavior in the Y-maze (**Figures 3 and 4**). Further validation of the test with *melanogaster* memory mutants (e.g., Rutabaga, *rut¹*)¹² has confirmed that this patterned exploration is predicated on functional short-term working memory (**Figure 4**). Some of this work is now under consideration at the journal *Animal Behaviour* (2017).

By and large, the data we have collected over the past year suggest that *ananassae* offer us a powerful model by which we can establish links between circadian decay and age-related

cognitive impairment and rule sets for using light to rehabilitate the brain's aging clock. We are now working systematically to achieve the long-term deliverables described in **Aims 5 & 6**.

Figure 1: A Phase Response Curve (PRC) to Light in *Drosophila Ananassae*. Flies were loaded into individual activity monitors and maintained under a standard 12:12 light-dark cycle with controlled temperature and humidity. After two-and-a-half days of entrainment to this schedule, separate groups of animals received a 15-min light pulse at one of the 11 circadian hours associated with the subjective night (i.e., circadian time, CT 13-23) and then placed in constant darkness. The acrophase of the animals' activity period, the point of the subjective day



where they are most highly active, was used as a phase marker to calculate the magnitude of light-induced phase shifts. Delays are plotted in hours with negative numbers, while advances are plotted with positive numbers. The largest delays were achieved when the light pulses were delivered at CT 15; the largest advances were observed with light delivery at CT20. These data replicate previous PRCs done in *Drosophila* and rodents. (Inserts) Embedded within the PRC graph are raw population actograms showing how the distribution of locomotor activity was shifted in groups of flies that were either: 1. put in constant darkness without any light treatment (DD; free-running conditions) or 2. exposed to light treatment at CT13, 14, 15, 21, 22, or 23 and then placed in DD. Light treatment causes clear shifts in the animals' wake-sleep schedule, while DD alone does not affect the timing of their acrophase.

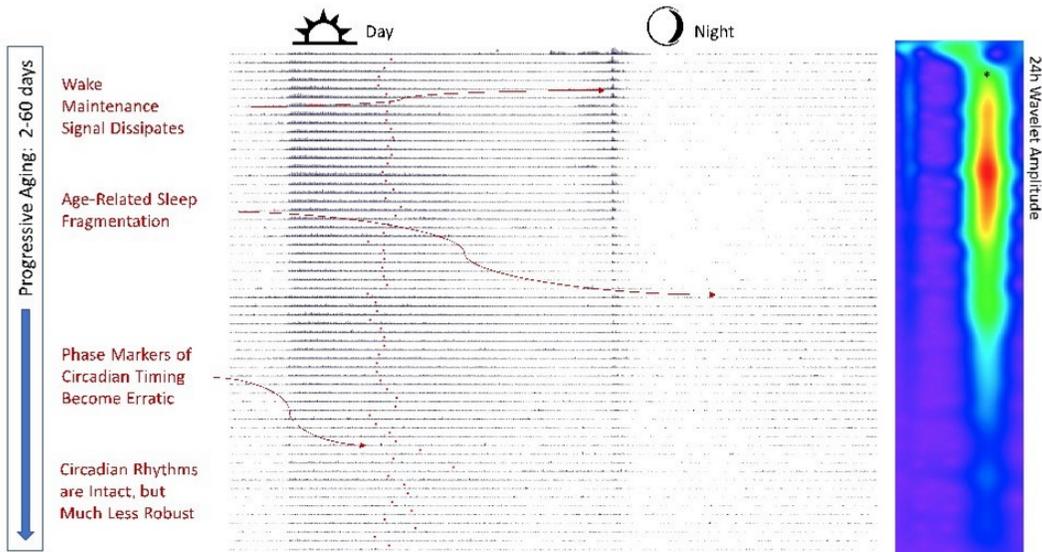


Figure 2: *Drosophila Ananassae* - Breakdown of Circadian Rhythms and Sleep Across Aging. Population actogram showing how behavioral activity (dark blue ticks) is distributed across the day and night in a large cohort of ananassae (n=64). Data are shown as the flies aged from 2-day old young adults to 60-day old “seniors,” with one day’s activity record plotted per line, top-to-bottom. Four major sleep-circadian phenotypes associated with aging in humans are evident upon visual inspection of the fly actogram. First, the wake maintenance “arousal” signal that the circadian system typically sends at night right before the sun goes down (or as the lights go out in the vivarium) is diminished in the animals as they exit the first few weeks of life. Loss of early nighttime arousal tends to cause older individuals to retire to bed earlier than younger individuals. Second, nighttime activity begins to appear in the actogram as the animals pass 1-month of age, a stretch associated with middle adulthood. These data suggest that the animals are waking at night and are not consolidating sleep as well as they did when they were younger. Third, phase markers associated with circadian timekeeping—such as daytime acrophase (marked by red circles)—are no longer stable from one day to the next near the tail-end of the recording period. This suggests that the circadian system is having trouble entraining to the light-dark cycle, an assertion that is supported by a subsequent quantitative analysis of circadian robustness (phenotype 4). Lomb-Scargle (LS) time series analysis and intradaily stability (IS) measures show that 24h patterns of behavioral activity are still there in older animals. However, these measures are significantly reduced when comparing the animals’ first month of life to their second (LS and IS values, 2-31 days = 6215 and 0.779, respectively; LS and IS values, 32-60 days = 3839 and 0.448, respectively). (**Far right**) Wavelet analysis marking the amplitude of the 24h oscillation in the ananassae activity record. Twenty-four hour patterns of activity dissipate across age.

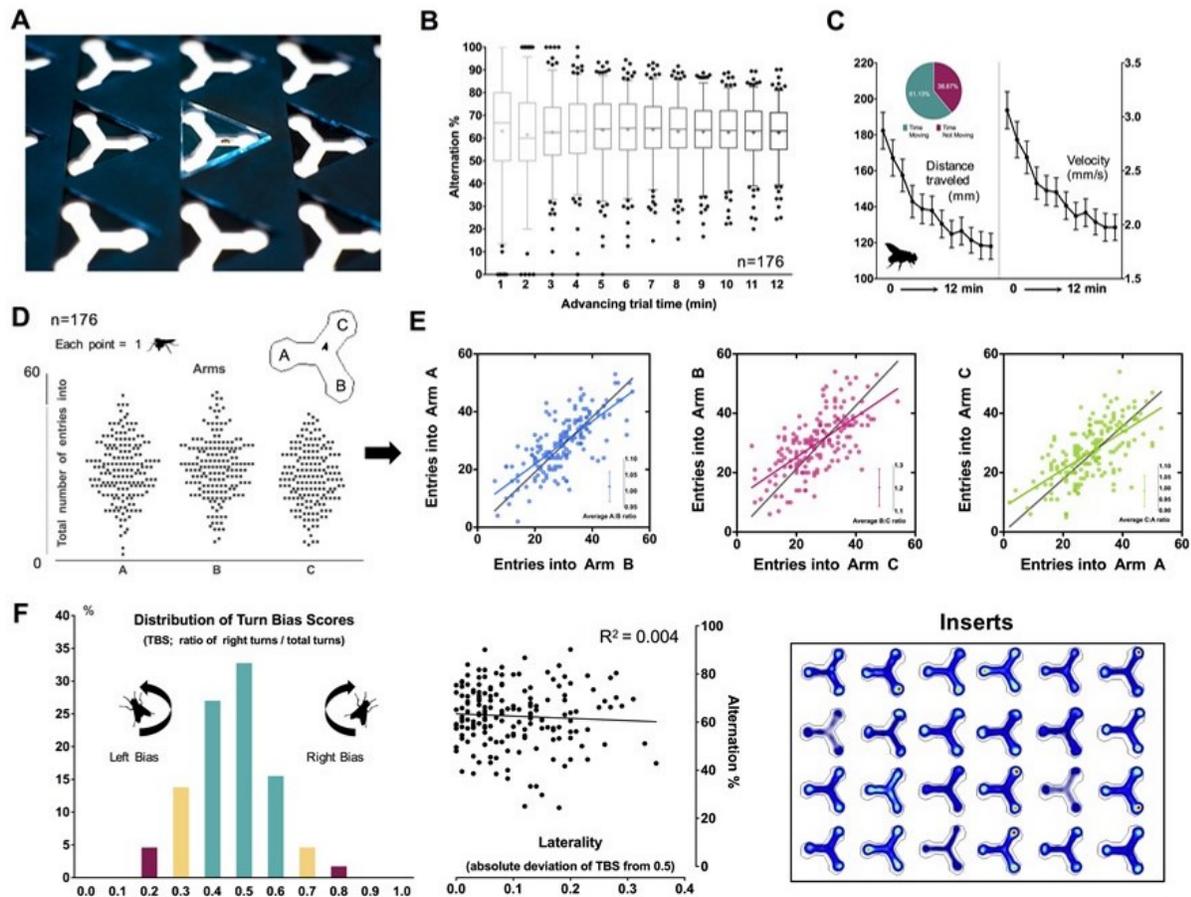
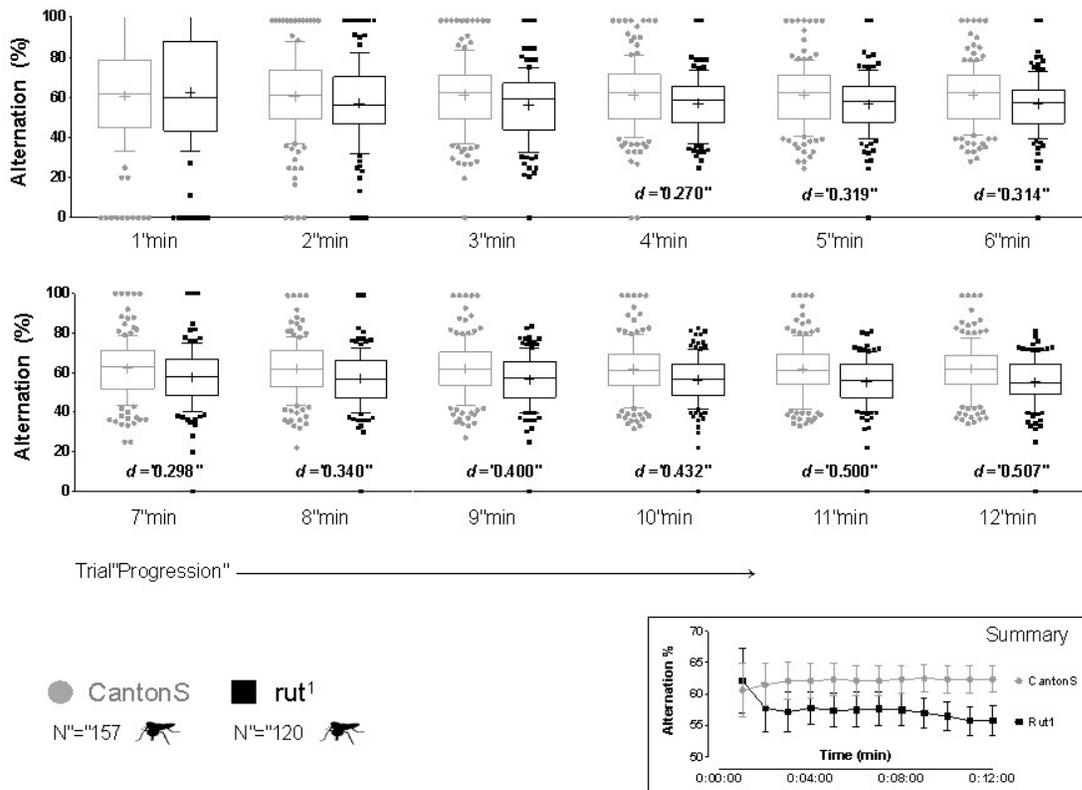


Figure 3: Spontaneous Alternation Behavior in *Drosophila Ananassae*. (A) Photo taken of *Drosophila ananassae* placed in the testing apparatus (courtesy of Bevin Christina Photography). (B) Minute-by-minute snapshots of the alternation percentage accrued at different stages of the Y-maze task by populations of *ananassae* from Southeast Asia ($n=176$). Data are shown in a series of box and whiskers plots. The hinges of the plots extend to the 25th-75th percentile for the group's alternation scores, while the whiskers represent the 5th-95th percentiles. Individual flies with performance outside of this range are singled out by black dots. The average alternation rate is marked with a plus sign (final score by 12 min, $62.49 \pm 12.63\%$; last plot, darkened outline), the median with a straight horizontal line. The proximity of these central tendency measures suggests that the distribution of scores was symmetrical (skewness calculated with the g1 method for the full trial across 12 min, -0.439). (C) Average distance traveled (mm; left panel) and walking speed (mm/s; right panel) for Asian *ananassae* in the Y-maze. The animals walked less as the trial progressed from 1 minute to the next, $F(4.739, 819.8) = 69.98$, $p < 0.0001$, and slowed their movement, $F(4.717, 816.1) = 70.84$, $p < 0.0001$. Both locomotor indices decreased by approximately 35% (Distance traveled from 0-1 min, 182.3 ± 68.18 mm; Velocity from 0-1 min, 3.06 ± 1.15 mm/s; Distance traveled from 11-12 min, 118.0 ± 48.38 mm; Velocity from 11-12 min, 1.98 ± 0.81 mm/s), suggesting that the flies were habituating to the enclosure. (D) Scatter plot of entries made into arms A (29.12 ± 9.66), B (29.8 ± 9.70), and C (26.79 ± 9.42). Each gray dot represents a value from an individual fly. Chi-square analysis indicates that the distribution of entries to each location was not statistically different from one animal to the next, $X^2(346) = 385.9$, $p = 0.069$. (E) Simple linear regressions were calculated for the number of entries each fly made into each combination of arms accessible in the Y-maze.

Regression lines forced through a zero intercept are shown in gray; those not forced through a zero intercept are shown in color, along with a scatter plot of individual arm-entry ratios (ArmA/ArmB = blue; ArmB/ArmC = light magenta; ArmC/ArmA = lime green). The number of times an animal visited one arm significantly predicted how many times it would visit another arm ($R^2s = 0.42-0.55$, $Fs(1, 172) > 123$, $ps < 0.0001$). Testing the ratio of visits to each combination of arms against a hypothetical value of 1 (parity) suggested that, relative to A, arms B and C were equally frequented (mean ArmA/ArmB ratio = 1.01, $t(173) = 0.585$, $p = 0.559$; mean ArmC/ArmA ratio = 0.99, $t(173) = 0.319$, $p = 0.750$). However, relative to B, fewer entries were made into arm C (mean ArmB/ArmC ratio = 1.2, $t(173) = 4.963$, $p < 0.0001$). (F, left panel) Histogram of turn bias scores exhibited by Asian ananassae in the Y-maze. Turn bias was measured by quantifying the probability of the animals turning right while traveling about the arena (i.e., the ratio of right turns to total turns). Approximately 75% of the flies in our sample had turn bias scores not far removed from 0.5 (e.g., ratios between 0.4-0.6; green bars), indicating that they were turning right in the maze about as often as they were turning left. However, approximately one-quarter of the animals did express significant locomotor handedness, defined by turning right greater than 70% of the time or less than 30% of the time (yellow and red bars). (F, right panel) Linear regression suggests that spontaneous alternation in the Southeast Asian cohort was not a byproduct of directional persistence (i.e., “laterality,” measured by quantifying the absolute deviation of their turn bias scores from 0.5). (Inserts) Heat maps visualizing the travel area of flies in the Y-maze. Twenty-four representative trials are illustrated that track the entire run. All values are reported as mean \pm SD.

Figure 4: Canton-S and *rut*¹: Alternation Performance. By the end of the 12-min test in the Y-maze, Canton-S flies (gray, gray circles) had accrued an alternation rate that was significantly higher than the rate seen in *rut*¹ flies (black, black squares) (Mann-Whitney $U = 6932$, n_1 , Canton-S = 157, n_2 , *rut*¹ = 120, $p = 0.0002$, two-tailed; Canton-S median = 62.18%, *rut*¹ median = 55.56%).



ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Interventions to improve memory and executive function in older adults in real-world settings. Elizabeth L. Glisky, PhD, Matt Grilli, PhD, Lee Ryan, PhD, Meredith Hay, PhD, Nancy Sweitzer, MD, PhD, Jane Mohler, PhD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims:

Specific Aim 1: To explore the cognitive benefits of social engagement in a population of truly socially-isolated older adults.

Specific Aim 2: To design and test the effectiveness of a prospective memory training program for older adults with heart failure.

Background and Significance:

Aim 1. Previous correlational studies have suggested that older adults who remain socially active and cognitively engaged have better cognitive function than those who are isolated and disengaged. In a direct test of this hypothesis, we previously taught older people how to use Facebook, which they continued to use for a period of 8 weeks, and we found improvements in a specific component of executive function—updating and monitoring of working memory. We suggested that benefits were most likely attributable to the social interaction involved in the use of Facebook. Another possible interpretation, however, is that the Facebook task was more cognitively challenging than the non-social computerized diary task that served as a cognitive control. Other investigators have claimed that the benefits are attributable to the combined effects of cognitive training and social engagement, and have suggested that social interaction alone does not provide cognitive gains. In all of these studies, the older adults were community residents who volunteered to participate in a research study and were able to travel to a central site for training purposes. Although the participants in our Facebook study for the most part lived alone, they were not truly isolated, and many lived in retirement communities that offered considerable opportunities for social engagement. The present study will focus on older people who are truly socially isolated, do not leave their homes often, tend to have lower income, and do not participate in group activities. This group of older people are at greatest risk for cognitive decline, and we expect that they will show even greater benefits of Facebook training and subsequent use. In addition, to assess the extent to which the benefits are attributable to the social or cognitive aspects of Facebook, we will have a second group of socially-isolated older adults who will interact socially in their homes with two undergraduates over the same period of time, but they will not be engaged in any cognitive training. Our goal here is to assess the extent to which cognitive benefits are achieved primarily as a function of cognitive activity or as a function of social engagement, or both. We are currently working with social service and health-related agencies in the community to identify these individuals, and recruit them for the study.

Aim 2. Cognitive impairment, particularly in memory, is a common complaint in heart failure patients, and may be a significant contributor to medication non-compliance, repeated hospitalizations, and poor clinical outcomes. Remembering to take medications requires prospective memory (PM), i.e., remembering to do things in the future, and there have been relatively few intervention studies that have focused on improving PM in memory-impaired

populations, and none in heart failure (HF) patients. Our program will teach HF patients specific strategies designed to help them remember to take their medications at the appropriate times—a real-world PM task. These strategies have been found effective at improving PM in both young and older adults and in some clinical populations in the laboratory. To our knowledge, however, no studies have directly trained PM and measured medication adherence in a real-world setting. The establishment of an efficacious protocol for training medication adherence in patients could have far-reaching benefits for the treatment of diseases in multiple clinical fields, particularly for older adults, many of whom will experience cognitive decline as they age.

Preliminary Data: Both of these studies are in the early development stages and as yet we have no preliminary data. However, these studies are follow-ups to previous studies that we have already published, including the previous Facebook study (Myhre et al., 2016) and several studies of PM strategies relevant to Aim 2 (Grilli & McFarland, 2011; McFarland et al., 2009, 2011, 2012).

Proposed One-Year and Long-Term Outcomes: The one-year goal for the Facebook study is to complete data collection and analysis. The one-year goal for the PM study is to design the intervention and to collect pilot data to support a larger-scale clinical trial looking at the combined effects of a pharmacologic therapy and the PM training. An R21 grant has been submitted to support the PM component of that study, but it will not be reviewed until this fall. Both studies will inform the design of future interventions and future grant submissions, highlighting the importance of and methods for translating laboratory findings into real-world contexts.

Year End Progress Summary: The project associated with Specific Aim 1—to explore the cognitive benefits of social engagement in a population of truly socially-isolated older adults—is underway. Recruitment of socially-isolated participants has been difficult, but we are working with several agencies in the community. Sixty-seven people have been screened for the study, but because they constitute a low-income and low-education group often with mental health issues, enrollment in the study has been slow and attrition has been a problem. Of the 67, only 26 were eligible and were enrolled, and 8 of those subsequently dropped out. Currently, 18 (of the required 36) participants are enrolled, 8 of those have completed, 3 are in progress and 7 will be starting shortly.

The project outlined in Specific Aim 2 is on hold. The R21 grant was not successful and the prospective memory component of the clinical trial is on hold while other preliminary tests of the pharmacological therapy and pilot data for the imaging component are being carried out. The PM training component is expected to be included in another grant submission, but the collection of pilot data will not go forward until other issues are resolved. The RA who was to be assigned to that project has been assisting on the Microbiome and Cognition project with Carol Barnes and will be assisting on the study with socially-isolated older adults outlined in Specific Aim 1.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

The influence of physical activity, genetic susceptibility, and their interaction, on risk for Alzheimer's disease. Yann Klimentidis, PhD, Gene Alexander, PhD, David Raichlen, PhD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims:

Aim 1: Test cross-sectional and prospective association of several types and patterns of self-reported and objective physical activity measurements with cognitive function and neurodegenerative disease.

Aim 2: Perform agnostic and candidate-gene tests of genetic interaction with physical activity using cross-sectional and prospective analysis of cognitive function and neurodegenerative disease.

Background and Significance: With an aging population, neurodegenerative diseases represent a growing health and financial burden. Improving prevention and treatment of these diseases may help stem this tide. Since there is an substantial heritable component to these diseases, identifying the specific genes and how they interact with environmental factors, such as lifestyle and behavior, can help us understand the pathophysiological pathways. In turn, elucidating the pathways can lead to more precise methods for preventing or halting the disease process.

Physical activity is known to be associated with protection against neurodegenerative disease. Less is known about the specific type and pattern most strongly associated with this protection. Clarifying the relationship between exercise and brain health can help us to optimize prevention efforts, and also to better understand the mechanisms through which physical activity is protective.

In addition, little is known about whether physical activity may be more or less effective according to an individual's genetic profile. Using a large-scale genomic approach, interaction analyses can also identify new loci that have context-dependent effects on neurodegenerative disease. Ultimately, this may enable us to identify individuals most likely to benefit from physical activity, and as mentioned above, to uncover mechanisms through which physical activity is protective.

Preliminary Data: The PI has previously performed multiple studies examining the interaction of genetic susceptibility factors with physical activity, inactivity, and other lifestyle and phenotypic factors. Major findings include 1) a weaker association of physical activity with incident type-2 diabetes among individuals at greater genetic risk for insulin resistance, and 2) a similarly weaker effect of physical activity among those with high genetic risk for obesity in a randomized 1-year exercise intervention. These findings suggest that physical activity can modulate the effect of genetic risk factors, and conversely that one's genetic profile may modulate the extent to which health benefits are derived through physical activity.

Proposed One-Year and Long-Term Outcomes: Results from this project will be submitted for publication in peer-reviewed journals, and for presentation at scientific conferences. In addition, this project will generate critical preliminary data to support a planned application for

an R01 grant from the NIH. Areas of focus for the new proposed external grant application include the use of brain imaging, biomarkers, and more detailed objective physical activity measurements through accelerometry, in order to deepen our understanding of the mechanisms of currently known susceptibility genes, identify novel genes that are implicated in cognitive decline and Alzheimer's disease susceptibility, and clarify exercise prescriptions for healthy brain aging.

Year End Progress Summary: In the past seven months, we have made progress on the above aims on several fronts. We have downloaded cognitive function data from the Atherosclerosis Risk in Communities study, and performed preliminary analyses on the interaction of self-reported physical activity with specific measures of genetic susceptibility to Alzheimer's disease (individual genetic markers and genetic risk scores), in relation to cross-sectional and longitudinal changes in cognitive function. We continue to explore and analyze this data, by analyzing other genetic markers, for example.

We have also obtained approval from the UK Biobank for this project, and have downloaded the main dataset, and have completed or initiated the downloading of bulk imaging and accelerometry data. We have examined specific variables of interest such as cognitive function, physical activity, and specific genetic markers, and have constructed several genetic risk scores. We have also begun to evaluate bivariate relationships among these variables, and performed preliminary interaction analyses.

Throughout this time, Drs. Klimentidis, Alexander, and Raichlen have been meeting on a weekly basis to discuss these datasets, preliminary data analyses, and to develop an NIH R01 grant proposal for submission, which is tentatively planned for this summer. We have begun to draft this proposal, including tables detailing the data that we will be able to analyze, and the potential specific aims.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Memory functioning in heart failure patients with risk for Alzheimer's Disease. Lee Ryan, PhD, Meredith Hay, PhD, Nancy Sweitzer, MD, PhD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims:

Aim 1: To expand a pilot study to evaluate the safety and efficacy of Ang 1-7 to enhance cognitive function in participants with mild to moderate heart failure.

Aim 2: To evaluate hippocampal and perirhinal function in a group of HF patients compared to age-matched controls using functional MRI.

Background and Significance: Heart failure (HF) is a chronic, progressive disease that disproportionately occurs in older adults. Cognitive impairment is common among HF patients. It is estimated that 25-85% of people with HF experience significant cognitive impairment than healthy persons of similar age. Cognitive impairment is associated with poor clinical outcomes in HF patients, including decreased ability to carry out self-care activities essential to management of their disease, more hospitalizations, higher mortality rates, and poorer medication adherence. Older adults (ages 60 or older) with HF experience faster age-related memory decline, and are at higher risk for developing Alzheimer's disease. Mechanisms thought to contribute to memory impairment in patients with HF include changes in cerebral blood flow (CBF), altered cerebrovascular autoregulation, microembolism, and inflammation. Increases in systemic inflammatory factors such as IL-1 and IL-6 in HF patients are strongly correlated with a decrease in cognitive performance.

Thus, there is an important clinical need for a safe and effective therapy for the treatment of cognitive impairment in patients with heart failure. The peptide Ang (1-7) is known to decrease brain ROS production and inflammation in pre-clinical models and is known to be safe in preliminary studies in humans. Ang (1-7) is an endogenous peptide hormone of the renin-angiotensin system with endogenous receptors known to be located in brain regions involved in memory. Studies in our laboratories have shown that Ang 1-7 reverses HF-induced memory impairment in an animal model of heart failure.

Preliminary Data: Several key steps have been completed that will allow us to carry out a larger treatment trial. Drug stability testing, a requirement for FDA approval, has been completed to 30 days. The IND for Ang1-7 was approved by the FDA in August 2015 for cardiac bypass surgery patients (IND 125320) and has been amended to include MCI patients with and without heart failure, based on work completed during this project. Enrollment in the treatment trial is ongoing. Preliminary data from the project has already resulted in two major donations from the Gooter Foundation (\$25,000) and an anonymous donor (\$8,900).

Proposed One-Year and Long-Term Outcomes: Data and findings from this proposed project will be submitted for presentation at relevant scientific conferences and in peer-reviewed manuscripts. In addition, the results will be used support submission of an RO1 to fund a large-

scale clinical trial of Ang 1-7 in heart failure patients and healthy older adults with Mild Cognitive Impairment.

Year End Progress Summary: To date, we have obtained full data from three patients who have completed the 3 month treatment trial, and another three patients have been enrolled and will be completing their treatment within the next few months. The results to date are promising. Patients tolerated the drug regimen without adverse events. In each patient, some measures of executive functioning and memory functioning improved, and depression measures improved in all three patients.

The second proposed study evaluating HF patients and matched controls on hippocampal and perirhinal functions is ongoing. Data collection should be completed by June, 2017.

Preliminary data from the HF treatment trial will be presented at the Spring Hippocampus Conference in Taormina, Italy in June, 2017. We are currently preparing an ROI that will be submitted to NIA/NINDS in March 2017.

We are very pleased to report that our first UO1 grant submitted to NIH/NHLBI has been awarded, and the official approval letter for the grant will be sent this week. This grant included safety and preliminary data obtained through the pilot study funded by AAC. In this study, patients undergoing cardiac bypass surgery will be treated with Angiotensin 1-7 or saline placebo for three weeks from the date of surgery, with the hypothesis that Ang 1-7 will decrease post-surgical delirium and cognitive impairment. The study will be conducted at Sarver Heart Center (University of Arizona) and the NIH Clinical Center in Bethesda.

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

Enhanced delivery of therapy to the brain in Alzheimer's mice. Ted Trouard, PhD, Marek Romanowski, PhD, Terry Matsunaga, PhD, Robert Erickson, MD. University of Arizona, Arizona Alzheimer's Consortium.

Specific Aims: The ability to deliver therapeutic drugs to the brain is often hindered by the blood brain barrier (BBB) and, because of this, therapies that work in cell cultures often do not provide benefit in humans. A relatively new technology that uses focused ultrasound (FUS) in conjunction with magnetic resonance imaging (MRI) guidance (referred to as **Magnetic Resonance-guided Focused Ultrasound, MRgFUS**), has potential to address this problem. When used in conjunction with FDA-approved **microbubble (μB)** ultrasound contrast agents, MRgFUS has been shown to be able temporarily make the BBB permeable to drugs in the vascular system. In this one-year project, we propose to evaluate the method for delivering cyclodextrin to the brains of Alzheimer's mice. We will also complete development of a new nanoparticle drug delivery system that will allow more efficient delivery of drugs to the brain while minimizing their exposure to peripheral organs.

Specific Aim 1. Evaluate MRgFUS for the delivery of drug in Alzheimer's mice. Building on our previous work, we will evaluate MRgFUS for delivery of hydroxypropyl-beta-cyclodextrin in the PS1/APP double transgenic mouse model of AD. MRI will be used to evaluate the BBB opening to molecules of a similar molecular weight of HP β CD.

Specific Aim 2. Evaluate liposome- μB nanoparticles for enhancing the delivery of therapy to the brain. Liposomes that can be internally loaded with therapeutics, e.g. HP β CD, will be conjugated to the lipid monolayer of μBs . These liposome- μB nanoparticles will then be used in the MRgFUS experiments to deliver therapy to the brain.

Background and Significance: The effectiveness of drugs for treating neurological diseases is continually hindered by the inability of drugs to cross the blood brain barrier (BBB) and unless significant advancements are made to safely deliver drugs to the brain, drug development for neurological disease will remain limited. Existing techniques for circumventing the BBB have had limited success. Intraventricular infusion is a poor method of delivering drugs to the brain because it requires skull penetration and introduces the risk of infection. In addition, drugs are rapidly cleared by the CSF and diffusion of drugs into tissue can be minimal. MRgFUS techniques use focused ultrasound (FUS) in combination with FDA-approved intravascular microbubble (μB) contrast agents to locally and temporarily open the BBB to intravenous drugs. In combination with magnetic resonance imaging (MRI), local regions of FUS delivery can be accurately determined so that drug delivery to specific brain regions can be achieved.

Preliminary Data: Over the last three years, we have developed the capability to carry out MRgFUS in mice to deliver drugs to the brain. We have designed and built an MRI compatible FUS system in collaboration with Synergy Electronics (Tempe, AZ) that fits within the bore of our 7T small-animal MRI magnet and will allow targeting and monitoring of the delivery of FUS and the opening of the BBB. Bench top experiments have been carried out that demonstrate the ability of the FUS system and the MRgFUS technique to safely and consistently open the BBB in

mice and monitor the opening to MRI contrast agent. We have also miniaturized the system to work within the 7T magnet.

We have also initiated development of more efficient drug delivery systems to be used in MRgFUS. In our initial work in this area, we have developed the facilities and techniques necessary to fabricate liposome- μ B nanoparticles. Their structure has been verified by microscopy and we have utilized them in preliminary MRgFUS experiments in mice.

Proposed One-Year and Long-Term Outcomes: We aim to collect the data necessary to publish two papers on this work. A R01 grant has been re-submitted to the NIH and we expect that the data obtained from this project will jump-start the experiments in the R01 grant to ensure quick progress and ongoing funding.

Year End Progress Summary: We have made significant progress on Aim 2 of the project. Through work carried out in this project, we submitted an invention disclosure on a novel nanoparticle drug delivery system which the University of Arizona subsequently filed an international patent application PCT US16/62728 on 11/20/2016. Title of Invention: PHASE CHANGE NANODROPLET CONJUGATES FOR TARGETED DELIVERY. Also, Ryan Rath, a graduate student in Biomedical Engineering, received his MS degree in January of 2017 from his work on this project. Because of the emphasis on Aim 2, because of the need to generate data for the patent application, the work on Aim 1 has been delayed. The R01 grant that was resubmitted to the NIH was unfortunately unscored on resubmission and we are working on a plan to reformulate the application into a new submission. One manuscript involving mouse brain imaging has been submitted for publication and a second manuscript is in preparation for submission by April, 2017.

Project Progress Reports
University of Arizona
College of Medicine – Phoenix

ARIZONA ALZHEIMER'S CONSORTIUM

2016-2017 Scientific Progress Report

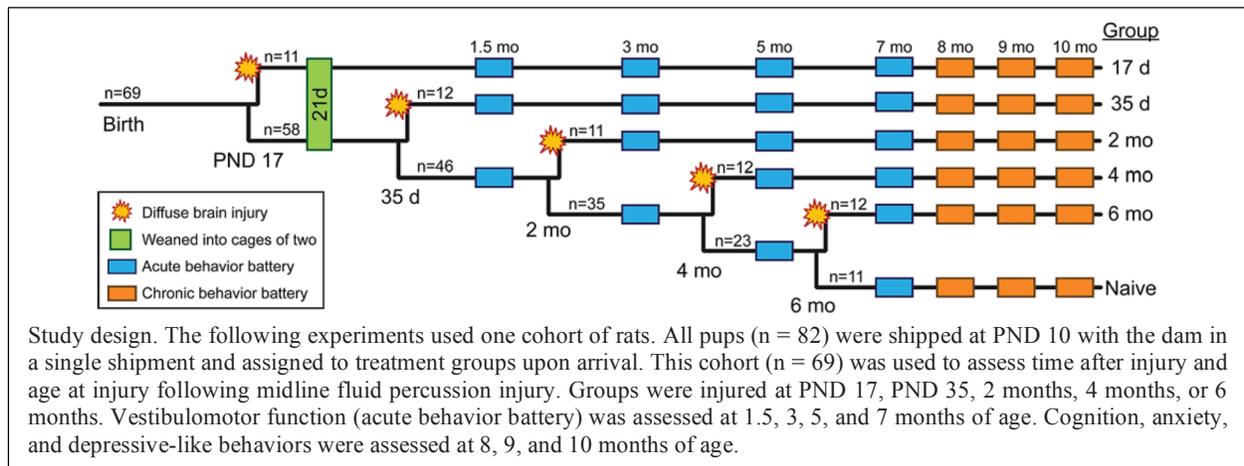
Aging with traumatic brain injury. Jonathan Lifshitz, PhD. UA College of Medicine – Phoenix, Barrow Neurological Institute at Phoenix Children's Hospital, Phoenix Veterans Administration Healthcare System, Arizona Alzheimer's Consortium.

Scope: In our Translational Neurotrauma Research Program, we investigate the consequences of TBI across the lifespan and the contributing factors of inflammation in the process. We continue investigating brain injury across the lifespan of the rodent, while mechanistically exploring the role of microglia in injury-related inflammation. The long term goal is determining key mechanisms responsible for accelerated age-related decline in neurological performance due to TBI.

Project Aims:

Project 1: Age-at-injury effects on behavioral outcome

Development and aging are influenced by external factors with the potential to impact health throughout the life span. Traumatic brain injury (TBI) can initiate and sustain a lifetime of physical and mental health symptoms. Over 1.7 million TBIs occur annually in the USA alone, with epidemiology suggesting a higher incidence for young age groups. Additionally, increasing life spans mean more years to age with TBI. While there is ongoing research of experimental pediatric and adult TBI, few studies to date have incorporated animal models of pediatric, adolescent, and adult TBI to understand the role of age-at-injury across the life span. **Here, we explored repeated behavioral performance between rats exposed to diffuse TBI at five different ages.** Our aim was to follow neurological morbidities across the rodent life span with respect to age-at-injury. A single cohort of male Sprague-Dawley rats (n = 69) was received at postnatal day (PND) 10 (Study Design below). Subgroups of this cohort (n = 11–12/group) were subjected to a single moderate midline fluid percussion injury at age PND 17, PND 35, 2 months, 4 months, or 6 months. A control group of naïve rats (n = 12) was assembled from this cohort. The entire cohort was assessed for motor function by beam walk at 1.5, 3, 5, and 7 months of age. Anxiety-like behavior was assessed with the open field test at 8 months of age. Cognitive performance was assessed using the novel object location task at 8, 9, and 10 months



of age. Depression-like behavior was assessed using the forced swim test at 10 months of age. Age at injury and time since injury differentially influenced motor, cognitive, and affective behavioral outcomes. Motor and cognitive deficits occurred in rats injured at earlier developmental time points, but not in rats injured in adulthood. In contrast, rats injured during adulthood showed increased anxiety-like behavior compared to uninjured control rats. A single diffuse TBI did not result in chronic depression-like behaviors or changes in body weight among any groups. The interplay of age-at-injury and aging-with-injury are translationally important factors that influence behavioral performance as a quality of life metric. More complete understanding of these factors can direct rehabilitative efforts and personalized medicine for TBI survivors.

Project 2: Age-at-injury effects on pathophysiology

Traumatic brain injury (TBI) is more than a singular event, such that a disease state with evolving pathophysiology ensues over an extended timeframe following the initial insult. Effective recovery from injury and enduring neuropathology may depend on many parameters, including the biological age-at-injury. A vulnerable population of TBI survivors includes children and adolescents whose on-going developmental processes are diverted by TBI pathophysiology. Throughout “normal” aging, neuroinflammatory status is in constant flux and homeostatic processes oscillate. TBI activates inflammatory processes, such that the sustained inflammatory state disrupts neurovascular units, including microglia, that can contribute to injury-induced behavioral morbidities. Given the comparable inflammatory profiles of the aged and the injured brain, **we hypothesize that TBI accelerates pathological hallmarks of aging in the brain.** In the same cohort as Project 1 above, histopathology was conducted at 10 months of age to assess the consequences of prior exposure to brain injury. A skeleton analysis method was used to quantify microglial ramification in Iba-1 stained cortical tissue. There was no overall difference in number of microglia endpoints per cell or process length per cell among treatment groups at 10mo of age. For the first time, rod microglia, an activated morphology identified in brain tissue following diffuse brain injury, Alzheimer’s disease, and aging, were identified in rats that were injured at PND17, PND35, and 6mo. The interplay of age-at-injury and aging with an injury are translationally important factors and continued investigation of injury-induced neuropathology, including astrocytes and neurons are ongoing. More complete understanding of these factors can direct therapeutic interventions and personalized medicine for TBI survivors.

Impact: The impact of the proposed work relates to the world-wide prevalence of TBI in the context of increasing lifespan. Modern lifestyle adaptations and biomedical advancements have increased lifespan around the world. Living longer means that more individuals are susceptible to age-related neurological conditions, particularly cognitive decline, dementia and Alzheimer’s disease. In addition, over this lifetime, recreational and organized activities have inherent and hidden risks for sustaining a TBI, where over 1-2 million TBIs occur annually in the United States alone. For professional football players, 25-30 years worth of helmet-to-helmet contact in practice and competition have shown adverse outcome in terms of suicide, depression and cognitive decline. The ravages of TBI resemble the histological hallmarks of Alzheimer’s disease, suggesting a correlation between TBI and age-related neurological conditions. The same processes may be at play for any single TBI, whether a result of car accident, fall, sports, or combat. For these reasons, we explore the progressive behavioral performance and terminal

histopathology in rodents exposed to TBI at different times throughout the lifespan. Additional projects have emerged as outlined below.

Remainder of proposed work: The Arizona Alzheimer's Consortium support for the UA College of Medicine – Phoenix was experimental two years ago. After an initial year of funding (FY14-15) and the subsequent gap year (FY15-16), the current funding (FY16-17) has resulted in one publication on the behavioral outcomes and a growing dataset on histopathological findings. Initial results indicate a sustained, and bi-modal, inflammatory profiles with respect to age-at-injury. Remaining work includes additional histological and immunohistochemical stains. In our group, we seek objective, quantitative analysis procedures to represent the pathological burden, as reported for the skeletal analysis. These techniques will be applied to digital micrographs acquired by light, fluorescent or confocal microscope of the cortex, hippocampus and thalamus. These regions of interest have been chosen due to involvement in cognitive function and the presence of neuropathology following diffuse TBI. Histological results are targeting the extent of cellular senescence and injury-induced inflammation as a function of age-at-injury.

Long term plan: At the conclusion of Project 2, both neurological behavior and chronic histopathology directed toward inflammation will be complete. New questions and opportunities have emerged that will pursue the role of inflammation in the development of neurological impairments and associated pathology. Four potential projects are as follows:

A. Behavioral and histopathological outcome from TBI in the absence of microglia

We propose a novel approach to evaluate the role of microglia in TBI. **Our objective is the transient depletion and repopulation of microglia from the brain to evaluate the impact on behavioral performance and histological outcome.** To this end, young adult rodents will be microglia depleted before or after diffuse TBI, evaluated on a battery of behavioral tasks, and examined histologically. Microglia depletion will be accomplished by dietary supplementation of PLX3397, an inhibitor of the colony stimulating factor (CSF) 1 receptor. As such, timing of brain injury and PLX3397 diet can differentially remove microglia from post-injury pathophysiology. Behavioral and histological outcomes will mirror those in Project 1 and 2 above. We anticipate pilot data to support subsequent grant submissions (e.g. NIH R21) in determining the role for existing microglia, re-populated microglia, and the consequences therein.

B. Phenotype profile and regulation of circulating monocytes and macrophages

This study aims to determine a non-invasive, nontoxic, and universally available method to prevent chronic neurological impairment in TBI. Remote ischemic conditioning (RIC) protocols restrict blood flow to a limb to generate pro-resolving molecules that can minimize damage to a vital organ, such as the brain. The exact mechanism(s) of RIC is poorly understood, with evidence for reducing inflammation. Surprisingly, central brain injury induces a peripheral macrophage response, which can amplify and sustain the inflammatory pathophysiology. **Thus, we hypothesize that RIC modulates the peripheral immune response to ameliorate TBI-induced neuroinflammation and behavioral deficits.** Specific aims may include whether (a) RIC modulates the peripheral inflammatory response post-injury, and (b) early or repeated RIC are effective treatments for chronic neurological and histological outcomes of TBI. The macrophage response will be quantified by flow cytometry of blood, spleen, and brain samples. Cognitive outcomes will be measured chronically using Barnes maze, Y maze, and novel object

recognition testing. These studies will illuminate the role of peripheral, chronic inflammation in TBI, thereby identifying a therapeutic target and treatment mechanism for TBI and neurodegenerative conditions. We continue to apply for pilot research funds through local and state organizations.

C. Exploration of cognitive performance and late rehabilitation

The protracted disease course of TBI dissuades continued pharmacological interventions in favor of rehabilitation strategies to alleviate neurological impairment. Rehabilitation strategies have reported mixed results, as most do not focus on specific symptomatology. Experimental diffuse TBI causes clinically-relevant impairment in short term, long term, and working memory, as measured in novel object recognition tasks. Therefore, laboratory studies provide a platform to evaluate efficacy and mechanism of rehabilitation strategies to mitigate neurological symptoms after diffuse brain injury. **We test the hypothesis that effective brain injury rehabilitation depends on neuronal activation and circuit reorganization.** Aim 1 addresses whether tactile exploration and spatial navigation through a peg forest rehabilitation environment for three weeks at 1 month post-injury restores cognitive performance months post-injury. Aim 2 addresses whether the efficacy of rehabilitation depends on neuronal activation, using local infusions of muscimol. Aim 3 quantifies rehabilitation-related structural and functional changes in cognitive circuitry. Expected outcomes would show therapeutic efficacy of peg forest rehabilitation on cognitive performance, which depended on neuronal activation and structural change in circuitry. The impact of these studies is a firm foundation to promote rehabilitation strategies, rather than rest, in the recovery from TBI. Ongoing extramural grant submissions (NIH R21, VA Merit) call for pilot data to support the applications.

D. Chronic vascular injury

TBI induces acute and long term cerebrovascular dysfunction, where cardiovascular risk factors are strongly linked with dementia. In fact, vascular dysfunction leading to cerebral hypoperfusion is critical in the early and late stages of AD. **We hypothesize that an important etiopathologic basis of TBI-related late cognitive dysfunction is TBI-induced cerebrovascular dysfunction and vascular inflammation that lead to chronic brain hypoperfusion.** We will evaluate the extent and mechanisms of cerebrovascular dysfunction and inflammation in rats exposed to experimental diffuse TBI and establish the relationship to cognitive function. Following TBI or sham surgery, we will compare 180-day in-vivo cerebral flow (CBF) and cerebrovascular reactivity using MRI, and ex-vivo endothelial and smooth muscle-dependent function of isolated circle of Willis cerebral arteries from TBI versus uninjured rats and determine the relationship of vascular function with measures of cognitive function (novel object recognition tasks) and degree of neuropathology. The signaling pathways explored in this proposal will enhance our understanding of the interplay between cardiovascular risk factors and late cognitive dysfunction associated with early-life TBI. We seek to expand the preliminary data necessary for competitive VA Merit and NIH R01 funding.

2016– 2017
Publications, Manuscripts,
& Grants

2016 Publications and Manuscripts

Adler CH, Beach TG. Neuropathological basis of nonmotor manifestations of Parkinson's disease. *Mov Disord*. 2016 Aug;31(8):1114-9. doi: 10.1002/mds.26605. Review. PubMed PMID: 27030013; PubMed Central PMCID: PMC4981515.

Adler CH, Dugger BN, Hentz JG, Hinni ML, Lott DG, Driver-Dunckley E, Mehta S, Serrano G, Sue LI, Duffy A, Intorcchia A, Filon J, Pullen J, Walker DG, Beach TG. Peripheral Synucleinopathy in Early Parkinson's Disease: Submandibular Gland Needle Biopsy Findings. *Mov Disord*. 2016 Feb;31(2):250-6. doi: 10.1002/mds.26476. PubMed PMID: 26799362; PubMed Central PMCID: PMC4747813.

Adler CH, Hentz JG, Beach TG. Assessing medication role on neuropathological findings in Parkinson's disease. *Mov Disord*. 2016 May;31(5):613-4. doi: 10.1002/mds.26536. PubMed PMID: 26879513; PubMed Central PMCID: PMC4861676.

Aguirre-Acevedo DC, Jaimes-Barragan F, Henao E, Tirado V, Munoz C, Reiman EM, Tariot PN, Langbaum JB, Lopera F. Diagnostic accuracy of CERAD total score in a Colombian cohort with mild cognitive impairment and Alzheimer's disease affected by E280A mutation on presenilin-1 gene. *Int Psychogeriatr*. 2016;28(3):503-10. doi: 10.1017/S1041610215001660. PubMed PMID: 26478578.

Aguirre-Acevedo DC, Lopera F, Henao E, Tirado V, Muñoz C, Giraldo M, Bangdiwala SI, Reiman EM, Tariot PN, Langbaum JB, Quiroz YT, Jaimes F. Cognitive Decline in a Colombian Kindred With Autosomal Dominant Alzheimer Disease: A Retrospective Cohort Study. *JAMA Neurol*. 2016 Apr;73(4):431-8. doi:10.1001/jamaneurol.2015.4851. PubMed PMID: 26902171.

Aisen P, Touchon J, Andrieu S, Boada M, Doody R, Nosheny RL, Langbaum JB, Schneider L, Hendrix S, Wilcock G, Molinuevo JL, Ritchie C, Ousset PJ, Cummings J, Sperling RA, DeKosky S, Lovestone S, Hampel H, Petersen R, Legrand V, Egan M, Randolph C, Salloway S, Weiner M, Vellas B. Registries and cohorts to accelerate early phase Alzheimer's trials: A report from the E.U./U.S. Clinical Trials in Alzheimer's Disease Task Force. *J Alzheimer's Prevention*. 2016;3(2):67-74.

Ali H, Alquarni AS, Owayss AA, Hassan AM, Smith BH (2016) Osmotic concentration in three races of honey bee, *Apis mellifera* L. under environmental conditions of arid zone. *Saudi J Biological Sciences* online 23 Dec 2016

Allen AM, Burke AD, Dougherty J, Petitti DB, Burke WJ, Zamrini E, Anderson D, Howard J, Reiman EM, Tariot PN. Banner Alzheimer's Institute Dementia Care Initiative: Design and Implementation of the Pilot Phase. Poster presentation. AAIC annual meeting, Toronto, Canada, July 2016.

Alzheimer's Disease Neuroimaging Initiative, Li B, Shi J, Gutman BA, Baxter LC, Thompson PM, Caselli RJ, Wang Y. Influence of APOE Genotype on Hippocampal Atrophy over Time -

An N=1925 Surface-Based ADNI Study. PLoS One 2016; 11 (4):e0152901 Epub 2016 Apr 11 PMID:27065111 PMCID:4827849 DOI:10.1371/journal.pone.0152901

Anand K, Sabbagh MN. Amyloid imaging: poised for integration into medical practice. *Neurotherapeutics* . 2016 Aug 29. [Epub ahead of print] Review.

Ashley Levan A, Black G, Mietchen J, Baxter L, Kirwan CB, Gale SD. Right frontal pole cortical thickness and executive functioning in children with traumatic brain injury: the impact on social problems. *Brain Imaging and Behavior*, 2015, Nov 5. PMID: 26542618

Babiloni C, Del Percio C, Caroli A, Salvatore E, Nicolai E, Marzano N, Lizio R, Cavedo E, Landau S, Chen K, Jagust W, Reiman E, Tedeschi G, Montella P, De Stefano M, Gesualdo L, Frisoni GB, Soricelli A. Cortical sources of resting state EEG rhythms are related to brain hypometabolism in subjects with Alzheimer's disease: an EEG-PET study. *Neurobiol Aging*. 2016;48:122-34. doi: 10.1016/j.neurobiolaging.2016.08.021. PubMed PMID: 27668356.

Bahureksa L, Najafi, B., Saleh, A., Sabbagh, M., Coon, D.W., Mohler, J. & Schwenk, M. (in press). The impact of mild cognitive impairment on balance and gait: A systematic review and meta-analysis. *Gerontology*.

Bahureska L, Schwenk M, Sabbagh M, Mohler J, Saleh A, Najafi B. The Impact of Mild Cognitive Impairment on Balance and Gait: a Systematic Review and Meta-Analysis. *Gerontology* 2016; (DOI:10.1159/000445831)

Baxter, LC. Appetite Changes in Depression (Invited Editorial) *Am J Psychiatry* 2016; 173:1–2; doi: 10.1176/appi.ajp.2016.16010010.

Beach TG, Adler CH, Serrano G, Sue LI, Walker DG, Dugger BN, Shill HA, Driver-Dunckley E, Caviness JN, Intorcchia A, Filon J, Scott S, Garcia A, Hoffman B, Belden CM, Davis KJ, Sabbagh MN. Prevalence of Submandibular Gland Synucleinopathy in Parkinson's Disease, Dementia with Lewy Bodies and other Lewy Body Disorders. *J Parkinsons Dis*. 2016 Jan 9. [Epub ahead of print]

Beach TG, Adler CH, Sue LI, Serrano G, Shill HA, Walker DG, Lue L, Roher AE, Dugger BN, Maarouf C, Birdsill AC, Intorcchia A, Saxon-Labelle M, Pullen J, Scroggins A, Filon J, Scott S, Hoffman B, Garcia A, Caviness JN, Hentz JG, Driver-Dunckley E, Jacobson SA, Davis KJ, Belden CM, Long KE, Malek-Ahmadi M, Powell JJ, Gale LD, Nicholson LR, Caselli RJ, Woodruff BK, Rapsack SZ, Ahern GL, Shi J, Burke AD, Reiman EM, Sabbagh MN. Arizona Study of Aging and Neurodegenerative Disorders and Brain and Body Donation Program. *Neuropathology*. 2015 Jan 26. doi: 10.1111/neup.12189. [Epub ahead of print]

Beach TG, Corbillé AG, Letournel F, Kordower JH, Kremer T, Munoz DG, Intorcchia A, Hentz J, Adler CH, Sue LI, Walker J, Serrano G, Derkinderen P. Multicenter Assessment of Immunohistochemical Methods for Pathological Alpha-Synuclein in Sigmoid Colon of Autopsied Parkinson's Disease and Control Subjects. *J Parkinsons Dis*. 2016 Oct 19;6(4):761-770. PubMed PMID: 27589538.

Beach TG, Thal DR, Zanette M, Smith A, Buckley C. Detection of Striatal Amyloid Plaques with [18F]flutemetamol: Validation with Postmortem Histopathology. *J Alzheimers Dis.* 2016 Mar 31;52(3):863-73. doi: 10.3233/JAD-150732. PubMed PMID: 27031469.

Beck JS, Mufson EJ, Counts SE.: Evidence for mitochondrial UPR gene activation in familial and sporadic Alzheimer's disease. *Curr Alz. Res.* 37:147-53, 2016. 2015. PMID: 26687188
Counts, S, E, and E.J. Mufson: Regulator of Cell Cycle (RGCC) expression during the progression of Alzheimer's disease, Cell Transplantation, in press.

Berman BP, Pandey A, Li Z, Jeffries L, Trouard TP, Oliva I, Cortopassi F, Martin DR, Altbach MI, Bilgin A (2016) Volumetric MRI of the lungs during forced expiration. *Magnetic Resonance in Medicine*, 75:2295-2302.

Birch K, Ten Hope, M, Malek-Ahmadi M, O'Connor K, Schofield S, Coon D, Nieri W (2016). Cognitive function as a mediator in the relationship between physical activity and depression status in older adults. *Journal of Aging & Physical Activity*, 24, 450-546.

Birgiolas J, Jernigan CM, Smith BH, Crook S (2016) SwarmSight: Measuring the temporal progression of animal group activity levels from natural scene and laboratory videos. *Behavior Res Methods*. Apr 29 Epub

Biwer LA, D'souza KM, Abidali A, Tu D, Siniard AL, DeBoth M, Huentelman M, Hale TM. Hypertension research: official journal of the Japanese Society of Hypertension. 2016; 39(1):8-18. Time course of cardiac inflammation during nitric oxide synthase inhibition in SHR: impact of prior transient ACE inhibition. PMID: 26490086

Bluett B, Litvan I, Cheng S, Juncos J, Riley DE, Standaert DG, Reich SG, Hall DA, Kluger B, Shprecher D, Marras C, Jankovic J; ENGENSE PSP study.. Understanding falls in progressive supranuclear palsy. *Parkinsonism Relat Disord.* 2016 Dec 15. pii: S1353-8020(16)30490-4. doi: 10.1016/j.parkreldis.2016.12.009. [Epub ahead of print] PubMed PMID: 28007518.

Borad MJ, Egan JB, Condjella RM, Liang WS, Fonseca R, Ritacca NR, McCullough AE, Barrett MT, Hunt KS, Champion MD, Patel MD, Young SW, Silva AC, Ho TH, Halfdanarson TR, McWilliams RR, Lazaridis KN, Ramanathan RK, Baker A, Aldrich J, Kurdoglu A, Izatt T, Christoforides A, Cherni I, Nasser S, Reiman R, Cuyugan L, McDonald J, Adkins J, Mastrian SD, Valdez R, Jaroszewski DE, Von Hoff DD, Craig DW, Stewart AK, Carpten JD, Bryce AH (2016). Clinical implementation of integrated genomic profiling in patients with advanced cancers. *Scientific Reports* 6(1):25.

Braden BB, Andrews M.G., Acosta JI, Mennenga S.E., Lavery C., Bimonte-Nelson H.A. (2016) A comparison of progestins within three classes: Differential effects on learning and memory in the aging surgically menopausal rat. *Behav Brain Research.* S0166-4328(16)30417. Pubmed PMID: 27368418.

Braden BB, Dassel KB, Bimonte-Nelson HA, O'Rourke HP, Connor DJ, Moorhous S, Sabbagh MN, Caselli RJ, Baxter LC. Sex and post-menopause hormone therapy effects on hippocampal

volume and verbal memory. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* 2016 Jun 4; 1-20 Epub 2016 June 04 PMID:27263667

Braden BB, Pipe TB, Smith R, Glaspy TK, Deatherage BR, Baxter LC. Brain and behavior changes associated with an abbreviated four-week mindfulness-based stress reduction course in back pain patients. *Brain and Behavior*, March 2016, doi: 10.1002/brb3.443.

Brinton RD (2016) Neuroendocrinology: Oestrogen therapy affects brain structure but not function. *Nat Rev Neurol.*, 12:561-562.

Brody DM, Litvan I, Warner S, Riley DE, Hall DA, Kluger BM, Shprecher DR, Cunningham CR. Relationship between uric acid levels and progressive supranuclear palsy. *Mov Disord.* 2016 May;31(5):663-7. doi: 10.1002/mds.26535. PubMed PMID: 26890571.

Bryce AH, Borad MJ, Egan JB, Condjella RM, Liang WS, Fonseca R, McCullough AE, Hunt KS, Ritacca NR, Barrett MT, Patel MD, Young SW, Silva AC, Ho TH, Halfdanarson TR, Stanton ML, Chevillie J, Swanson S, Schneider DE, McWilliams RR, Baker A, Aldrich J, Kurdoglu A, Izatt T, Christoforides A, Cherni I, Nasser S, Reiman R, Cuyugan L, McDonald J, Adkins J, Mastrian SD, Von Hoff DD, Craig DW, Stewart AK, Carpten JD (2016). Comprehensive genomic analysis of metastatic mucinous urethral adenocarcinoma guides precision oncology treatment: Targetable EGFR amplification leading to successful treatment with Erlotinib. *Clin Genitourinary Canc* pii: S1558-7673(16)30337-8 [Epub ahead of print].

Burden CM, Elmore C, Hladun KR, Trumble JT, Smith BH. (2016) Acute exposure to selenium disrupts associative conditioning and long-term memory recall in honey bees (*Apis mellifera*). *Ecotoxicol. Environ. Safety* 127: 71-79.

Burke AD, Riggs G, Weidman D, Burke WJ, Brand H. Movements and Memories. *Prim Care Companion CNS Disord.* 2016;18 (1)

Burke AD, Tariot PN. Treatments for behavioural and psychological symptoms in Alzheimer's disease and other dementias, in Ames D, O'Brien J, Burns A. *Dementia*, Hodder Arnold Pub, 2016

Byron SA, Van Keuren-Jensen KR, Engelthaler DM, Carpten JD, Craig DW. Translating RNA sequencing into clinical diagnostics: opportunities and challenges. *Nature reviews. Genetics.* 2016; 17(5):257-71. PMID: 26996076

Caccamo A, Ferreira E, Branca C, Oddo S. p62 improves AD-like pathology by increasing autophagy. *Molecular Psychiatry* 2016, in press.

Chang P, Li X, Ma C, Zhang S, Liu Z, Chen K, Ai L, Chang J, Zhang Z. The Effects of an APOE Promoter Polymorphism on Human White Matter Connectivity during Non-Demented Aging. *J Alzheimers Dis.* 2016. doi: JAD160447 [pii];10.3233/JAD-160447 [doi].

Carasquillo M, Younkin SG, Jakobsdóttir J, Kauwe JS, Wilhelmsen KC, Rujescu D, Nöthen MM, Hofman A, Jones L, Haines JL, Psaty BM, Van Broeckhoven C, Holmans P, Launer LJ, Mayeux R, Lathrop M, Goate AM, Escott-Price V, Seshadri S, Pericak-Vance MA, Amouyel P, Williams J, van Duijn CM, Schellenberg GD, Farrer LA. A novel Alzheimer disease locus located near the gene encoding tau protein. *Molecular psychiatry*. 2016; 21(1):108-17. NIHMSID: NIHMS654371 PMID: 25778476 PMCID: PMC4573764

Caselli RJ, Dueck AC, Locke DE, Baxter LC, Woodruff BK, Geda YE. Sex-based memory advantages and cognitive aging: a challenge to the cognitive reserve construct? *J Int Neuropsychol Soc*. 2015 Feb;21(2):95-104. doi: 10.1017/S1355617715000016. Epub 2015 Feb 9. PubMed PMID: 25665170.

Caselli RJ, Dueck AC, Locke DE, Henslin BR, Johnson TA, Woodruff BK, Hoffman-Snyder C, Geda YE. Impact of Personality on Cognitive Aging: A Prospective Cohort Study. *J Int Neuropsychol Soc* 2016 Aug; 22 (7):765-76 Epub 2016 June 27 PMID:27346168 DOI:10.1017/S1355617716000527

Caselli RJ, Woodruff BK. Clinical Impact of Amyloid Positron Emission Tomography-Is It Worth the Cost? *JAMA Neurol* 2016 Dec 01; 73 (12):1396-1398 PMID:27802483 DOI:10.1001/jamaneurol.2016.3792

Caviness JC, Utanski RL, Hentz JL, Beach JG, Dugger BN, Shill HA, Driver-Dunckley E, Sabbagh MN, Mehta S, Adler CA. Differential Spectral Quantitative EEG Patterns between Control and Parkinson's disease Cohorts. *European Journal of Neurology* 2016 Feb;23(2):387-92. doi: 10.1111/ene.12878; PMID:26518336

Caviness JN, Lue LF, Hentz JG, Schmitz CT, Adler CH, Shill HA, Sabbagh MN, Beach TG, Walker DG. Cortical phosphorylated α -Synuclein levels correlate with brain wave spectra in Parkinson's disease. *Mov Disord*. 2016 Jul;31(7):1012-9. doi: 10.1002/mds.26621. PubMed PMID: 27062301; PubMed Central PMCID: PMC4931950.

Chen Y, Liu C, Parker WD, Chen H, Beach TG, Liu X, Serrano GE, Lu Y, Huang J, Yang K, Wang C. Mitochondrial DNA Rearrangement Spectrum in Brain Tissue of Alzheimer's Disease: Analysis of 13 Cases. *PLoS One*. 2016 Jun 14;11(6):e0154582. doi: 10.1371/journal.pone.0154582. PubMed PMID: 27299301; PubMed Central PMCID: PMC4907522.

Chen K, Sapidis N, Sarraga R, Strotman T, Wolters H, Xie Z. . A CAD tribute to Gerald Farin. *Journal Computer-Aided Design*. 2016. Epub July 2016. doi: doi: 10.1016/j.cad.2016.07.007.
Chen K XZ, Reiman E.M. Tribute to Prof. Gerald Farin: Remembering A Geometric Modeling Researcher and Educator. *Neuroscience and Biomedical Engineering*. 2016. Epub May. doi: 10.2174/221338520401160511100538.

Chen Y, Liu Z, Wang A, Zhang J, Zhang S, Qi D, Chen K, Zhang Z. Dysfunctional organization of default mode network before memory impairments in type 2 diabetes.

Psychoneuroendocrinology. 2016;74:141-8. doi: S0306-4530(16)30573-X [pii];10.1016/j.psyneuen.2016.08.012 [doi].

Chen Y, Liu Z, Zhang J, Chen K, Yao L, Li X, Gong G, Wang J, Zhang Z. Precuneus degeneration in nondemented elderly individuals with APOE varepsilon4: Evidence from structural and functional MRI analyses. *Hum Brain Mapp.* 2016. doi: 10.1002/hbm.23359 [doi].

Coon DW, Besst, D. A., Doucet, J. S., Chavez, A., Fenzi, M., Raach, K. et al. (2016). CarePRO: Embedding an evidence-based intervention for caregiver empowerment. *Journal of the Arizona Geriatrics Society*, 22, 9-13.

Corbillé AG, Letournel F, Kordower JH, Lee J, Shanes E, Neunlist M, Munoz DG, Derkinderen P, Beach TG. Evaluation of alpha-synuclein immunohistochemical methods for the detection of Lewy-type synucleinopathy in gastrointestinal biopsies. *Acta Neuropathol Commun.* 2016 Apr 4;4:35. doi: 10.1186/s40478-016-0305-8. PubMed PMID: 27044604; PubMed Central PMCID: PMC4820972.

Corenblum MJ, Ray S, Remley QW, Long M, Harder B, Zhang DD, Barnes CA, Madhavan L (2016) Reduced NrF2 expression mediates the decline in neural stem cell function during a critical middle-age period. *Aging Cell*, 4:725-736.

Craig DW, Nasser S, Corbett R, Chan SK, Murray L, Legendre C, Tembe W, Adkins J, Kim N, Wong S, Baker A, Enriquez D, Pond S, Pleasance E, Mungall AJ, Moore RA, McDaniel T, Ma Y, Jones SJ, Marra MA, Carpten JD, Liang WS. A somatic reference standard for cancer genome sequencing. *Scientific reports.* 2016; 6:24607. PMID: 27094764 PMCID: PMC4837349

Decourt B, Drumm-Gurnee D, Wilson J, Sandra Jacobson MD¹, Belden C, Sirrel S, Ahmadi M, Shill H, Powell J, Walker A, Gonzales A, Macias M, Sabbagh MN. Poor Safety and Tolerability Hamper Reaching a Potentially Therapeutic Dose in the Use of Thalidomide for Alzheimer's disease: Results from a Double-Blind, Placebo-Controlled Trial Current Alzheimer's Research 2015 (in press)

Decourt B, Lahiri DK, Sabbagh MN. Targeting Tumor Necrosis Factor Alpha for Alzheimer's Disease. *Curr Alzheimer Res.* 2016 Sep 30.

Delabar, JM, Allinquant B, Bianchi, D., Blumenthal, T., Dekker, A., Edgin, J., O'Bryan, J., Diersssen, M., Potier, M.C., Wiseman, F., Guedj, F., Creau, N., Reeve, R., Gardiner, K. and Busciglio, J. (2016) Changing Paradigms in Down Syndrome: The First International Conference of the Trisomy 21 Research Society. *Molecular Syndromology*, 7:251-261.

Delvaux E, D. Mastroeni, J. Nolz, P.D. Coleman, Novel method to ascertain chromatin accessibility at specific genomic loci from frozen brain homogenates and laser capture microdissected defined cells, *Neuroepigenetics*, 6 (2016) 1-9.

Dugger BN, Whiteside CM, Maarouf CL, Walker DG, Beach TG, Sue LI, Garcia A, Duncley T, Meechoovet B, Reiman EM, Roher AE. The Presence of Select Tau Species in Human

Peripheral Tissues and Their Relation to Alzheimer's Disease. *J Alzheimers Dis.* 2016;54(3):1249. doi: 10.3233/JAD-169007. PubMed PMID: 27716678.

Dugger BN, Malek-Ahmadi M, Monsell SE, Kukull WA, Woodruff BK, Reiman EM, Beach TG, Wilson J. A Cross-Sectional Analysis of Late-Life Cardiovascular Factors and Their Relation to Clinically Defined Neurodegenerative Diseases. *Alzheimer Dis Assoc Disord.* 2016 Jan 11. [Epub ahead of print] PMID: 26756386; PMCID: PMC4940303.

Engle J, Machado C, Permenter M, Vogt J, Maurer A, Bulleri A, Barnes, C.A. (2016) Network patterns associated with navigation behavior are altered in aged nonhuman primates. *Journal of Neuroscience*, 36:12217-12227.

Evans B & Coon DW (2016). The “Reckoning Point” as a marker for palliative and end of life care in Mexican American families. *Journal of Family Nursing.* 22, 606-630.

Evans B, Coon DW, Belyea MJ, Ume E (in press). Collective care: Multiple caregivers and multiple care recipients in Mexican American families. *Journal of Transcultural Nursing.*

Fa M, et al., *Extracellular Tau Oligomers Produce An Immediate Impairment of LTP and Memory.* *Sci Rep*, 2016. 6: p. 19393.

Fernandez F & Edgin JO (2016) Pharmacotherapy in Down syndrome: Which way forward? *Lancet Neurology*, 15:776-777.

Ferreira E, Shaw DM, Oddo S. Identification of learning-induced changes in protein networks in the hippocampi of a mouse model of Alzheimer's disease. *Transl Psychiatry.* 2016 Jul 5;6(7):e849.

Feyma T, Ramsey K, Huentelman MJ, Craig DW, Padilla-Lopez S, Narayanan V, Kruer MC. Dystonia in ATP2B3-associated X-linked spinocerebellar ataxia. *Movement disorders : official journal of the Movement Disorder Society.* 2016; 31(11):1752-1753. PMID: 27653636

Filon JR, Intorcchia AJ, Sue LI, Vazquez AE, Wilson J, Davis KJ, Sabbagh MN, Belden CM, Caselli RJ, Adler CH, Woodruff BK, Rapsack SZ, Ahern GL, Burke AD, Jacobson S, Shill HA, Driver-Dunckley E, Chen K, Reiman EM, Beach TG, Serrano GE. Gender Differences in Alzheimer Disease: Brain Atrophy, Histopathology Burden, and Cognition. *J Neuropathol Exp Neurol.* 2016. doi: nlw047 [pii];10.1093/jnen/nlw047 [doi]. PMID: 27297671.

Fonseca MI, S. Chu, A.L. Pierce, W.D. Brubaker, R.E. Hauhart, D. Mastroeni, E.V. Clarke, J. Rogers, J.P. Atkinson, A.J. Tenner, Analysis of the Putative Role of CR1 in Alzheimer's Disease: Genetic Association, Expression and Function, *PLoS One*, 11 (2016) e0149792.

Gale S, Baxter L, Thompson J. Greater memory impairment in dementing females than males relative to sex matched health controls. *J Clin Exp Neuropsychol.* 2016 Jan 6:1-7. PMID:26735615

George SJ*, Hernandez JA*, Jimenez-Vicente E, Echavarri-Erasun C, & Rubio LM. (2016) Mo biochemistry during FeMo-co synthesis: EXAFS reveals two Mo sites in NifQ. *Chem. Commun. (Camb)*. 52(79):11811-11814. (*both first authors).

Gray DT, Smith, A.C., Burke, S.N., Gazzaley, A. and Barnes, C.A. (2016) Attentional updating and monitoring and affective shifting are impacted independently by aging in the macaque monkeys. *Behavioral Brain Research*, in press. doi: 10.1016/j.bbr.2016.06.056.

Grilli MD & Verfaellie M (2016) Experience-near but not experience-far autobiographical facts depend on the medial temporal lobe for retrieval: evidence from amnesia. *Neuropsychologia*, 81: 108-185.

Hamlett E, Edward Goetzl, PHD; Heather A Boger, PhD; Aurelie Ledreux, PHD; Angela LaRosa, MD; David Clark, MD; Steven L Carroll, MD/PhD; Elliott Mufson, MD/PhD; Marwan Sabbagh, MD/PhD; Abdul Mohammed, PhD; Dean Hartley, PhD; Juan Fortea, MD; Eric Doran, PhD; Ira Lotta, MD/PhD; Ann-Charlotte (Lotta) Granholm, Neuronal exosome biomarkers reveal early Alzheimer pathology in Down syndrome. *Alzheimer's & Dementia* 2016 2016 Oct 15. pii: S1552-5260(16)32892-8. doi: 10.1016/j.jalz.2016.08.012

Han P, Serrano G, Beach TG, Caselli RJ, Yin J, Zhuang N, Shi J.A Quantitative Analysis of Brain Soluble Tau and the Tau Secretion Factor. *J Neuropathol Exp Neurol*. 2017 Jan 9

Han P, Trinidad BJ, Shi J. Hypocalcemia-Induced Seizure: Demystifying the Calcium Paradox. *ASN Neuro*. 2015 Mar 24;7(2).

He Zhou¹, PhD, Marwan Sabbagh^{2,3}, MD, Rachel Wyman³, BS., Carolyn Liebsack³, Mark E. Kunik^{4,5,6}, MD, MPH, Bijan Najafi¹, PhD Instrumented Trail-Making Task (iTMT) to Differentiate Persons with No Cognitive Impairment, Mild Cognitive Impairment, Alzheimer's Disease - Proof of Concept Study. *Gerontology* 2016 Nov 18. [Epub ahead of print]

Henderson-Smith A, Corneveaux JJ, De Both M, Cuyugan L, Liang WS, Huentelman M, Adler C, Driver-Dunckley E, Beach TG, Dunckley TL. Next-generation profiling to identify the molecular etiology of Parkinson dementia. *Neurol Genet*. 2016 May 24;2(3):e75. doi: 10.1212/NXG.0000000000000075. PubMed PMID: 27275011; PubMed Central PMCID: PMC4881621.

Hibar DP, Adams HH, Jahanshad N, Chauhan G, Stein JL, et al.. Novel genetic loci associated with hippocampal volume. *Nature communications*. 2017; 8:13624. PMID: 28098162 PMCID: PMC5253632.

Hiroi R, Carbone D.L., Zuloaga D.G., Bimonte-Nelson H.A., and Handa R.J. (2016) Sex-dependent programming effects of prenatal glucocorticoid treatment on the developing serotonin system and stress-related behaviors in adulthood. *Neuroscience*. 320:43-56. Pubmed PMID: 26844389.

Hiroi R, Weyrich G., Koebele S.V., Mennenga S.E., Talboom J.S., Hewitt L.T., Lavery C.N., Mendoza P., Jordan A., Bimonte-Nelson HA. (2016) Benefits of hormone therapy estrogens depend on estrogen type: 17 β -estradiol and conjugated equine estrogens have differential effects on cognitive, anxiety-like, and depressive-like behaviors and increase tryptophan hydroxylase-2 mRNA levels in dorsal raphe nucleus subregions. *Frontiers in Neuroscience*. Dec 8;510:517. PMID: 28008302.

Hoss AG, Labadorf A, Beach TG, Latourelle JC, Myers RH. microRNA Profiles in Parkinson's Disease Prefrontal Cortex. *Front Aging Neurosci*. 2016 Mar 1;8:36. doi: 10.3389/fnagi.2016.00036. PubMed PMID: 26973511; PubMed Central PMCID: PMC4772525.
Hu LS, Kelm Z, Korfiatis P, Dueck AC, Elrod C, Ellingson B, Kaufmann TJ, Eschbacher JM, Karis JP, Nakaji P, Pafundi D, Baxter LC, Erickson BJ. Impact of gadolinium preload dosing and software modeling on the accuracy of perfusion MRI in glioma. *AJNR Am J Neuroradiol*. in press.

Hu LS, Kelm Z, Korfiatis P, Dueck AC, Elrod C, Ellingson BM, Kaufmann TJ, Eschbacher JM, Karis JP, Smith K, Nakaji P, Brinkman D, Pafundi D, Baxter LC, Erickson BJ. Impact of Software Modeling on the Accuracy of Perfusion MRI in Glioma. *AJNR Am J Neuroradiol* 2015, Sep 10. PMID 26359151

Hu LS , Ning S, Eschbacher JM, Baxter LC, Gaw N, Ranjbar S, Plasencia J, Dueck AC, Peng S, Smith KA, Nakaji P, Karis JP, Quarles C, Wu T, Loftus J, Jenkins R , Sicotte H, Kollmeyer TM, O'Neill BP, Elmquist W, Hoxworth JM, Frakes D, Sarkaria J, Swanson KR , Tran N, Li J, Mitchell JR. Radiogenomics to Characterize Regional Genetic Heterogeneity in Glioblastoma Neuro-Oncology 2016 (in press).

Hu LS, Ning S, Eschbacher JM, Gaw N, Dueck AC, Smith KA, Nakaji P, Plasencia J, Ranjbar S, Price SJ, Tran N, Loftus J, Jenkins R, O'Neill BP, Elmquist W, Baxter LC, Gao F, Frakes D, Karis JP, Zwart C, Swanson KR, Sarkaria J, Wu T, Mitchell JR, Li J. Multi-Parametric MRI and Texture Analysis to Visualize Spatial Histologic Heterogeneity and Tumor Extent in Glioblastoma. *PLOS One* 2015, Nov 24; 10(11) PMID: 26599106

Huntington Study Group FS, Testa CM, Stamler D, Kayson E, Davis C, et al. Effect of Deutetrabenazine on Chorea Among Patients With Huntington Disease: A Randomized Clinical Trial. *JAMA Neurol*. 2016;3016(1):40-50. doi: 10.1001/jama2016.8655. PMID 273803420.

Ikonomovic MD, Buckley CJ, Heurling K, Sherwin P, Jones PA, Zanette M, Mathis CA, Klunk WE, Chakrabarty A, Ironside J, Ismail A, Smith C, Thal DR, Beach TG, Farrar G, Smith AP. Post-mortem histopathology underlying β -amyloid PET imaging following flutemetamol F 18 injection. *Acta Neuropathol Commun*. 2016 Dec 12;4(1):130. PubMed PMID: 27955679; PubMed Central PMCID: PMC5154022.

Jankovic J, Jimenez-Shahed J, Budman C, Coffey B, Murphy T, Shprecher D, Stamler D. Deutetrabenazine in Tics Associated with Tourette Syndrome. *Tremor Other Hyperkinet Mov (N Y)*. 2016 Nov 7;6:422. PubMed PMID: 27917309; PubMed Central PMCID: PMC5133390.

Jun G, Ibrahim-Verbaas CA, Vronskaya M, Lambert JC, Chung J, et al. Alzheimer's Disease Risk Polymorphisms Regulate Gene Expression in the ZCWPW1 and the CELF1 Loci. *PLoS one*. 2016; 11(2):e0148717. PMID: 26919393 PMCID: PMC4769299

Karim R, Dang H, Henderson VW, Hodis HN, St. John J, Brinton RD, Mack WJ (2016) Effect of Reproductive History and Exogenous Hormone Use on Cognitive Function in Mid- and Late Life. *J Am Geriatr Soc.*, 64:2448-2456.

Khurana HS, Groves RH, Jr., Simons MP, Martin M, Stoffer B, Kou S, Gerkin R, Reiman E, Parthasarathy S. Real-Time Automated Sampling of Electronic Medical Records Predicts Hospital Mortality. *Am J Med*. 2016;129(7):688-98 e2. doi: 10.1016/j.amjmed.2016.02.037. PubMed PMID: 27019043; PMCID: PMC4916370.

Kious BM, Jimenez-Shahed J, Shprecher DR. Treatment-refractory Tourette Syndrome. *Prog Neuropsychopharmacol Biol Psychiatry*. 2016 Oct 3;70:227-36. doi: 10.1016/j.pnpbp.2016.02.003. Review. PubMed PMID: 26875502.

Klimentidis YC & Arora A (2016) Interaction of Insulin Resistance and Related Genetic Variants with Triglyceride-Associated Genetic Variants. *Circulation. Cardiovascular Genetics*, 9:154-161.

Klimentidis YC, Arora A, Chougule A, Zhou J, Raichlen D (2016) FTO association and interaction with time spent sitting. *International Journal of Obesity*, 40:411-416.

Klimentidis YC, Arora A, Zhou J, Kittles R, Allison DB (2016) The genetic contribution of West-African ancestry to protection against central obesity in African-American men but not women: results from the ARIC and MESA studies. *Frontiers in Genetics*, <https://doi.org/10.3389/fgene.2016.00089>.

Klimentidis YC, Bea, J.W., Thompson, P., Klimecki, W.T., Hu, C., Wu, G., Nicholas, S. and Ryckman, K.K., CHARGE Consortium Musculoskeletal Working Group, Chen, Z. (2016) Genetic Variant in ACVR2B Is Associated with Lean Mass. *Medicine & Science in Sports & Exercise*, 48:1270-1275.

Koebele SV, Bimonte-Nelson H.A. (2016) Modeling menopause: The utility of rodents in translational behavioral endocrinology research. *Maturitas*. 87:5-17. Pubmed PMID: 27013283.

Koebele SV, Bimonte-Nelson HA (2016) The endocrine-brain-aging triad where many paths meet: female reproductive hormone changes at midlife and their influence on circuits important for learning and memory. *Experimental Gerontology*. S0531-5565(16)30584-30588. PMID: 27979770.

Lemas DJ, Klimentidis YC, Aslibekyan, S., Wiener, H.W., O'Brien, D.M., Hopkins, S.E., Stanhope, K.L., Havel, P.J., Allison, D.B., Fernandez, J.R., Tiwari, H.K. and Boyer, B.B. (2016) Polymorphisms in stearoyl CoA desaturase and sterol regulatory element binding protein interact

with N-3 polyunsaturated fatty acid intake to modify associations with anthropometric variables and metabolic phenotypes in Yup'ik people. *Mol Nutr Food Res*, 6:2642-2653.

Locatelli FF, Fernandez PC, Smith BH. (2016) Learning About natural variation of odor mixtures drives plasticity in early olfactory processing. *J Exp Biology*. pii: jeb.141465. [Epub ahead of print]

López-Torrejón G, Jiménez-Vicente E, Buesa JM, Hernandez JA, Verma HK & Rubio LM. (2016) Expression of a functional oxygen-labile nitrogenase component in the mitochondrial matrix of aerobically grown yeast. *Nature Communications* 7:11426.

Lozano AM, Fosdick L, Chakravarty MM, Leoutsakos JM, Munro C, Oh E, Drake KE, Lyman CH, Rosenberg PB, Anderson WS, Tang-Wai DF, Pendergrass JC, Salloway S, Asaad WF, Ponce FA, Burke AD, Sabbagh MN, Wolk DA, Baltuch G, Okun MS, Foote KD, McAndrews MP, Giacobbe P, Targum SD, Lyketsos CG, Smith G. A Phase II Study of Fornix Deep Brain Stimulation in Mild Alzheimer's Disease. *Journal of Alzheimer's Disease* 2016: 54 (2)

Lue LF, Schmitz CT, Snyder N, Chen K, Walker DG, Caviness J, Adler C, Sabbagh M, Shill. Converging mediators from immune and trophic pathways to identify Parkinson's disease dementia. *Neurology: Neuroimmunology & Neuroinflammation* 2016;3:e193

Lyketsos CG, Gwenn Smith, Lisa Fosdick, Jeannie-Marie Leoutsakos, Cynthia Munro, Esther Oh, Kristen Drake, Paul B. Rosenberg, William S. Anderson, Stephen Salloway, Cara Pendergrass, Anna Burke, David A. Wolk, David F. Tang-Wai, Francisco A. Ponce, Wael F. Asaad, Marwan Sabbagh, Michael S. Okun, Gordon Baltuch, Kelly D. Foote, Steven D. Targum, and Andres M. Lozano. A phase 2 study of fornix deep brain stimulation in mild Alzheimer dementia. *J Alzheimer's Dis*. 2016 Jul 18. [Epub ahead of print]

Ma C, Wang J, Zhang J, Chen K, Li X, Shu N, Chen Y, Liu Z, Zhang Z. Disrupted Brain Structural Connectivity: Pathological Interactions Between Genetic APOE epsilon4 Status and Developed MCI Condition. *Mol Neurobiol*. 2016. doi: 10.1007/s12035-016-0224-5. PubMed PMID: 27785756.

Ma C, Zhang Y, Li X, Zhang J, Chen K, Liang Y, Chen Y, Liu Z, Zhang Z. Is there a significant interaction effect between apolipoprotein E rs405509 T/T and ε4 genotypes on cognitive impairment and gray matter volume? *Eur J Neurol*. 2016 Sep;23(9):1415-25. doi: 10.1111/ene.13052. PubMed PMID: 27259692; PubMed Central PMCID: PMC4987229.

Ma C, Zhang Y, Li X, Chen Y, Zhang J, Liu Z, Chen K, Zhang Z. The TT allele of rs405509 synergizes with APOE epsilon4 in the impairment of cognition and its underlying default mode network in non-demented elderly. *Curr Alzheimer Res*. 2016;13(6):708-17. PubMed PMID: 26825091.

Malek-Ahmadi M, Kora, K., O'Connor, K., Schofield, S., Coon, D. & Nieri, W. (2016). Longer self-reported sleep duration is associated with decreased performance on the Montreal Cognitive

Assessment in older adults. *Aging Clinical and Experimental Research*, 28, 333-337. DOI: 10.1007/s40520-015-0388-2.

Malek-Ahmadi M, Perez SE, Chen K, Mufson EJ. Neuritic and Diffuse Plaque Associations with Memory in Non-Cognitively Impaired Elderly. *J Alzheimers Dis*. 2016;53(4):1641-52. doi: JAD160365 [pii];10.3233/JAD-160365 [doi].

Mahoney DF, Coon DW, Lozano C (2016). Latino/Hispanic Alzheimer's caregivers' experiencing dressing issues at home: Corroboration of the preservation of self model and reactions to using a "Smart Dresser" computer-based dressing aid. *Digital Health*, 2, 1-12.

Marquine MJ, Grilli, M.D., Rapcsak, S.Z., Kaszniak, A.W., Ryan, L., Walther, K., Glisky, E.L. (2016) Impaired personal trait knowledge, but spared other-person trait knowledge, in an individual with bilateral damage to the medial prefrontal cortex. *Neuropsychologia*, 89:245-253.

Martirosyan NL, Turner GH, Kaufman JA, Patel AA, Belykh E, Kalani MY, Theodore N, & Preul MC. (2016) Manganese-enhanced MRI offers correlation with severity of spinal cord injury in experimental models. *Open Neuroimag J* 10: 139-147 DOI: 10.2174/1874440001610010139.

Mastroeni D. An Epigenetic Perspective on Diseases of the Central Nervous System., Chapter 11; in: J. Cummings and J. Pillai (Ed.) *Neurodegenerative Diseases: Unifying Principles*, Oxford University Press 2016.

Mastroeni D, L. Chouliaras, D. Van den Hove, J. Nolz, B. Rutten, E. Delvaux, P. Coleman, Increased 5-hydroxymethylation levels in the sub ventricular zone of the Alzheimer's brain. *Neuroepigenetics*, 6 (2016).

Mastroeni D, O.M. Khmour, E. Delvaux, J. Nolz, G. Olsen, N. Berchtold, C. Cotman, S.M. Hecht, P.D. Coleman. Nuclear but not mitochondrial-encoded OXPHOS genes are altered in aging, mild cognitive impairment, and Alzheimer's disease, *Alzheimers Dement*, DOI 10.1016/j.jalz.2016.09.003(2016).

Mennenga SE, Gerson JE, Koebele SV, Kingston ML, Tsang CW, Engler-Chiurazzi EB, Baxter LC, Bimonte-Nelson HA. Understanding the cognitive impact of the contraceptive estrogen Ethinyl Estradiol: tonic and cyclic administration impairs memory, and performance correlates with basal forebrain cholinergic system integrity. *Psychoneuroendocrinology*. 2015 Apr;54:1-13. doi: 10.1016/j.psyneuen.2015.01.002. Epub 2015 Jan 12. PubMed PMID: 25679306; PubMed Central PMCID: PMC4433884.

Mittelman-Smith MA, Krajewski-Hall SJ, McMullen NT, Rance NE (2016) Ablation of KNDy neurons results in hypogonadotropic hypogonadism and amplifies the steroid-induced LH surge in female rats. *Endocrinology*, 157:2015-2027.

Moskowitz AM, Belnap N, Siniard AL, Szelinger S, Claasen AM, Richholt RF, De Both M, Corneveaux JJ, Balak C, Piras IS, Russell M, Courtright AL, Rangasamy S, Ramsey K, Craig

DW, Narayanan V, Huentelman MJ, Schrauwen I. A de novo missense mutation in ZMYND11 is associated with global developmental delay, seizures, and hypotonia. *Cold Spring Harbor molecular case studies*. 2016; 2(5):a000851. PMID: 27626064 PMCID: PMC5002929

Mufson EJ, Malek-Ahmadi M, Perez SE, Chen K. Braak staging, plaque pathology, and APOE status in elderly persons without cognitive impairment. *Neurobiol Aging*. 2016;37:147-53. doi: 10.1016/j.neurobiolaging.2015.10.012. PubMed PMID: 26686670; PMCID: PMC4687022.

Mufson EJ, Malek-Ahmadi M, Snyder N, Ausdemore J, Chen K, Perez SE. Braak stage and trajectory of cognitive decline in noncognitively impaired elders. *Neurobiol Aging*. 2016;43:101-10. doi: 10.1016/j.neurobiolaging.2016.03.003. PubMed PMID: 27255819; PMCID: PMC4894536.

Myhre JW, Mehl MR, Glisky EL (2016) Cognitive benefits of online social networking in healthy older adults. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*. doi: 10.1093/geronb/gbw025.

Nowicki BA, Hamada MA, Robinson GY, Jones DC. (2016) Adverse effects of bisphenol A (BPA) on the dopamine system in two distinct cell models and corpus striatum of the Sprague-Dawley rat. *J Toxicology and Environmental Health A*. 79(20):912-24. PMID: 27494678

Norambuena A, Wallrabe H, McMahon L, Silva A, Swanson E, Khan SS, Baerthlein D, Kodis E, Oddo S, Mandell JW, Bloom GS. mTOR and neuronal cell cycle reentry: How impaired brain insulin signaling promotes Alzheimer's disease. *Alzheimers Dement*. 2016, in press.

Nguyen LA, Haws KA, Fitzhugh, M.C., Torre, G.A., Hishaw, G.A. and Alexander, G.E. (2016) Interactive effects of subjective memory complaints and hypertension on learning and memory performance in the elderly. *Neuropsychol Dev Cogn B Aging Neuropsychol Cognition*, 23:154-170.

Nguyen TV, Frye JB, Zbesko JC, Stepanovic K, Hayes M, Urzua A, Serrano G, Beach TG, Doyle KP. Erratum to: Multiplex immunoassay characterization and species comparison of inflammation in acute and non-acute ischemic infarcts in human and mouse brain tissue. *Acta Neuropathol Commun*. 2016 Sep 26;4(1):104.

O'Connor K, Coon, D. W., Malek-Ahmadi, M., Bugger, B. N., Schofield, S., & Nieri, W. (2016). Description and cohort characterization of the Longevity Study: Learning from Our Elders. *Aging Clinical and Experimental Research*, 28, 863-869. doi:10.1007/s40520-015-0488-z.

Okun, A., McKinzie, D.L., Witkin, J.M., Remeniuk, B., Husein, O., Gleason, S.D., Oyarzo, J., Navratilova, E., McElroy, B., Cowen, S.L., Kennedy, J.D. and Porreca, F. (2016) Hedonic and motivational responses to food reward are unchanged in rats with neuropathic pain. *Pain* 157:2731–2738.

Orel N & Coon DW (2016). The challenges of change: How can we meet the care needs of the ever-evolving LGBT family. *Generations*, 40, 41-45.

Pal GD, Ouyang B, Serrano G, Shill HA, Goetz C, Stebbins G, Metman LV, Driver-Dunckley E, Mehta SH, Caviness JN, Sabbagh MN, Adler CH, Beach TG; Arizona Study of Aging Comparison of neuropathology in Parkinson's disease subjects with and without deep brain stimulation. *Neurodegenerative Disorders. Mov Disord.* 2016 Dec 2

Peng Q, Schork A, Bartsch H, Lo MT, Panizzon MS, Westlye LT, Kremen WS, Jernigan TL, Le Hellard S, Steen VM, Espeseth T, Huentelman M, Håberg AK, Agartz I, Djurovic S, Andreassen OA, Dale AM, Schork NJ, Chen CH. Conservation of Distinct Genetically-Mediated Human Cortical Pattern. *PLoS genetics.* 2016; 12(7):e1006143. PMID: 27459196 PMCID: PMC4961377

Penner MR, Parrish RR, Hoang LT, Roth TL, Lubin FD, Barnes CA (2016) Age-related changes in *Egr1* transcription and DNA methylation within the hippocampus. *Hippocampus*, 26:1008-1020.

Perkins M, Wolf AB, Chavira B, Shonebarger D, Meckel JP, Leung L, Ballina L, Ly S, Saini A, Jones TB, Vallejo J, Jentarra G, Valla J. (2016) Altered energy metabolism pathways in the posterior cingulate in young adult apolipoprotein E ϵ 4 carriers. *Journal of Alzheimer's Disease* 53(1), 95-106.

Powell J, Lendrum J, Huff R, Belden C, Sabbagh MN. The Mild Cognitive Impairment of Primary Progressive Aphasia: A case series. *Current Aging Science* 2016 Oct 24. [Epub ahead of print]

Raichlen DA, Bharadwaj PK, Fitzhugh MC, Haws KA, Torre GA, Trouard T, Alexander GE (2016) Differences in resting state functional connectivity between young adult endurance athletes and healthy controls. *Frontiers in Human Neuroscience*, 10:610.

Reiman EM. Alzheimer's disease: Attack on amyloid-beta protein. *Nature.* 2016;537(7618):36-7. doi: 10.1038/537036a. PubMed PMID: 27582214.

Reiman EM, Langbaum JB, Tariot PN, Lopera F, Bateman RJ, Morris JC, Sperling RA, Aisen PS, Roses AD, Welsh-Bohmer KA, Carrillo MC, Weninger S. CAP--advancing the evaluation of preclinical Alzheimer disease treatments. *Nat Rev Neurol.* 2016;12(1):56-61. doi: 10.1038/nrneurol.2015.177. PubMed PMID: 26416539; PMCID: PMC4847536.

Reiman EM, Langbaum JB, Tariot PN, Lopera F, Bateman RJ, Morris JC, Sperling RA, Weninger S, Carrillo MC, Dunn B, Aisen PS, Bateman RJ, Kotz JD, Langbaum JB, Mills SL, Reiman EM, Sperling R, Santacruz AM, Tariot PN, Welsh-Bohmer KA. Collaboration for Alzheimer's Prevention: Principles to guide data and sample sharing in preclinical Alzheimer's disease trials. *Alzheimers Dement.* 2016 May;12(5):631-2. doi: 10.1016/j.jalz.2016.04.001. PubMed PMID: 27157073.

Reinhardt M, Parigi AD, Chen K, Reiman EM, Thiyyagura P, Krakoff J, Hohenadel MG, Le DS, Weise CM. Deactivation of the left dorsolateral prefrontal cortex in Prader-Willi syndrome after meal consumption. *Int J Obes (Lond)*. 2016;40(9):1360-8. doi: ijo201675 [pii];10.1038/ijo.2016.75 [doi]. PMID: 27121248.

Rettberg JR, Dang, H., Hodis, H.N., Henderson, V/W/, St. John, J.A., Mack, W.J. and Brinton, R.D. (2016) Identifying postmenopausal women at risk for cognitive decline within a healthy cohort using a panel of clinical metabolic indicators: Potential for detecting an at-Alzheimer's risk metabolic phenotype, *Neurobiology of Aging*. 40:155-163.

Reynolds RJ, Vazquez, A.I., Srinivasasainagendra, V., Klimentidis, Y.C., Bridges, S.L. Jr, Allison, D.B. and Singh, J.A. (2016) Serum urate gene associations with incident gout, measured in the Framingham Heart Study, are modified by renal disease and not by body mass index. *Rheumatology International*, 36:263-270.

Ridge PG, Hoyt KB, Boehme K, Mukherjee S, Crane PK, Haines JL, Mayeux R, Farrer LA, Pericak-Vance MA, Schellenberg GD, Kauwe JS. Assessment of the genetic variance of late-onset Alzheimer's disease. *Neurobiology of aging*. 2016; 41:200.e13-20. NIHMSID: NIHMS773714 PMID: 27036079 PMCID: PMC4948179

Riedel BC, Thompson, P.M., Brinton, R.D. (2016) Age, APOE and Sex: Triad of Risk of Alzheimer's Disease. *J Steroid Biochem Mol Biol*. 160:134-147.

Rogalski E, Sridhar J, Rader B, Martersteck A, Chen K, Cobia D, Thompson CK, Weintraub S, Bigio EH, Mesulam MM. Aphasic variant of Alzheimer disease: Clinical, anatomic, and genetic features. *Neurology*. 2016;87(13):1337-43. doi: WNL.0000000000003165 [pii];10.1212/WNL.0000000000003165 [doi].

Roher AE, CL Maarouf and TA Kokjohn. (2016) Familial presenilin mutations and sporadic Alzheimer's disease pathology: Is the assumption of biochemical equivalence justified? *J. Alzheimers Dis*. 50(3):645-658.

Roher AE, Maarouf CL, Kokjohn TA, Belden C, Serrano G, Sabbagh MS, Beach TG. Chemical and neuropathological analyses of an Alzheimer's disease patient treated with solanezumab. *Am J Neurodegener Dis*. 2016 Aug 26;5(4):158-170.

Rowe RK, Ziebell JM, Harrison JL, Law LM, Adelson PD, Lifshitz J. Aging with Traumatic Brain Injury: Effects of Age at Injury on Behavioral Outcome following Diffuse Brain Injury in Rats. *Dev Neurosci*. 38: 195-205, 2016.

Sabbagh MN, Editorial Introduction, *Neurol Ther*. 2016 Nov 9. [Epub ahead of print]

Sabbagh, M. and Edgin, J. (2016) Clinical assessment of cognitive decline in adults with Down syndrome. *Current Alzheimer Research*, 13:30-34.

Sabbagh MN, Schäuble B, Anand K, Richards D, Beach TG, Murayama S, Akatsu H, Takao M, Rowe CC, Masters CL, Barthel H, Gertz H-J, Peters O, Rasgon N, Jovalekic A, Booth DR, Sabri O, Schulz-Schaeffer WJ, Seibyl J. Histopathology and florbetaben PET in patients incorrectly diagnosed with Alzheimer's disease. *Journal of Alzheimer's Disease* 2016 [Epub ahead of print]

Sabbagh M, Han S, Kim S, Na H, Lee J, Kandiah N, Phanthumchinda K, Suthisisang C, Senanarong V, Pai M, Narilastrri D, Sowani AM, Ampil E, Dash A, for the Asia Pacific EXpert Panel (APEX) for Donepezil 23 mg Clinical recommendations for the use of donepezil 23 mg in moderate-to-severe Alzheimer's disease in the Asia-Pacific region. *Dement Geriatr Cogn Disord Extra* 2016;6:382-395 (DOI:10.1159/000448214)

Schwenk M, Sabbagh, M., Lin, I., Morgan, P., Grewal, G., Mohler, J., Coon, D.W., & Najafi, B. (in press). Sensor-based balance training with motion feedback in people with mild cognitive impairment. *Journal of Rehabilitation Research and Development*.

Smith R, Baxter LC, Thayer JF, Lane RD. Disentangling introspective and exteroceptive attentional control from emotional appraisal in depression using fMRI: A preliminary study. *Psychiatry Res.* 2016 Feb 28;248:39-47. Epub 2016 Jan 8.

Schwartz M, Geidy Serrano, Thomas G. Beach, Andrew Tsai, Michael Malek-Ahmadi, Sandra Jacobson, Lucia I. Sue, Kathryn Davis, Marwan N. Sabbagh. Neurofibrillary Tangle Predominant Dementia: Clinical and Pathological Description in a Case Series. *J Alzheimer's Disease and Parkinsonism* 2016; 6:1, <http://dx.doi.org/10.4172/2161-0460.1000204>

Schwenk M, Sabbagh MN, Lin I, Morgan P, Grewal G, Mohler J, Coon, DW, Najafi B. Exergaming improves balance in people with mild cognitive impairment: a randomized controlled pilot study. *JRRD* 2015 (in press)

Schrauwen I, Hasin-Brumshtein Y, Corneveaux JJ, Ohmen J, White C, Allen AN, Lusic AJ, Van Camp G, Huentelman MJ, Friedman RA. A comprehensive catalogue of the coding and non-coding transcripts of the human inner ear. *Hearing research.* 2016; 333:266-74. NIHMSID: NIHMS726101 PMID: 26341477 PMCID: PMC4775449

Seibyl J, Catafau AM, Barthel H, Ishii K, Rowe CC, Leverenz JB, Ghetti B, Ironside JW, Takao M, Akatsu H, Murayama S, Bullich S, Mueller A, Koglin N, Schulz-Schaeffer WJ, Hoffmann A, Sabbagh M, Stephens AW, Sabri O. Impact of training method on the robustness of the visual assessment of 18Fflorbetaben PET scans - results from a phase 3 study. *J Nucl Med.* 2016 Jan 28. pii: jnumed.115.161927. [Epub ahead of print] PMID:26823561

Shefner JM, Sabbagh MN, An Appraisal of Novel Biomarkers for Evaluating and Monitoring Neurologic Diseases: Editorial Introduction. *Neurotherapeutics.* 2016 Dec 8. [Epub ahead of print] No abstract available.

Sherman SM, Buckley, T.P., Baena, E., Ryan, L. (2016). Caffeine Enhances Memory Performance in Young Adults during Their Non-optimal Time of Day. *Front Psychol*, 14:1757:1764.

Shi J, Zhang W, Tang M, Caselli RJ, Wang Y. Conformal invariants for multiply connected surfaces: Application to landmark curve-based brain morphometry analysis. *Medical Image Analysis*. 2016 Sep; DOI:10.1016/j.media.2016.09.

Shill HA, Joseph G. Hentz, MS,³ John N. Caviness, MD,³ Erika Driver-Dunckley, MD,³ Sandra Jacobson, MD,^{1,2} Christine Belden, PsyD,¹ Marwan N. Sabbagh, MD,^{1,2} Thomas G. Beach, MD, PhD,¹ Charles H. Adler, MD PhD³ Unawareness of Hyposmia in Elderly People With and Without Parkinson's Disease. *Movement Disorders Clinical Practice*. 2016 doi:10.1002/mdc3.12220: 43-47

Smith R, Baxter LC, Thayer JF, Lane RD. Disentangling introspective and exteroceptive attentional control from emotional appraisal in depression using fMRI: A preliminary study. *Psychiatry Res*. 2016 Feb 28;248:39-47. doi: 0.1016/j.psychres.2016.01.009. Epub 2016 Jan 8.

Snyder H, Asthana, S., Bain, L., Brinton, R., Craft, S., Dubal, D., Espeland, M., Gatz, M., Mielke, M., Raber J, et al. (2016) Sex biology contributions to vulnerability to Alzheimer's disease: A think tank convened by the Women's Alzheimer's Research Initiative. *Alzheimer's & Dementia*, 12:1186-1196.

Sommen M, Schrauwen I, Vandeweyer G, Boeckx N, Corneveaux JJ, van den Ende J, Boudewyns A, De Leenheer E, Janssens S, Claes K, Verstreken M, Strenzke N, Predöhl F, Wuyts W, Mortier G, Bitner-Glindzicz M, Moser T, Coucke P, Huentelman MJ, Van Camp G. DNA Diagnostics of Hereditary Hearing Loss: A Targeted Resequencing Approach Combined with a Mutation Classification System. *Human mutation*. 2016; 37(8):812-9. PMID: 27068579

Spanò G & Edgin JO (2016) Everyday memory in individuals with Down syndrome: Validation of the Observer Memory Questionnaire–Parent Form. *Child Neuropsychology*, 16:1-13.

Spencer B, et al., *alpha-synuclein conformational antibodies fused to penetratin are effective in models of Lewy body disease*. *Ann Clin Transl Neurol*, 2016. 3(8): p. 588-606.

Su J (2016) Single Molecule Detection with Microtoroid Optical Resonators. *Optics & Photonics News*, December 2016.

Su J (2016) Reply to Comment on Label-Free Single Exosome Detection Using Frequency-Locked Microtoroid Optical Resonators," *ACS Photonics*, 3:718.

Su Y, Blazey TM, Owen CJ, Christensen JJ, Friedrichsen K, et al.. Correction: Quantitative Amyloid Imaging in Autosomal Dominant Alzheimer's Disease: Results from the DIAN Study Group. *PLoS One*. 2016;11(9):e0163669. doi: 10.1371/journal.pone.0163669 [doi];PONE-D-16-36842 [pii]. PMID: 27649320.

Su J, Goldberg, A.F. and Stoltz, B.M. (2016) Label-Free Single Detection of Single Nanoparticles and Biological Molecules Using Microtoroid Optical Resonators, *Light: Science & Applications*, (Nature Publishing Group), 5, e1600.

Swanson MJ, Baribault ME, Israel, JN, and Bae, NS. (2016) Telomere protein RAP1 levels are affected by cellular aging and oxidative stress. *Biomedical Reports* 5:181-187.

Tanaka Y, Suzuki G, Matsuwaki T, Hosokawa M, Serrano G, Beach TG, Yamanouchi K, Hasegawa M, Nishihara M. Progranulin regulates lysosomal function and biogenesis through acidification of lysosomes. *Hum Mol Genet.* 2017 Jan 10. pii: ddx011. doi: 10.1093/hmg/ddx011.

Tariot PN, Langbaum JB, Reiman EM; Alzheimer's Prevention Initiative.. What are we willing to accept for preventing Alzheimer's disease? - Investigators' reply. *Lancet Neurol.* 2016 Jun;15(7):660-1. doi: 10.1016/S1474-4422(16)30055-2. PubMed PMID: 27302231.

Tariot PN, Langbaum JB, Reiman EM. What are we willing to accept for preventing Alzheimer's disease? *Lancet Neurol*, 15(7):660-1, 2016.

Thome A, Gray, D.T., Erickson, C.A., Lipa P. and Barnes, C.A. (2016) Memory impairment in aged primates is associated with region-specific network dysfunction. *Molecular Psychiatry*, 21:1257-1262.

Tiernan CT, S. D. Ginsberg, A.L. Guillozet-Bongaarts, S. M. Ward, B. He, N. M. Kanaan, E. J. Mufson, L. I. Binder, S. E. Counts: Protein homeostasis gene dysregulation in pretangle-bearing nucleus basalis neurons during the progression of Alzheimer's disease. *Neurobiol. Aging*, 42:80-90, 2016. PMID: 27143424. PMC-In Process. NIHMSID: 766697

Toosizadeh N, Najafi B, Reiman EM, Mager RM, Veldhuizen JK, O'Connor K, Zamrini E, Mohler J. Upper-Extremity Dual-Task Function: An Innovative Method to Assess Cognitive Impairment in Older Adults. *Front Aging Neurosci.* 2016;8:167. doi: 10.3389/fnagi.2016.00167. PubMed PMID: 27458374; PMCID: PMC4935727.

Turovsky E, Theparambil SM, Kasymov V, Deitmer JW, Del Arroyo AG, Ackland GL, Corneveaux JJ, Allen AN, Huentelman MJ, Kasparov S, Marina N, Gourine AV. Mechanisms of CO₂/H⁺ Sensitivity of Astrocytes. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2016; 36(42):10750-10758. PMID: 27798130 PMCID: PMC5083006

Umashankar A, Corenblum, M.J., Ray, S., Valdez, M., Yoshimaru, E.S., Trouard, T.P., Madhavan, L. (2016) Effects of the iron oxide nanoparticle Molday ION Rhodamine B on the viability and regenerative function of neural stem cells: relevance to clinical translation. *International Journal of Nanomedicine*, 11:1731-1748.

Utianski RL, John N. Caviness, Elisabeth C.W. van Straaten, Thomas G. Beach, Brittany N. Dugger, Holly A. Shill, Erika D. Driver-Dunckley, Marwan N. Sabbagh, Shyamal Mehta, Charles H. Adler, Joseph G. Hentz, Graph theory network function in Parkinson's disease

assessed with electroencephalography. *Clinical Neurophysiology*, May 2016, Volume 127 (5):2228–2236

Van Hoogmoed AH, Nadel, L., Spanò, G. and Edgin, J. O. (2016). ERP correlates of object recognition memory in Down syndrome: Do active and passive tasks measure the same thing? *Neuropsychologia*, 82:39-53.

Van Keuren-Jensen KR, Malenica I, Courtright AL, Ghaffari LT, Starr AP, Metpally RP, Beecroft TA, Carlson EW, Kiefer JA, Pockros PJ, Rakela J. microRNA changes in liver tissue associated with fibrosis progression in patients with hepatitis C. *Liver international: official journal of the International Association for the Study of the Liver*. 2016; 36(3):334-43. PMID: 26189820 PMCID: PMC5049661

Velazquez R, Shaw DM, Caccamo A, Oddo S. Pim 1 inhibition as a novel therapeutic strategy for Alzheimer's disease. *Mol Neurodegener*. 2016 Jul 13;11(1):52.

Walker DG, John Caviness, Charles Adler, Marwan Sabbagh, Holly Shill. Converging mediators from immune and trophic pathways to identify Parkinson's disease dementia. *Neurology: Neuroimmunology & Neuroinflammation* 2016;3:e193

Wang P, Chen K, Yao L, Hu B, Wu X, Zhang J, Ye Q, Guo X. Multimodal Classification of Mild Cognitive Impairment Based on Partial Least Squares. *J Alzheimers Dis*. 2016;54(1):359-71. doi: JAD160102 [pii];10.3233/JAD-160102 [doi].

Wang T, Xiao S, Chen K, Yang C, Dong S, Cheng Y, Li X, Wang J, Zhu M, Yang F, Li G, Su N, Liu Y, Dai J, Zhang M. Prevalence, Incidence, Risk and Protective Factors of Amnesic Mild Cognitive Impairment in the Elderly in Shanghai. *Curr Alzheimer Res*. 2016. PubMed PMID: 27875948.

Wang Y & Brinton, R.D. (2016) Triad of Risk for Late Onset Alzheimer's: Mitochondrial Haplotype, APOE Genotype and Chromosomal Sex. *Frontiers in Aging Neuroscience*, 8:232.

Weidman DA, Burke AD, Riggs GH, Brand H, Copeland JN, Burke WJ. At a Loss for Words. *Prim Care Companion CNS Disord*. 2016;18(4). doi: 10.4088/PCC.16alz01994. PubMed PMID: 27828698.

Weise CM, Piaggi P, Reinhardt M, Chen K, Savage CR, Krakoff J, Pleger B. The obese brain as a heritable phenotype-A combined morphometry and twin study. *Int J Obes (Lond)*. 2016. doi: 10.1038/ijo.2016.222. PubMed PMID: 27916985.

Weninger S, Carrillo MC, Dunn B, Aisen PS, Bateman RJ, Kotz JD, Langbaum JB, Mills SL, Reiman EM, Sperling R, Santacruz AM, Tariot PN, Welsh-Bohmer KA. Collaboration for Alzheimer's Prevention: Principles to guide data and sample sharing in preclinical Alzheimer's disease trials. *Alzheimers Dement*. 2016;12(5):631-2. doi: 10.1016/j.jalz.2016.04.001. PubMed PMID: 27157073; PMCID: PMC5111162.

Wiegand J-P, Gray, D.T., Schimanski, L.A., Lipa, P., Barnes, C.A., Cowen, S.L. (2016) Age is associated with reduced sharp-wave ripple frequency and altered patterns of neuronal variability. *The Journal of Neuroscience*, 36:5650-5660.

Williams SM, P. Schulz, and M.R. Sierks, *Oligomeric alpha-synuclein and beta-amyloid variants as potential biomarkers for Parkinson's and Alzheimer's diseases*. *Eur J Neurosci*, 2016. 43(1): p. 3-16.

Williams AA, White R, Siniard A, Corneveaux J, Huentelman M, Duch C. MECP2 impairs neuronal structure by regulating KIBRA. *Neurobiology of disease*. 2016; 91:284-91. PMID: 27015692

Witharana WKL, Cardiff, J., Chawla, M.K., Xie, J.Y., Alme, C.B., Eckert, M., Lapointe, V., Demchuk, A., Maurer, A.P., Trivedi, V., Sutherland, R.J., Guzowski, J.F., Barnes, C.A. and McNaughton, B.L. (2016) Nonuniform allocation of hippocampal neurons to place fields across all hippocampal subfields. *Hippocampus*, 26:1328-1344.

Wu X, Li Q, Yu X, Chen K, Fleisher AS, Guo X, Zhang J, Reiman EM, Yao L, Li R. A Triple Network Connectivity Study of Large-Scale Brain Systems in Cognitively Normal APOE4 Carriers. *Front Aging Neurosci*. 2016;8:231. doi: 10.3389/fnagi.2016.00231 [doi]. PMID: 27733827.

Xu L WX, Chen K, Yao L. Supervised Within-Class-Similar Discriminative Dictionary Learning for Face Recognition. *Journal of Visual Communication and Image Representation* 2016;38. Epub April. doi: 10.1016/j.jvcir.2016.04.003.

Yin J, Han P, Tang Z, Liu Q, Shi J. Sirtuin 3 mediates neuroprotection of ketones against ischemic stroke. *J Cereb Blood Flow Metab*. 2015 Jun 10. doi: 10.1038/jcbfm.2015.123. [Epub ahead of print]

Yin JX, Maalouf M, Han P, Zhao M, Gao M, Dharshaun T, Ryan C, Whitelegge J, Wu J, Eisenberg D, Reiman EM, Schweizer FE, Shi J. Ketones block amyloid entry and improve cognition in an Alzheimer's model. *Neurobiol Aging*. 2016 Mar;39:25-37. doi: 10.1016/j.neurobiolaging.2015.11.018. Epub 2015 Dec 7. PMID: 26923399

Zhang HG, Cao P, Teng Y, Hu X, Wang Q, Yeri AS, Zhuang X, Samykutty A, Mu J, Deng ZB, Zhang L, Mobley JA, Yan J, Van Keuren-Jensen K, Miller D. Isolation, identification, and characterization of novel nanovesicles. *Oncotarget*. 2016; 7(27):41346-41362. PMID: 27191656 PMID: 27191656 PMID: 27191656

Zhang J, Liu Z, Li Z, Wang Y, Chen Y, Li X, Chen K, Shu N, Zhang Z. Disrupted White Matter Network and Cognitive Decline in Type 2 Diabetes Patients. *J Alzheimers Dis*. 2016;53(1):185-95. doi: JAD160111 [pii];10.3233/JAD-160111 [doi].

Zhang J, Liu Z, Zhang H, Yang C, Li H, Li X, Chen K, Zhang Z. A Two-Year Treatment of Amnesic Mild Cognitive Impairment using a Compound Chinese Medicine: A Placebo

Controlled Randomized Trial. *Sci Rep.* 2016 Jul 4; 6:28982. doi: 10.1038/srep28982. PubMed PMID: 27373556; PubMed Central PMCID: PMC4931444.

Zhang W, Shi J, Stonnington C, Bauer RJ 3rd, Gutman BA, Chen K, Thompson PM, Reiman EM, Caselli RJ, Wang Y. Morphometric Analysis of Hippocampus and Lateral Ventricle Reveals Regional Difference Between Cognitively Stable and Declining Persons. *Proc IEEE Int Symp Biomed Imaging.* 2016 Apr; 2016:14-18. PubMed PMID: 27499828; PubMed Central PMCID: PMC4974021.

Zhang J, Stonnington C, Li Q, Shi J, Bauer RJ, III, Gutman BA, Chen K, Reiman EM, Thompson PM, Ye J, Wang Y. Applying sparse coding to surface multivariate tensor-based morphometry to predict future cognitive decline. *Proc IEEE Int Symp Biomed Imaging.* 2016;2016:646-50. doi: 10.1109/ISBI.2016.7493350 [doi]. PMID: 27499829.

Zhang J, Shi J, Stonnington C, Li Q, Gutman BA, Chen K, Reiman EM, Caselli RJ, Thompson PM, Ye J, Wang Y. Hyperbolic Space Sparse Coding with Its Application on Prediction of Alzheimer's Disease in Mild Cognitive Impairment. *Med Image Comput Comput Assist Interv* 2016 Oct; 9900:326-334 Epub 2016 Oct 02 PMID:28066843 PMCID:5217478

Zhang L, Trushin, S., Christensen, T., Bachmeier, B., Gateno, B., Schroeder, A., Yao, J., Itoh, K., Sesaki, H., Poon, W., Gylys, K., Patterson, E., Parisi, J., Brinton, R.D., Salisbury, J., Trushina, E. (2016) Altered brain energetics induces mitochondrial fission arrest in Alzheimer's Disease. *Scientific Reports*, 6:18725; doi: 10.1038/srep18725

Zhao L, Mao, Z., Woody, S.K. and Brinton, R.D. (2016) Sex differences in metabolic aging of the brain: insights into female susceptibility to Alzheimer's disease. *Neurobiol Aging*, 42:69-79.

2017 Publications and Manuscripts

Alexander GE (2017) An emerging role for imaging white matter in the preclinical risk for Alzheimer's disease - Linking beta-amyloid to myelin. *JAMA Neurology*, 74:17-19.

Beeson PM, Rising, K., DeMarco, A.T., Howard, T., and Rapcsak, S.Z. (2017) The nature and treatment of phonological text agraphia. *Neuropsychological Rehabilitation*, in press.

Beeson PM, Rising, K., and Rapcsak, S.Z. (2017) Acquired impairments of reading and writing. In Lapointe, LL & Stierwalt JAG (Eds). *Handbook of Aphasia and Brain-Based Cognitive-Language Disorders*. New York: Thieme Publishers, in press.

Branca C and Oddo S. Paving the way for new clinical trials for Alzheimer's disease. *Biological Psychiatry*, 2017, in press.

Chawla MK, Biwer, L.A., Turk, M., Hoang, L.T., Uprety, A.R., Fitzhugh, M.C., De Both, M., Coleman, P.D., Trouard, T.P., Alexander, G.E., Mitchell, K.D., Barnes, C.A., Hale, T.M. and

Huentelman, M. Gradual hypertension induction in middle-aged CYP1A1-REN2 transgenic rats produces significant impairments in spatial memory. Submitted.

Chen Z, Klimentidis, Y.C., Bea, J.W., Ernst, K.C., Hu, C., Jackson, R. and Thomson, C.A. (2017) Body Mass Index, Waist Circumference and Mortality in a Large Multiethnic Postmenopausal Cohort - Results from the Women's Health Initiative. *Journal of the American Geriatrics Society*, in press.

Decourt B, Drumm-Gurnee D, Wilson J, Jacobson S, Belden C, Sirrel S, Ahmadi M, Shill H, Powell J, Walker A, Gonzales A, Macias M, Sabbagh MN. Poor Safety and Tolerability Hamper Reaching a Potentially Therapeutic Dose in the Use of Thalidomide for Alzheimer's Disease: Results from a Double-Blind, Placebo-Controlled Trial. *Curr Alzheimer Res.* 2017;14(4):403-411.

Decourt B, Lahiri DK, Sabbagh MN. Targeting Tumor Necrosis Factor Alpha for Alzheimer's Disease. *Curr Alzheimer Res.* 2017;14(4):412-425.

DeMarco AT, Wilson, S.M., Rising, K, Rapcsak, S.Z. and Beeson, P.M. (2017). Neural substrates of sublexical processing for spelling. *Brain and Language*, 164:118-128.

Di Martino A, O'Connor D, Chen B, Alaerts K, Anderson J, Assaf M, Balsters J, Baxter L, Beggiano A, Bernaerts S, Blanken S, Bookheimer S, Braden B, Byrge L, Castellanos F, Dapretto M, Delorme R, Fair D, Fishman I, Fitzgerald J, Gallagher L, Keehn RJJ, Kennedy D, Lainhart J, Luna B, Mostofsky S, Müller RA, Nebel MB, Nigg J, O'Hearn K, Solomon M, Toro R, Vaidya C, Wenderoth N, White T, Craddock C, Lord C, Leventhal B, and Milham M. Enhancing studies of the connectome in autism using the Autism Brain Imaging Data Exchange II. *Scientific Data*, in press.

Edgin JO, Anand, P., Rosser, T., Pierpont, E.I., Figueroa, C., Hamilton, D., . . . Sherman, S. (2017) The Arizona Cognitive Test Battery for Down Syndrome: Test-retest Reliability and Practice Effects. *American Journal on Intellectual and Developmental Disabilities*, in press.

Edgin JO and Nadel, L. (2017) Hippocampus and Development. *Neuroscience and Biobehavioral Psychology*, in press.

Esbensen AJ, Hooper, S.R., Fidler, D., Hartley, S., Edgin, J., d'Ardhuy, X.L., . . . Urv, T. (2017) Outcome Measures for Clinical Trials in Down Syndrome. *American Journal on Intellectual and Developmental Disabilities*, in press.

Fernandez F, Nyhuis, C.C., Anand P., Demara, B.I., Ruby, N.F., Spanò, G., Clark C. and Edgin J.O. (2017) Young children with Down syndrome show normal development of circadian rhythms, but poor sleep efficiency: A cross-sectional study across the first 60 months of life. *Sleep Medicine*, in press.

Gibson CP, Nielsen C, Alex R, Cooper K, Farney M, Gaufin D, Cui JZ, van Breemen C, Broderick TL, Vallejo-Elias J, Esfandiarei M. 2017. Mild aerobic exercise blocks elastin fiber

fragmentation and aortic dilatation in a mouse model of Marfan syndrome associated aortic aneurysm. *J. Appl Physiol.* Epub ahead of print. PMID: 28385916 DOI:10.1152/jappphysiol.00132.2017

Grilli MD, Woolverton, C.B., Crawford, M.S., & Glisky, E.L. (2017) Self-reference and emotional memory effects in older adults at increased genetic risk of Alzheimer's disease. *Aging, Neuropsychology, and Cognition.* doi:10.1080/13825585.2016.1275508.

Hay, M., Vanderah, T.W., Samareh-Jahani, F., Constantopoulos, E., Uprety, A.R., Barnes, C.A., and Konhilas, J. (2017) Cognitive impairment in heart failure: A protective role for Angiotensin-(1-7). *Behavioral Neuroscience*, in press.

Han P, Serrano G, Beach TG, Caselli RJ, Yin J, Zhuang N, Shi J. A Quantitative Analysis of Brain Soluble Tau and the Tau Secretion Factor. *J Neuropathol Exp Neurol* 2017 Jan 09 [Epub ahead of print] PMID:28069930 DOI:10.1093/jnen/nlw105

Kern KC, Wright, C.B., Bergfield, K.L., Fitzhugh, M., Chen, K., Moeller, J.R., Nabizadeh, N., Elkind, M.S.V., Sacco, R.L., Stern, Y., DeCarli, C. and Alexander GE. Blood pressure control is linked to microvascular disease-associated regional cerebral atrophy. Revision under review.

Kun-Rodrigues C, Ross OA, Orme T, Shepherd C, Parkkinen L, et al. Analysis of C9orf72 repeat expansions in a large international cohort of dementia with Lewy bodies. *Neurobiol Aging.* 2017 Jan;49:214.e13-214.e15.

Lee JM, Derkinderen P, Kordower JH, Freeman R, Munoz DG, Kremer T, Zago W, Hutten SJ, Adler CH, Serrano GE, Beach TG. The Search for a Peripheral Biopsy Indicator of α -Synuclein Pathology for Parkinson Disease. *J Neuropathol Exp Neurol.* 2017 Jan 9.

Li H, Lv CL, Yang CS, Wei DF, Chen KW, Li SW, Zhang ZJ. SORL1 rs1699102 polymorphism modulates age-related cognitive decline and gray matter volume reduction in non-demented individuals. *Eur J Neurol.* 2017 Jan; 24(1):187-194. doi: 10.1111/ene.13182. PubMed PMID: 27779372; PubMed Central PMCID: PMC5177470.

Liang WS, Hendricks W, Kiefer J, Schmidt J, Sekar S, Carpten J, Craig DW, Adkins J, Cuyugan J, Manojlovic Z, Halperin RF, Helland A, Nasser S, Legendre C, Hurley LH, Sivaprakasam K, Johnson DB, Crandall H, Busam KJ, Zismann V, Deluca V, Lee J, Sekulic A, Ariyan CE, Sosman J, Trent J (2017). Integrated genomic analyses reveal frequent TERT aberrations in acral melanoma. *Gen Research* 27(4):524-532.

Liang WS, Sekar S, Nasser S, Adkins J, Cuyugan L, Enriquez D, Rangasamy S, Narayanan V (2017) Phenotypic variability and mTOR pathway gene aberrations in familial tuberous sclerosis. *J Ped Neurol* (accepted).

McGarry A, McDermott M, Kieburtz K, de Bleeck EA, Beal F, et al. Huntington Study Group 2CARE Investigators and Coordinators.. A randomized, double-blind, placebo-controlled trial of coenzyme Q10 in Huntington disease. *Neurology.* 2017 Jan 10;88(2):152-159. doi:

10.1212/WNL.0000000000003478. PubMed PMID: 27913695; PubMed Central PMCID: PMC5224719.

Neville J, Kopko S, Romero K, Corrigan B, Stafford B, LeRoy E, Broadbent ST, Cisneroz M, Wilson E, Reiman E, Vanderstichele H, Arneric SP, Stephenson DT. Accelerating drug development for Alzheimer's disease through the use of data standards, *Alzheimer's & Dementia: Translational Research & Clinical Interventions* (In Press, 2017)

Qian J, Wolters FJ, Beiser A, Haan M, Ikram MA, Karlawish J, Langbaum JB, Neuhaus JM, Reiman EM, Roberts JS, Seshadri S, Tariot PN, Wood BM, Betensky RA, Blacker D. APOE-related risk of mild cognitive impairment and dementia for prevention trials: an analysis of four cohorts. *PLoS Medicine*, in press, 2017.

Raichlen DA, Pontzer, H., Harris, J.A., Mabulla, A.Z., Marlowe, F.W., Snodgrass, J., Eick, G., Colette Berbesque, J., Sancio, A., Wood, B.M.. (2017) Physical activity patterns and biomarkers of cardiovascular disease risk in hunter-gatherers. *American Journal of Human Biology*, in press.

Rapcsak SZ (2017) Prosopagnosia. In Wenzel, AE. (Ed.), *The SAGE Encyclopedia of Abnormal and Clinical Psychology*, in press.

Rapcsak SZ, Hishaw, G.A., and Lin, T.P. (2017) Cortical blindness. In Kreutzer, JS, DeLuca, J, and Kaplan, B (Eds). *Encyclopedia of Clinical Neuropsychology*. 2nd Edition. New York: Springer-Verlag, in press.

Rios-Romenets S, Lopez H, Lopez L, Hincapie L, Saldarriaga A, Madrigal L, Piedrahita F, Navarro A, Acosta-Uribe J, Ramirez L, Giraldo M, Acosta-Baena N, Sanchez S, Ramos C, Munoz C, Baena A, Alzate D, Ospina P, Langbaum JB, Cho W, Tariot PN, Paul R, Reiman EM, Lopera F. The Colombian Alzheimer's Prevention Initiative (API) Registry. *Alzheimer's & Dementia*, in press.

Rowles J, Veltri C. Performance on Interdisciplinary Topics in an Integrated Pharmacy Course. *Inov Pharm*. 2017;8(1): Article 6. <http://pubs.lib.umn.edu/innovations/vol8/iss1/6>.

Sabbagh M, Decourt B. Editorial: Current and Emerging Therapeutics in AD. *Curr Alzheimer Res*. 2017; 14(4): 354-355

Shi J, Zhang W, Tang M, Caselli RJ, Wang Y. Conformal invariants for multiply connected surfaces: Application to landmark curve-based brain morphometry analysis. *Med Image Anal* 2017 Jan; 35:517-529 Epub 2016 Sept 06 PMID:27639215 PMCID:5099092 DOI:10.1016/j.media.2016.09.001

Spanò G, Clark C., and Edgin J.O. (2017) Young children with Down syndrome show normal circadian development, but poor sleep efficiency: A cross-sectional study across the first 60 months of life. *Sleep Medicine*, in press.

Spano G, Intraub, H. and Edgin, J.O. (2017) Testing the “Boundaries” of Boundary Extension: Anticipatory Scene Representation Across Development and Disorder. *Hippocampus*, in press.

Stonnington CM, Lee, W., Thiyyagura, P., Bauer, R.J., Sharieff, S., Chen, Y., Alexander, G.E., Caselli, R.J., Locke, D.E.C., Reiman, E.M. and Chen, K. Predicting progression to the clinical stages of Alzheimer’s disease using volumetric magnetic resonance imaging and fluorodeoxyglucose positron emission tomography. Submitted.

Thome A, Marrone, D.F., Ellmore, T.M., Chawla, M.K., Lipa, P., Ramirez-Amaya, V., Lisanby, S.H., McNaughton, B.L. and Barnes, C.A. (2017) Evidence for an evolutionarily conserved memory coding scheme in the mammalian hippocampus. *The Journal of Neuroscience*, in press.

Williams SM, et al., *TDP-43 protein variants as biomarkers in amyotrophic lateral sclerosis*. *BMC Neurosci*, 2017. 18(1): p. 20.

Zissimopoulos JM, Barthold, D., Brinton, R.D. and Joyce, G. (2017) Sex and Race Differences in the Association Between Statin Use and the Incidence of Alzheimer Disease. *JAMA Neurol*, in press.

Current and Pending Grants

Current Grants

R01 Grant, R01 AG028084 9/01/13 - 5/31/18
Renewal: from National Institute on Aging \$1,609,782 Total Costs
PI: Heather Bimonte-Nelson
Variations in hormones during menopause: effects on cognitive and brain aging.

R01 AG028084
National Institute on Aging 10/07 - 9/12
PI: Heather Bimonte-Nelson \$1,653,769 Total Costs
Variations in hormone therapy: effects on cognition and markers of brain aging.

National Science Foundation (NSF) Graduate Research Fellowship
Co-mentors: Heather Bimonte-Nelson, Rachael Sirianni (Barrow Neurological Institute),
Student: Alesia Prakapenka
Development of targeted delivery of estrogen to examine its effect on cognitive function.

Alzheimer's Disease Center Grant (Arizona)
NIH 2P30AG19610 7/1/01 -6/30/21
PI: Eric Reiman \$8,811,853 Total Costs
Co-Directors of the Research Education Component: Heather Bimonte-Nelson and Yonas Geda (Mayo).
Arizona Alzheimer's Disease Core Center

R01 NS097537
NIH-HHS (NINDS) 7/1/2016 – 5/31/2021
PI: Jason Newbern \$1,731,322
Co-Investigator: Heather Bimonte-Nelson
Functions of ERK/MAPK Signaling in GABAergic Circuit Development

Postdoctoral T32 Training Grant 5/01/2016 – 4/30/2021
NIA (NIH) T32AG044402 \$1,237,680 Total Costs
PI: Carol Barnes (U of A), Paul Coleman (ASU), Eric Reiman (Banner Alzheimer's Institute)
Associate Directors: Heather Bimonte-Nelson, Matthew Huentelman (TGEN)
Postdoctoral Training, Neurobiology of Aging and Alzheimer's Disease

Mayo MEGA Award 1/1/2017 – 6/31/2019
Cognitive effects of ovarian hormones in menopause \$100,000 Total Costs
PI: Juliana Kling (Mayo)
Co-Investigators: Heather Bimonte-Nelson (ASU), Virginia Miller (Mayo), Cynthia Stonnington (Mayo), Leslie Baxter (Barrow Neurological Institute), Anita Mayer (Mayo), Paru David (Mayo), Julia Files (Mayo), Dona Locke (Mayo), Yonas Geda (Mayo), Hamy Temkit (Mayo)

Mayo Ken and Linda Morris Weight and Wellness Solutions Program Awards Obesity in menopause: The role of estrogen therapy on the gut microbiome and host energy balance after surgically-induced menopause Co-PIs: Heather Bimonte-Nelson (ASU, Psychology), Julia Files (Mayo); Anita Mayer (Mayo); Marcia Ko (Mayo), Rosy Krajmalnik-Brown (ASU, Biodesign)	2/1/2017 – 1/31/2018 \$50,000 Total Costs
Coon, David: Principal Investigator EPIC: A Group Intervention for Early-Stage AD Dyads in Diverse Communities. National Institute on Aging. Total ASU Budget \$3.6 million.	
Coon, David: Principal Investigator (subcontract) Music and Memory II Phoenix Symphony	10/01/16 – 09/30/17 \$85,000
Coon, David: Co-Principal Investigator Empowering Caregiver Self-Care HHS-HRSA Teri Kennedy, Co-PI of ASU subcontract.	07/01/15 – 06/30/18 \$200,000
Coon, David: Core Leader Outreach & Recruitment Core Arizona Alzheimer's Disease Core Center P30 National Institute on Aging Total Award: \$8.8 million.	07/01/16 – 06/30/21 \$459,230 ORE Core Total
Coon, David: Principal Investigator (subcontract) Arizona Healthy Brain Initiative Collaborating Center Centers for Disease Control	9/30/14-05/13/16 \$20,068
Coon, David: Principal Investigator (subcontract) Nevada's ACL: Dementia Capability for Persons with Alzheimer's Disease and Related Dementias U.S. Administration for Community Living/U.S. Administration on Aging	09/01/14 – 08/30/17 \$20,000
NIRG-14-321390 (Mastroeni,Diego, PI) Alzheimer's Association Profiling the Gliome in Alzheimer's Disease	12/1/2015-11/30/2017 \$34,436.00
ADHS16-104646 (Mastroeni,Diego, PI) ABRC A Novel Compound to Protect Mitochondria against Oligomeric Abeta Toxicity Implications for the Synapses	7/1/2015-1/21/2017 \$107,739.00
CH 07/19/16 (Mastroeni,Diego, PI) AADC	7/1/2016-6/30/2017 \$30,000.00

FY17: Arizona Alzheimer's Consortium

2R01AG037637-07 NIH – NIA Molecular interplay between Abeta, tau and mTOR: Mechanisms of neurodegeneration Role: Principal Investigator	08/2016 - 07/2021 \$1,250,000 Total DC
1R21NS096375-01A1 NIH – NINDS Tau conditional knockout mice to elucidate the function of tau in the adult brain Role: Principal Investigator	08/2016 - 07/2018 \$450,000
Arizona Alzheimer's Consortium Assessing the role of necroptosis in Alzheimer's disease Role: Principal investigator	07/2016 - 06/2017 \$40,000 Total DC
National Science Foundation Elucidating the molecular mechanisms linking maternal choline supplementation to healthy cognitive aging Role: Mentor (PI: Ramon Velazquez)	08/2016 - 07/2018 \$143,613 Total DC
Alzheimer's Association Pim1 inhibition as a therapeutic strategy for Alzheimer's disease Role: Mentor (PI: Ramon Velazquez)	10/2016 - 09/2019 \$175,000 Total DC
Alzheimer's Drug Discovery Foundation Testing of selective DYRK1A inhibitors as a novel treatment of AD Role: Collaborator (PI: Travis Dunckley)	04/2016 - 10/2017 \$175,000 Total DC
Project #: AARG-17-503765 Alzheimer's Association Molecular mechanisms of cognitive decline in Alzheimer's disease Role: Principal Investigator (PI: Travis Dunckley)	02/2017 - 01/2020 \$175,000 Total DC
Human Frontiers Science Program Odor Background Segregation	7/1/15-6/30/18 \$337,500
NIH Multiscale Model of Exploitation Exploration Tradeoff	9/1/15-5/31/19 \$1,918,606
NSF Ideas Lab Collaborative Research Y 4	11/1/15-10/31/18 \$900,000

AZ Alzheimer's Cons Mouse olfactory Learning	7/1/16-6/30/17	
	\$43,208	
Decourt, Boris (PI)	12/1/15-11/30/19	
NIH (NIA) K01 Career Development Award	\$110,000 Annual DC	
Pre-clinical testing of lenalidomide as pleiotropic therapeutics for Alzheimer's.		
Decourt, Boris (PI)	07/01/16-06/30/17	
Arizona Alzheimer's Research Consortium (AARC)	\$30,000 Annual DC	
Identification of the amyloidogenic targets altered by lenalidomide.		
D'Souza, Gary (PI)	09/01/16-08/31/19	
Arizona Alzheimer's Research Consortium (AARC)	\$50,000 Annual DC	
Development of composite cognitive endpoints for presymptomatic AD trials.		
2P30AG019610 (Reiman)	7/1/2016-6/30/2021	0.36 Calendar
NIH/NIA	\$71,503 Annual Direct Costs	
Arizona Alzheimer's Disease Core Center – Clinical Core		
Role: Site PI		
Burke, Anna MD		
AARC (Reiman)	7/1/16-6/30/17	0.12 Calendar
State of Arizona	\$55,000 Annual Direct Costs	
Native American Outreach and Native American Clinical Core		
Role: Core PI		
P30 AG019610 (Reiman)	7/1/2016-6/30/2021	0.31 Calendar
NIH/NIA	\$1,682,234 Annual Direct Costs	
Arizona Alzheimer's Disease Core Center		
Role: Site PI		
2 R01 AG031581 (Reiman)	5/01/2008-3/31/2019	3.0 calendar
NIH/NIA	\$1,110,690 Annual Direct Costs	
Bain Imaging, APOE, & the Preclinical Course of Alzheimer's Disease		
Role: Co-Investigator		
R01AG031581-17S1 (Reiman)	9/15/2016-3/31/2019	0.60 calendar
NIH/NIA	\$352,478 Annual Direct Costs	
Administrative Supplement to Brain Imaging, APOE & the Preclinical Course of Alzheimer's Disease		
Role: Co-Investigator		
W81XWH-12-2-0012 (Weiner)	2/21/13-9/29/2018	0.01 Calendar
NIH/Northern California Institute Res & Educ.	\$30,278 Annual Direct Costs	

Effects of traumatic brain injury and post-traumatic stress disorder on Alzheimer's disease (AD) in Veterans using ADNI.

Role: Co-Investigator

W81XWH-13-1-0259 (Weiner) 9/30/13-9/29/17 0.00 calendar
NIH/Northern California Institute Res & Educ. \$5,448 Annual Direct Costs

Effects of traumatic brain injury and post-traumatic stress disorder on Alzheimer's disease (AD) in Veterans with Mild Cognitive Impairment (MC) using the Alzheimer's disease neuroimaging initiative (ADNI).

Role: Co-Investigator

Arizona Alzheimer's Research Consortium (Reiman) 7/1/2011-6/30/2017 0.60 Calendar
State of Arizona \$75,000 Annual Direct Costs

Advanced Image Analysis Techniques for the Detection and Tracking of Alzheimer's disease and its prevention

Role: Project PI

1RF1AG041705-01A1 (Reiman/Tariot/Lopera) 5/18/2012-4/30/2017 1.20 Calendar
NIH/NIA \$12,302,690 Total NIH Direct Costs

Alzheimer's Prevention Initiative

Role: Co-Investigator

1UF1AG046150-01 (Reiman/Tariot) 9/1/2013-8/31/2018 0.60 Calendar
NIH/NIA \$22,280,073 Total NIH Direct Costs

Alzheimer's Prevention Initiative APOE4 Trial

Role: Co-Investigator

4UH3TR000967-03 (Strittmatter/Van Dyck) 6/18/2013-5/31/2017 0.12 Calendar
NIH/Yale University \$38,187 Annual Direct Costs

Fyn Inhibition by AZD0530 for Alzheimers Disease

Role: Co-Investigator

ADHS14-00003606 (Sabbagh) 10/23/2015-10/22/2017 1.8 calendar
Arizona Biomedical Research Commission (ABRC)/HHS \$161,707 Annual Direct Costs

Prime award received by St. Joseph's Hospital & Medical Center. Subaward agreement pending execution.

Longitudinal Assessment of Florbetapir PET, FDG PET, and MRI in Down Syndrome Individuals with and without Alzheimer's Dementia

Role: Co-Site PI

R01 (Handen) 9/30/2015-9/30/2020 0.60 calendar
NIH via University of Pittsburgh \$263,470 Annual Direct Costs

Neurodegeneration in Aging Down Syndrome (NiAD): A Longitudinal Study of Cognition and Biomarkers of Alzheimer's Disease

Subaward agreement pending execution.

Role: Co-Investigator

CMMI-1505260 (Huang) NSF via University of Washington Collaborative Research: Data-Driven Smart Monitoring of Alzheimer's Disease via Data Fusion, Personalized Prognostics, and Selective Sensing. Role: Co-Investigator	9/1/2016-8/31/2017 0.74 calendar \$13,671 Annual Direct Costs
2 R01 AG031581 (Reiman) NIH/NIA Bain Imaging, APOE, & the Preclinical Course of Alzheimer's Disease Role: Principal Scientist	5/01/08-3/31/19 0.12 calendar \$1,110,690 Annual Direct Costs
Arizona Alzheimer's Research Consortium (Langbaum) State of Arizona Arizona Alzheimer's Registry Role: Principal Investigator	7/1/11-6/30/17 0.0 Calendar \$12,149 Annual Direct Costs
1RF1AG041705-01A1 (Reiman/Tariot/Lopera) NIH/NIA Alzheimer's Prevention Initiative Role: Co-Investigator	5/18/12-4/30/17 3.0 Calendar \$12,302,690 Total NIH DC
1UF1AG046150-01 (Reiman/Tariot) NIH/NIA Alzheimer's Prevention Initiative APOE4 Trial Role: Co-Investigator	9/20/13-8/31/18 6.0 Calendar \$22,280,073 Total Project DC
Alzheimer's Association/GHR/FBRI(Reiman/Tariot/Langbaum) Alzheimer's Prevention Initiative APOE4 Trial Role: Co-Principle Investigator	1/1/16-12/31/20 0.0 Calendar \$10,000,000 Total Project Costs
Flinn Foundation (Reiman/Tariot/Langbaum) Alzheimer's Prevention Initiative Role: Co-Principal Investigator	1/1/14-12/31/18 0.6 Calendar
5 P30 AG19610 (Reiman) NIH/NIA Arizona Alzheimer's Disease Core Center Role: PI	7/1/2016-6/30/2021 1.2 calendar \$1,682,232 Annual Direct Costs
2 R01 AG031581 (Reiman) NIH/NIA Brain Imaging, APOE, & the Preclinical Course of Alzheimer's Disease Role: PI	5/01/2008-3/31/2019 1.2 calendar \$1,110,690 Annual Direct Costs
3R01AG031581-17S1 (Reiman) NIH/NIA	6/15/2016-3/31/2019 0.36 calendar \$352,478 Annual Direct Costs

Administrative Supplement to Brain Imaging, APOE & the Preclinical Course of Alzheimer's Disease

Role: PI

1RF1AG041705-01A1 (Reiman/Tariot/Lopera) 5/18/2012-4/30/2017 1.8 calendar
NIH/NIA \$12,302,690 Total Project DC
Alzheimer's Prevention Initiative
Role: Co-PI

1UF1AG046150-01 (Reiman/Tariot) 9/20/2013-8/31/2018 1.8 calendar
NIH/NIA \$22,280,073 Total Project DC
Alzheimer's Prevention Initiative APOE4 Trial
Role: Co-PI

TGen Professional Services Agreement (Reiman) 7/1/2008-12/31/2017 0.6 calendar
Translational Genomics Research Institute \$30,330 Annual Direct Costs
Role: PI

W81XWH-12-2-0012 (Weiner) 2/21/2013-9/29/2018 0.0 calendar
NIH/Northern California Institute Res & Educ. \$30,278 Annual Direct Costs
Effects of traumatic brain injury and post-traumatic stress disorder on Alzheimer's disease (AD) in Veterans using ADNI.
Role: Site PI

W81XWH-13-1-0259 (Weiner) 9/30/2013-9/29/2017 0.0 calendar
NIH/Northern California Institute Res & Educ. \$5,448 Annual Direct Costs
Effects of traumatic brain injury and post-traumatic stress disorder on Alzheimer's disease (AD) in Veterans with Mild Cognitive Impairment (MC) using the Alzheimer's disease neuroimaging initiative (ADNI).
Role: Site PI

4UH3TR000967-02 (Strittmatter/Van Dyck) 6/18/2013-5/31/2017 0.12 calendar
NIH/Yale University \$38,187 Annual Direct Costs
Fyn Inhibition by AZD0530 for Alzheimer's Disease
Role: Site PI

U01NS093334-01 (Cummings/Reiman/Shenton/Stern) 12/15/2015-11/30/2022 0.6 calendar
Boston University via Mayo Clinic Arizona \$436,318 Total Project Direct Costs
Chronic Traumatic Encephalopathy: Detection, Diagnosis, Course and Risk Factors
Role: Co-PI

Alzheimer's Association/GHR/FBRI 1/1/2016-12/31/2020
(Reiman/Tariot/Langbaum) 0.0 Calendar
Alzheimer's Prevention Initiative APOE4 Trial \$10,000,000 Total Project Costs
Role: Co-PI

Flinn Foundation (Reiman/Tariot/Langbaum) 1/1/2014-12/31/2018 0.0 Calendar
Alzheimer's Prevention Initiative
Role: Co-PI

ADHS14-00003606 (Sabbagh) 10/23/2015-10/22/2017
0.24 calendar
Arizona Biomedical Research Commission (ABRC)/HHS \$161,707 Annual Direct Costs
Prime award received by St. Joseph's Hospital & Medical Center.. Subaward agreement pending execution.
Longitudinal Assessment of Florbetapir PET, FDG PET, and MRI in Down Syndrome Individuals with and without Alzheimer's Dementia
Role: Co-Site PI

1UG3OD023171-01 (Reiman) 7/1/2016-6/30/2021 0.6 calendar
NIH/ University of Arizona \$389,364 Annual Direct Costs
University of Arizona-Banner Health Precision Medicine Initiative Cohort Enrollment Center
Role: PI

R01 (Handen) 9/30/2016-9/30/2020 0.6 calendar
NIH via University of Pittsburgh \$263,470 Annual Direct Costs
Neurodegeneration in Aging Down Syndrome (NiAD): A Longitudinal Study of Cognition and Biomarkers of Alzheimer's Disease
Prime award received by the University of Pittsburgh. Subaward agreement pending execution.
Role: Site PI

Beach, Thomas (Core Leader)
P30 AG019610 (Reiman) 7/1/16 to 6/30/21
NIH/NIA \$1,682,235 Annual DC
Arizona Alzheimer's Disease Core Center

Beach, Thomas
U01 (Scherzer) 9/30/12-8/31/15
Brigham and Women's Hospital \$22,598 Annual DC
Biomarkers for early detection and intervention in Parkinson's disease

Beach, Thomas
MJFF 2015 6270.04 (Beach/Adler) 10/1/15-4/30/16
Michael J. Fox Foundation for Parkinson's Research \$10,866 Total Costs
Submandibular Gland Needle Biopsy for the Diagnosis of Early Parkinson's Disease Supplement

Beach, Thomas
MJFF 2015 9035.01 (Beach/Derkinderen, Kordower/Munoz) 10/1/15-4/30/16
Michael J. Fox Foundation for Parkinson's Research \$31,542 Total Costs
Follow-up Autopsy Study: Evaluation of alpha-synuclein immunohistochemical methods for the detection of Lewy-type synucleinopathy in gastrointestinal biopsies

Beach, Thomas MJFF 2013 9035 (Beach/Derkinderen) Michael J. Fox Foundation for Parkinson's Research Evaluation of alpha-synuclein immunohistochemical methods for the detection of Lewy-type synucleinopathy in gastrointestinal biopsies.	11/25/13-11/30/15 \$132,686 Annual DC
Beach, Thomas MJFF (Beach) Michael J. Fox Foundation for Parkinson's Research Resources Utilization Grants Program	1/01/14 – 12/31/16 \$36,250 Total Costs
Beach, Thomas MJFF (Multi PI Cuenca, Beach, Walker, Adler) MJFF Retinal Pathology in Parkinson's Disease: Implications for Vision and Biomarkers	9/1/14-4/30/16 \$90,120 Annual DC
Beach, Thomas GE Healthcare (Beach) Postmortem Correlation for Amyloid Imaging Ligand GE-067-007	8/1/10 to present
Beach, Thomas Schering-Bayer Pharmaceuticals, Inc. (Beach) Schering-Bayer Pharmaceuticals, Inc. Postmortem Correlation for Amyloid Imaging Ligand Bay 94-9172	11/1/09 to present
Beach, Thomas Avid Radiopharmaceuticals, Inc. Beach (PI) Postmortem Correlation for the Amyloid Imaging Ligand AV45-A07 and AV45-A16.	1/10/09 to present
Beach, Thomas Navidea Biopharmaceuticals Dr. Beach is Leader of the Central Neuropathology Site for this imaging-to-autopsy Phase III clinical trial of an amyloid imaging agent for diagnostic usage.	4/01/2014 to present
Beach, Thomas Janssen Research & Development A Brain Donation Study for Subjects Who Participated in Clinical Trials for the Alzheimer Immunotherapy Program	9/01/2014 to present
Beach, Thomas R01 AG044372-02 (PI: Kanaan) NIH via Michigan State University Tau-induced axonal degeneration in Alzheimer's disease and tauopathies	9/30/14-4/30/19 \$12,300 Annual DC
Beach, Thomas R01 AG044723-02 (PI: Migrino)	9/15/14-8/31/16

NIH via Phoenix VA Human Vascular model to study Alzheimer's Disease	\$2,532 Annual DC
Beach, Thomas 1R21NS093222 (Huentelman) NIH via TGEN Identification of pathogenic mechanisms important in multiple system atrophy	7/1/14 - 6/30/17 \$16,000 Annual DC
Beach, Thomas ABRC ESI (Mastroeni) Arizona Biomedical Research Commission A Novel Compound to Protect Mitochondria against Oligomeric Abeta Toxicity, Implications for the Synapse	10/23/14-10/22/17 \$68,167 Annual DC
Beach, Thomas AARC (Reiman, Project PI: Beach) AZ DHS via AARC Developing a Shared Resource of CSF, Plasma, Serum , PBMC samples from Arizona's Longitudinal Brain and Body Donation and APOE4 Gene Dose Program	7/1/16– 6/30/17 \$304,987 Annual DC
Beach, Thomas AARC (Reiman, Project PI: Beach) AZ DHS via AARC Towards Single-Cell Analysis in Human Brain Neurodegenerative Disease: A Pilot Study	7/1/16– 6/30/17 \$111,611 Annual DC
Beach, Thomas AARC (Reiman, Project PI: Shprecher) AZ DHS via AARC Population Survey and Clinicopathological Study Initiation for Incidental REM Sleep Behavior Disorder in Sun City, Arizona	7/1/16 – 6/30/17 \$73,403 Annual DC
Belden, Christine P30 AG019610 (Reiman) NIH/NIA Arizona Alzheimer's Disease Core Center – Clinical Core	7/1/16 to 6/30/21 \$1,682,235 Annual DC
Serrano, Geidy New Investigator Grant (Serrano) ABRC The effects of APOE genotype on APP/A β levels in human liver and brain	11/1/14-10/31/17 \$68,030 Annual DC
Serrano, Geidy U01 (Scherzer) Brigham and Women's Hospital Biomarkers for early detection and intervention in Parkinson's disease	9/30/12-8/31/15 \$22,598 Annual DC

Serrano, Geidy
MJFF 2015 6270.04 (Beach/Adler) 10/1/15-4/30/16
Michael J. Fox Foundation for Parkinson's Research \$10,866 Total Costs
Submandibular Gland Needle Biopsy for the Diagnosis of Early Parkinson's Disease Supplement

Serrano, Geidy
MJFF 2015 9035.01 (Beach/Derkinderen, Kordower/Munoz) 10/1/15-4/30/16
Michael J. Fox Foundation for Parkinson's Research \$31,542 Total Costs
Follow-up Autopsy Study: Evaluation of alpha-synuclein immunohistochemical methods for the detection of Lewy-type synucleinopathy in gastrointestinal biopsies

Serrano, Geidy
MJFF 2013 9035 (Beach/Derkinderen) 11/25/13-11/30/15
Michael J. Fox Foundation for Parkinson's Research \$132,686 Annual DC
Evaluation of alpha-synuclein immunohistochemical methods for the detection of Lewy-type synucleinopathy in gastrointestinal biopsies.

Serrano, Geidy
MJFF (Multi PI Cuenca, Beach, Walker, Adler) 9/1/14-4/30/16
MJFF \$90,120 Annual DC
Retinal Pathology in Parkinson's Disease: Implications for Vision and Biomarkers

Serrano, Geidy
Avid Radiopharmaceuticals, Inc. Beach (PI) 1/10/09 to present
Postmortem Correlation for the Amyloid Imaging Ligand AV45-A07 and AV45-A16.

Serrano, Geidy
Navidea Biopharmaceuticals 4/01/2014 to present
Dr. Beach is Leader of the Central Neuropathology Site for this imaging-to-autopsy Phase III clinical trial of an amyloid imaging agent for diagnostic usage.

Serrano, Geidy
Janssen Research & Development 9/01/2014 to present
A Brain Donation Study for Subjects Who Participated in Clinical Trials for the Alzheimer Immunotherapy Program

Serrano, Geidy
R01 AG044372-02 (PI: Kanaan) 9/30/14-4/30/19
NIH via Michigan State University \$12,300 Annual DC
Tau-induced axonal degeneration in Alzheimer's disease and tauopathies

Grant (Shprecher) 7/2013-present
Sun Health Foundation \$97,300 Annual Direct Costs
Feasibility study of an early wellness program in Parkinson's disease and impact on quality of life

P30 AG019610 (Reiman) NIH/NIA Arizona Alzheimer's Disease Core Center – Clinical Core	7/1/16 to 6/30/21 \$1,682,235 Annual DC
Zamrini, Edward AARC (Reiman, Project PI: Beach) AZ DHS via AARC Developing a Shared Resource of CSF, Plasma, Serum , PBMC samples from Arizona's Longitudinal Brain and Body Donation and APOE4 Gene Dose Program	7/1/16 – 6/30/17 \$304,987 Annual DC
AARC (Reiman, Project PI: Saner/Chen) AZ DHS via AARC Development of a Centralized Data Management Program for the ADCC, BBDP, and APOE4 Gene Dose Program	7/1/16-6/30/17 \$280,000 Annual DC
Department of Defense (Baxter PI) W81XWH-14-ARP-IDA “Cognitive and Neural Correlates of Aging in Autism Spectrum Disorder”	6/01/15- 5/30/18 \$358,757 (DC)
Baxter, Leslie (PI) Institute for Mental Health Research “Depression and anxiety in the aging autism spectrum disorders cohort” Baxter, Leslie (Co-Pi PI: Pipe, Schmainda) RO1 CA092500	7/1/15-8/1/17 \$20,000 (DC) 2012-2017 \$28,373 (DC)
“MRI contrast agent methods in GBM” Baxter, Leslie (co-PI; Reiman PI) NIA AG019610 Arizona Alzheimer's Disease Core Center	07/01/11 – 06/30/16 \$ 175,097 (TC)
Sabbagh MN (PI) ADHS14-00003606 Arizona Biomedical Research Commission Longitudinal Assessment of Florbetapir FET, FDG PET, and MRI in Down Syndrome	10/23/14-10/22/17 \$223,816 Annual DC
Mufson EJ (PI) PO1AG14449 Neurobiology of Mild Cognitive Impairment in the Elderly	12/01/2014 – 1/31/2019 \$341,627.79 (DC)
Mufson, E.J. (PI) RO1AG43775 Cellular and Molecular Medial Temporal lobe pathology in elderly with PreMCI	\$538,953.78 (DC) 1/15/2015 – 5/31/2018
Mufson, E.J. (PI) Barrow and Beyond Genetic signature of cortical neurons in sporadic AD	07/01/2015 – 6/30/2018 \$50,000 (DC)

Mufson, EJ (site PI) Cifu, D. (PI, Virginia Commonwealth University) Department of Defense PT108802-SC106187 Chronic Effects of Neurotrauma	9/30/2014 – 8/31/2019 \$594,745.46 (DC)
Han, Peng Cheng (PI) BNI-UA-Phoenix Joint Translation Neuroscience PACAP deficit in AD.	2013-2015 \$50,000 (DC)
Shi, Jiong (PI) Eli Lilly Effect of LY2062430, an Anti-Amyloid Beta Monoclonal Antibody, on the Progression of Alzheimer's Disease as Compared with Placebo (H8A-MC-LZAM).	2009-2015 \$510,036 (TC)
Shi, Jiong (PI) Avanir Pharmaceuticals, Inc A Prospective, Open-label Study to Assess the Safety and Efficacy of Nuedexta (Dextromethorphan/Quinidine) in the Treatment of Pseudobulbar Affect (PBA) in Patients with Alzheimer's Disease.	2013-2015 \$180,000 (TC)
Shi, Jiong (PI) Merck & Co. A Phase III, Randomized, Placebo-Controlled, Parallel-Group, Double Blind Clinical Trial to Study the Efficacy and Safety of MK-8931 (SCH900931) in Subjects with Amnesic Mild Cognitive Impairment due to Alzheimer's Disease (prodromal AD).	2014-2016 \$460,500 (TC)
Shi, Jiong (PI) GE Healthcare A Principal Open-label Study to Assess the Prognostic Usefulness of Flutemetamol (18F) Injection for Identifying Subjects with Amnesic Mild Cognitive Impairment Who Will Convert to Probable Alzheimer's Disease.	2010-2015 \$86,437 (TC)
Shi, Jiong (PI) Navidea Biopharmaceuticals A Phase 2 Clinical Trial to Evaluate the Efficacy and Safety of [¹⁸ F] AZD4694 PET in the Detection of Beta Amyloid in Subjects with Probable Alzheimer's Disease, Older Healthy Volunteers, and Young Healthy Volunteers.	2012-2015 \$307,625 (TC)
Department of Defense (Baxter PI) W81XWH-14-ARP-IDA "Cognitive and Neural Correlates of Aging in Autism Spectrum Disorder"	6/01/2015 – 5/30/2018 \$358,757 (DC)
Baxter, Leslie (PI) Institute for Mental Health Research "Depression and anxiety in the aging autism spectrum disorders cohort"	7/1/15-8/1/16 \$20,000 (DC)

Baxter, Leslie (co-PI; Theodore BNI PI; Sierkes PI) Oligomeric Neuronal Protein Aggregates as Biomarkers for Traumatic Brain Injury (TBI) and Alzheimer's disease (AD)	9/25/12-6/24/15 \$339,424 27,999 (BNI: DC)
Baxter, Leslie (co-PI; Reiman PI) NIA AG019610 Arizona Alzheimer's Disease Core Center	07/01/11 – 06/30/16 \$53,272 (TC)
Baxter, Leslie (Co-Pi PI: Pipe, Schmainda) RO1 CA092500 "MRI contrast agent methods in GBM"	2012-2017 \$28,373 (DC)
Baxter, Leslie (PI), Jiong Shi, Elliot Mufson State of Arizona/Barrow Subcontract Alzheimer's Disease and Aging Studies at BNI	07/01/14 - 06/30/15 \$150,000 (TC) \$150,000 (BNI Match)
ADHS14-052688 State of Arizona, DHS (Caselli) Arizona Alzheimer's Research Center (Consortium) Normal and Pathological Aging (Preclinical Alzheimer's Disease) Role: Principle Investigator	7/1/11 – 6/30/17 1.2 calendar \$360,000
P30 AG019610 (Reiman) National Institute on Aging Alzheimer's Disease Clinical Core B Center Role: Associate Director and Clinical Core Director	8/15/11 - 6/30/21 1.8 calendar \$120,616
P30 AG019610 (Reiman) National Institute on Aging Alzheimer's Disease Administrative Core A Role: Core Director	8/15/11 - 6/30/21 0.60 calendar \$10,916
P30 AG019610 (Reiman) National Institute on Aging Arizona Alzheimer's Disease Center Plasma/Serum Storage at MCA Bio-repository Role: Core Director	7/1/13 – 6/30/21 0.12 calendar \$19,584
R01 AG031581 (Reiman and Caselli) National Institute of Neurological Disorders and Stroke PET, APOE & the Preclinical Course of Alzheimer Disease Role: Co-Investigator	5/1/14 – 4/30/19 1.44 calendar \$73,956

R01 AG054048 (Sierks)	7/1/16 – 6/30/21
	0.60 calendar
National Institute of Aging, NIH	\$24,257
Protein variants as blood based biomarkers for diagnosing and staging AD	
Role: Co-Investigator	
MK-8931-017 (Caselli)	11/1/13 – 10/31/20
	0.12 calendar
Merck & Co., Inc.	\$69,573
13-000419/Protocol No. MK-8931-017-02 A Randomized, Placebo Controlled, Parallel-Group, Double Blind Efficacy and Safety Trial of MK-8931 in Subjects with Mild to Moderate Alzheimer's Disease	
Role: Principal Investigator	
MK-8931-019 (Caselli)	11/1/13 – 10/31/20
	0.24 calendar
Merck & Co., Inc.	\$81,781
IRB 13-006525/Protocol No. MK-8931-019 A Phase III, Randomized, Placebo-Controlled, Parallel-Group, Double-Blind Clinical Trial	
Role: Principal Investigator	
1P01 AG052350-01A1 (Zlokovic)	4/1/16-3/31/21 1.20 calendar
	11/1/13 – 10/31/20 0.24 calendar
National Institute of Aging, NIH	\$50,662
Vascular Contributions to Dementia Effects of Genetic Risk Factors for Alzheimer's Disease	
Role: Co-Investigator	
U01 AG019676 (Reiman)	8/01/16 – 7/31/17 0.12 calendar
National Institutes of Health	\$10,481
Retrospective measurement of repeated head impact exposure	
Role: Co-Investigator	
R01 AG041232 (Myers)	07/01/2013 - 04/30/2018 1.2 calendar mos.
NIH/NIA	\$125,000/year direct costs
APOEomic: Searching for APOE interacting risk factors using omics data	
Role: Co-Investigator	
UH2/UH3TR0000891 (Huentelman)	08/01/2013 - 07/31/2018 1.2 calendar mo.
NIH/Trans-NIH Research	\$242,183/year direct costs
exRNA signatures predict outcomes after brain injury	
Role: Principal Investigator	
AAC - DHS (Huentelman)	07/01/2016 – 06/30/2017 0.6 calendar mo.
State of Arizona, DHS	\$70,000 /year direct costs
AARC FY 17: Alzheimer's Projects	
Role: Principal Investigator	

R0AG048907 (Huentelman/Barnes) 09/15/2014 – 09/14/2018 1.2 calendar mos.
NIH/NIA \$245,145/year direct costs
CATT: Development and Application of a Neuronal Cell Activity-Tagging Toolbox
Role: Principal Investigator (multi-PI)

RO1 AG049465 (Barnes) 08/01/2014 – 03/31/2019 1.2 calendar mos.
NIH/NIA \$162,203/year direct costs
Neural System Dynamics and Gene Expression Supporting Successful Cognitive Aging
Role: Co-Investigator

1 RO1 AG049464 (Coleman/Barnes/Alexander) 08/01/2014 – 05/31/2019 1.2 calendar mos.
NIH/NIA \$178,013/year direct costs
Epigenetic, Neuroimaging and Behavioral Effects of Hypertension in the Aging Brain
Role: Co-Investigator

R21NS093222 (Huentelman) 09/01/2015 – 08/30/2017 0.6 calendar mo.
NIH \$119,500/year direct costs
Identification of pathogenic mechanisms important in multiple system atrophy
Role: Principal Investigator

Flinn Foundation Grant 12/23/2014 – 12/31/2017 1.2 calendar mos.
\$171,275/year direct costs
Role: Co Investigator

Grant (Sabbagh) 04/01/2016 – 03/31/2019 0.48 calendar mo.
Alzheimer's Association \$37,829/year direct cots
Treatment for AD in individuals with Down's Syndrome
Role: Co Investigator

P30 AG019610 (Reiman) Competitive renewal 07/01/2016 - 06/30/2021
0.48 calendar mo.
NIH/NIA \$12,190/year direct cots
Arizona Alzheimer's Disease Core Center
Role: Co-Investigator

UG30D023313 (Deoni) 09/21/2016 – 08/31/2018
NIH 0.6 & 2.4 calendar mos.
The Developing Brain: Influences and Outcomes \$70,000/year direct costs
Role: Co-Investigator

Contract (Jonathan Keats) 09/01/2011 -08/31/2019 1.8 calendar mos.
MMRF \$1,364,526
Longitudinal, Observation Study in Newly Diagnosed Multiple Myeloma (MM) Patients to
Assess the Relationship between Patient Outcomes, Treatment Regimens and Molecular Profiles
(The MMRF Longitudinal Study)
Role: Co-Investigator

Liang, Winnie SU2C-AACR-DT0612 (Jeff Trent/Patricia LoRusso) American Association for Cancer Research Personalized Medicine for Patients with BRAF wild-type (BRAFWt) Cancer Role: Co-Investigator	04/01/2012 – 06/30/2017 \$30,000
Liang, Winnie R01 (Trent/Weissman/ NIH The tumor suppressor role of SMARCA4 in SCCOHT Role: Co-Investigator	04/01/2015 – 03/31/2020 \$223,475
Liang, Winnie Grant (Matt Huentelman) AzDHS TGEN AARC FY 17: Alzheimer's disease Research Role: Co-Investigator	07/01/2016 - 06/30/2017 \$65,000
Liang, Winnie Contract (Andrew Little) Dignity Health Genomics Characterization of Chordomas Role: Principal Investigator	04/01/2016 – 2/28/2017 \$50,000
Liang, Winnie P30CA016058-40S4 (Caligiuri, Shields, Trent) NIH/NC Cancer Center Support Grant Supplement Immunogenomic Profiling of Canine Melanoma and Osteosarcoma Role: Co-Investigator	09/01/2016 – 11/30/2017 \$137,230
Michael J Fox Foundation for Parkinson's Research Planning Grant RNAseq in PPMI Planning grant Planning Director for the RNASeq strategies for the PPMI Role: PI (Van Keuren-Jensen)	05/01/2016 – 4/31/2017 \$4,701
Michael J Fox Foundation for Parkinson's Research Grant 12401 Pre-analytical extracellular vesicle enrichment for increased reliability for alpha-synuclein detection in plasma and CSF Role: Multi-PI (Van Keuren-Jensen/El-Agnaf)	05/01/2016 – 9/31/2018 \$91,224
4UH3TR000891 03 NIH/Trans-NIH Research exRNA signatures predict outcomes after brain injury	08/01/2013 - 07/31/2018 \$336,309

Role: Multi-PI (Van Keuren-Jensen/Huentelman/Adelson/Kalani)

ALS Association Grant 08/01/2015 – 07/31/2018
Grant ID: 16-IIP-255 \$80,000
Assessment of extracellular vesicle contents in patients with ALS
Role: PI (Van Keuren-Jensen)

Arizona Department of Health Services Grant 07/01/2016 - 06/30/2017
TGen Arizona Alzheimer's Consortium FY17 Projects \$60,000
Role: Co-I (PI: Eric Reiman)

R03NS09001301A1 06/01/2015 – 05/31/2017
NIH/NINDS \$11,490
Gene Expression of Foci of TBI Neuropathology and Rod Microglia Interactions
Role: Subaward PI (PI: Jonathan Lifshitz)

Flinn Foundation Grant 10/22/2014 – 12/30/2017
Flinn Grant \$200,000
Role: Co-I (PI: Jeffery Trent)

NIH/NIA P30 AG019610 07/01/16 – 06/30/17
Arizona Alzheimer's Disease Core Center (UA Clinical Core) \$43,084 Annual DC

Ahern, Geoff (co-investigator) 07/01/16 – 06/30/17
State of Arizona, DHS Grant \$7,500 Annual DC
Arizona Alzheimer's Consortium - Patient Recruitment and Outreach for Alzheimer's Disease and Related-Disorders

Ahern, Geoff (PI) 2013 – present
Eisai \$107,194/patient
A Placebo-controlled, Double-blind, Parallel-group, Bayesian Adaptive Randomization Design and Dose Regimen-finding Study to Evaluate Safety, Tolerability and Efficacy of BAN2401 in Subjects With Early Alzheimer's Disease. Protocol # BAN2401-G000-201.

Ahern, Geoff (PI) 2013 – present
Lilly Pharmaceuticals \$32,863/patient
Effect of Passive Immunization on the Progression of Mild Alzheimer's Disease: Solanezumab (LY2062430) versus Placebo. Protocol # H8A-MC-LZAX.

Ahern, Geoff (PI) 2013 – present
EnVivo Pharmaceuticals \$37,069/patient
A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, 26-Week, Phase 3 Study of Two Doses of EVP-6124 or Placebo in Subjects with Mild to Moderate Alzheimer's Disease Currently or Previously Receiving an Acetylcholinesterase Inhibitor Medication. Protocol # EVP-6124-025

Ahern, Geoff (PI) EnVivo Pharmaceuticals A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, 26-Week, Phase 3 Study of Two Doses of EVP-6124 or Placebo in Subjects with Mild to Moderate Alzheimer's Disease Currently or Previously Receiving an Acetylcholinesterase Inhibitor Medication. Protocol # EVP-6124-025	2013 – present \$27,944/patient
Alexander, Gene (PI's: Coleman, Barnes, Alexander) Co-I's: Billheimer, Huentelman, Trouard NIH/NIA 1 RO1 AG049464 Epigenetic, Neuroimaging and Behavioral Effects of Hypertension in the Aging Brain	08/01/14 – 03/31/19 \$447,322 Annual DC
Alexander, Gene (PI, multi-PI) NIH/NIA RO1 AG054077 Augmenting Cognitive Training in Older Adults	09/01/16 – 04/30/21 \$184,020 Annual DC
Alexander, Gene (UA PI, co-I's Hirschaw, Trouard) McKnight Brain Research Foundation McKnight Inter-institutional Neuroimaging Core and Brain Aging Registry	01/01/15 – 12/31/18 \$310,587 Annual DC
Alexander, Gene (PI, co-I's: Glisky, Ryan) McKnight Brain Research Foundation McKnight Inter-institutional Cognitive Aging Assessment Core	12/17/15 – 10/17/18 \$266,667 Annual DC
Alexander, Gene (PI) State of Arizona, DHS Grant Arizona Alzheimer's Consortium – Risk Factors for Brain Aging and Preclinical Alzheimer's Disease	07/01/16 – 06/30/17 \$69,000 Annual DC
Barnes, Carol (PI) (co-I: Alexander, Billheimer, Huentleman, Trouard) NIH/NIA 1 RO1 AG049465 Neural System Dynamics and Gene Expression Supporting Successful Cognitive Aging	08/01/14 – 3/31/19 \$581,747 Annual DC
Barnes, Carol (PI) NIH/NIA 1 R01 AG050548 Cell Assemblies, Brain Adaptation and Cognitive Brain	09/1/15 – 05/31/20 \$307,382 Annual DC
Barnes, Carol (PI) NIH/NIA 1 RO1 AG003376 Neurobehavioral Relations in Senescent Hippocampus	01/01/16 – 11/30/20 \$618,272 Annual DC
Barnes, Carol (PIs: Barnes, Huentelman; co-I: Okuno) NIH/NIA 1 RO1 AG048907 CATT: Development and Application of a Neuronal Cell Activity-Tagging Toolbox	09/30/14 – 05/31/18 \$261,045 Annual DC
Barnes, Carol (PI; coI: Reiman, Coleman, Bimonte-Nelson, Huentelman)	05/15/16 – 04/30/21

NIA/NIA T32 AG044402 Postdoctoral Training, Neurobiology of Aging and Alzheimer's Disease	\$223,320 Annual DC
Barnes, Carol (co-investigator) NIH/NIA 5 P30 AG019610 Arizona Alzheimer's Disease Core Center Ad Hoc Review Program	08/15/16 – 06/30/121 \$15,945 Annual DC
Barnes, Carol (PI) State of Arizona, DHS Grant Arizona Alzheimer's Consortium – Exploring the microbiome-gut brain axis: impact of microbial communities and their genes in aging.	07/01/16 – 06/30/17 \$7,500 Annual DC
Barnes, Carol (mentor; NRSA to Daniel Gray) NIH/NIA 1 F31 AG055263 Neurobiological Basis of Age-Related Deficits in Attentional Shifting and Monitoring	01/01/17 – 12/31/19 \$35,014 Annual DC
Brinton, Robbie D. (PI) NIH/NIA1 PO1 AG026572 Perimenopause in Brain Aging and Alzheimer's Disease	09/30/16 – 03/31/21 \$1,617,953 Annual DC
Brinton, Robbie D. (PI) The Woman's Alzheimer's Movement Bioinformatic Analysis to Find Current Drug Therapies that can Prevent or Delay Alzheimer's Disease	11/14/16 – 11/14/17 \$60,000 Annual DC
Brinton, Robbie D. (PI) State of Arizona, DHS Grant ApoE4: Accelerator of Bioenergetic Aging in Female Brain and Risk of Alzheimer's Disease	07/01/16 – 06/30/17 \$25,000 Annual DC
Edgin, Jamie O. (UA PI) NIH/NICHHD 1 RO1 HD074346 Express Language Sampling as an Outcome Measure	04/01/13 – 02/28/17 \$75,427 Annual DC
Edgin, Jamie O. (PI) LuMind Foundation Brain Development, Sleep and Learning in Down Syndrome	07/01/15 – 06/30/17 \$222,182 Annual DC
Edgin, Jamie O. (PI) NIH/NICHHD 1 RO1 HD088409 Memory Measure for Clinical Trials in Down Syndrome and Fragile X Syndrome	09/22/16 – 06/30/21 \$512,284 Annual DC
Edgin, Jamie O. (PI) Bill and Melinda Gates Foundation Sleep Quality as a Marker of Early Brain Development	04/30/14 – 10/01/2016 \$101,317 DC
Edgin, Jamie O. (PI); Fernandez, Fabian (co-I)	07/01/16 – 06/30/17

State of Arizona, DHS Grant Sleep and Circadian Function as Biomarkers of Alzheimer's Disease Risk in Down Syndrome	\$13,000 Annual DC
Fernandez, Fabian (PI) State of Arizona, DHS Grant Establishing circadian biomarkers for age-related working memory impairment	07/01/16 – 06/30/17 \$15,000 Annual DC
Fernandez, Fabian (PI) National Science Foundation 2016 Bisgrove Scholar Program	08/01/16 – 07/31/18 \$200,000 TDC
Glisky, Elizabeth (PI) State of Arizona, DHS Grant Arizona Alzheimer's Consortium – Interventions to Improve Memory and Executive Function in Older Adults in Real-World Settings	07/01/16 – 06/30/17 \$32,000 Annual DC
Glisky, Elizabeth (mentor) Mind and Life Institute Developing an Objective Measure of Mindfulness in Daily Life (Polsinelli training award)	06/01/15 – 05/31/17 \$14,760 Annual DC
Grilli, Matt (PI; co-I: Ryan) NIH Autobiographical Memory Specificity Training: A Novel Cognitive Intervention for Older Adults	04/01/17 – 03/31/19 \$275,000 Total DC
Raichlen, David (PI) The National Science Foundation BCS-1440867 The Evolutionary Basis of Human Inactivity	12/01/14 – 12/31/17 \$208,854 Total DC
Raichlen, David (PIs: Alexander, Raichlen) TLA Wheelhouse UA15-011 Evaluation of the aerobic and cognitive training system for enhancing cognitive performance in older adults	2/5/15-06/01/17 \$106,769 Total DC
Rapcsak, Steve (PI) State of Arizona, DHS Grant Arizona Alzheimer's Consortium - Patient Recruitment and Outreach for Alzheimer's Disease and Related-Disorders	07/01/16 – 06/30/17 \$7,500 Annual DC
Rapcsak, Steve (co-investigator) NIH/NIA 5 P30 AG019610 Arizona Alzheimer's Disease Core Center (UA Clinical Core)	07/01/16 – 06/30/21 \$43,073 Annual DC
Rapcsak, Stephen (co-investigator) NIH/NIDCD 5 RO1 DC07646 Developing Evidence-Based Treatment Continuum for Spoken and Written Language	02/04/11 – 01/31/16 \$282,858 Annual DC

Rapcsak, Stephen (co-investigator) U.S. Department of Veteran Affairs Medial Temporal Lobe Contribution to Future Thinking: Evidence from Amnesia	01/20/14 – 01/19/17 \$44,614 Total Costs
Rapcsak, Stephen (co-investigator) NIH/NIDC R01 DC013270 Neural Correlates of Recovery from Aphasia after Acute Stroke	06/01/14 – 05/31/19 \$297,230 Annual DC
Rapcsak, Stephen (PI) Southern Arizona VA Health Care System Medial Temporal Lobe Contributions to Future Thinking: Evidence From Amnesia	01/20/16 – 01/19/17 \$12,614 DC
Ryan, Lee (co-investigator) NIH Evaluation of the Safety and Efficacy of angiotensin 1-7 to Enhance Cognitive Function in Participants Undergoing Coronary Artery Bypass (CABG) Surgery	03/01/17 – 02/28/22 \$2,003,195 Total DC
Ryan, Lee (PI) State of Arizona, DHS Grant Arizona Alzheimer's Consortium – Memory Functioning in Heart Failure Patients with Risk for Alzheimer's Disease	07/01/16-06/30/17 \$69,500 Annual DC
Su, Judith (co-investigator) NIH/NIMH R21 MH111109 Label-Free, Highly-Specific, Small Molecule Detection Using Microtoroid Optical Resonators	08/01/16 – 07/31/18 \$150,000 Annual DC
Su, Judith (co-investigator; PI: Jones) NSF 7696080 Partial Support for the Optics and Photonics Winter School and Workshop to be held at the College of Optical Sciences January 3-7, 2017	09/01/2016 – 02/28/2017 \$15,000 Annual DC
Su, Judith (PI) Barrett Cancer Imaging Grant, UA Cancer Center Ultra-Sensitive and Label-Free Detection of Circulating Tumor DNA using Microtoroid Optical Resonators	3/1/2016 – 7/31/2017 \$22,500 Annual DC
Su, Judith (PI) UA Internal Faculty Seed Grant Development of a Highly-Specific Small-Molecule Biosensor for Screening Drugs to Regulate Reward Signals in the Brain	08/01/2016 – 07/31/2017 \$10,000 Annual DC
Su, Judith (PI) Partnership for Clean Competition Sensitive and Rapid Detection of Performance Enhancing Drugs Using Microtoroid Optical Resonators	03/01/2017 – 02/28/2018 \$124,005 Annual DC

Su, Judith (co-investigator; PI: McLeod) UA TRIF Team LINK Award Rationally Designed Photonic Nanostructures for More Sensitive and Robust Biochemical Optical Resonator Sensors	1/1/2017 – 06/30/2017 \$20,371 Annual DC	
Trouard, Ted (co-investigator) NIH/NICHHD R01 HD079498 Intense Physiotherapies to Improve Function in Young Children with Cerebral Palsy	05/01/14 – 04/31/19 \$451,781 Annual DC	
Trouard, Ted (co-investigator) Department of Defense W81XWH-12-1-0386 Model for Predicting Cognitive and Emotional Health from Functional Neurocircuitry	04/15/15 – 04/04/19 \$553,327	
Trouard, Ted (co-investigator) Department of Defense W81XWH-16-1-0062 Refinement and Validation of a Military Emotional Intelligence Training Program	04/15/16 – 04/14/20 \$909,150 Annual DC	
Trouard, Ted (co-investigator) State of Arizona, DHS Grant Arizona Alzheimer's Consortium – Enhanced Delivery of Therapy to the Brain of Alzheimer's Mice	07/01/16 – 06/30/17 \$58,000 Annual DC	
Trouard, Ted (member) NIH/NIBIB T32 EB000809 Graduate Training in Biomedical Imaging and Spectroscopy	07/01/06 – 06/30/18	
R21 NS096515 (Lifshitz) NIH/NINDS Remote ischemic conditioning mitigates diffuse TBI via specialized pro-resolving mediators (SPM) Role: PI	08/15/2016 – 07/31/2018 \$451,965	1.2 calendar
(Lifshitz) Arizona Alzheimer's Consortium Enduring inflammation in the wake of TBI in the rodent Role: PI	07/01/2016 – 06/30/2017 \$35,000	0.48 calendar
R03 NS090013 (Lifshitz) NIH/NINDS Gene expression of foci of TBI neuropathology and rod microglia interactions Role: PI	06/01/2015 – 05/31/2017 \$59,881	0.48 calendar
ADHS14-3606 (Thomas) AZ Biomed. Res. Commission Experimental TBI-Induced Endocrine Dysfunction: Timing, Mechanisms and Treatment Role: Mentor	10/23/2014 – 10/22/2017 \$68,182	0.6 calendar

Neurotrauma (Lifshitz) 11/15/2013 – 11/14/2018 0.0 calendar
 Diane & Bruce Halle Foundation \$100,000
 Translational Neurotrauma Research Program
 Role: PI

PI: Al-Nakkash, Layla, Consultant: Kaufman, Jason A. 01/01/17- 12/31/17
 Diabetes Action Research & Education Foundation \$20,000
 Title: Genistein: Understanding its ability to ameliorate intestinal dysfunction

Pending Grants

Coon, David: Co-Principal Investigator
 An Early Palliative & End of Life Care Intervention
 with Hispanic/Latino Families
 Co-Principal Investigator, Bronwynne Evans, PhD, RN
 National Institute for Nursing Research. Total ASU Budget \$3.79 million.

Coon, David: Project Co-Lead
 Parkinson’s Partners in Care Project (PPCP)
 Patient Center Outcomes Research Institute (PCORI). Total ASU Budget \$50,000.

(Mastroeni, Diego. PI) 4/1/2017-3/31/2022
 GRANT12178995 HHS-NIH \$3,175,034.00
 Dissecting the molecular mechanisms involved in the pathogenesis of Alzheimer’s disease

(Mastroeni, Diego, Co-PI) 7/1/2017-6/30/2022
 FP00009244 Mastroeni UCLA (AT LOS ANGELES) \$422,319.00
 Epigenetic biomarkers of brain aging in Alzheimer’s disease.

(Mastroeni, Diego. PI) 7/1/2017-6/30/2022
 HHS-NIH-NIA \$1,994,498.00
 Can Species-, Age, and Disease state-appropriate 3D brain organoids recapitulate aging and Alzheimer’s diseases in a dish?

(Mastroeni, Diego, PI) 7/1/2017-6/30/2022
 HHS-NIH \$2,863,130.00
 The effect of intraneuronal oligomeric amyloid beta on the transport of nuclear mitochondrial mRNAs

(Mastroeni, Diego, PI) 7/1/2017-6/30/2019
 3979665 HHS-NIH \$434,842.00
 Can peripheral macrophages be used to determine brain inflammation in Alzheimer’s disease brain?

(Mastroeni, Diego, PI) 5/1/2016-4/30/2019
 2016ALZ000SAGA000116508 ALZHEIMER'S ASSN \$249,923.00

Regional and Gender Effects on the Aging Brain, A Hormonal-Synaptic Theory

(Mastroeni, Diego, PI) 7/1/2016-7/30/2019
AZ160043 Department of Defense \$1,297,292.00
Predicting Alzheimer's Disease in At-Risk Populations Using Novel Blood Test.

(Mastroeni, Diego, Co-PI) 7/1/2017-6/30/2019
3979665 HHS-NIH \$437,244.00
Intraneuronal oligomeric amyloid beta and the effect on Epigenetics to the Synapse in Alzheimer's disease.

NIH

Necroptosis as a novel mechanism of neurodegeneration in Alzheimer's disease 08/2017 - 07/2022
\$ 3,045,556 Total Costs
Role: Principal Investigator (Oddo)

Alzheimer's Drug Discovery Foundation 08/2017 – 07/2019
Targeting S6K1 as a novel approach to treat Alzheimer's disease \$300,000 Total Costs
Role: Principal Investigator

R01 AG055436-01

NIH 08/2017 – 07/2022
Targeting S6K1 as a novel approach to treat Alzheimer's disease \$ 3,595,608
Role: Principal Investigator

USDA Evaluation of the dose-response of honey bees to carboximide and strobilurin fungicides: from cellular mechanism to integrated management 4/1/17-3/21/20
Role: co-PI

Decourt, Boris (Consultant) 07/01/17-06/30/18
Arizona Alzheimer's Research Consortium (AARC) \$30,000 Annual DC
Restoring vascular health to prevent Alzheimer's disease in overweight animals.
PI: Dr. Karen Sweazea, Ph.D., ASU School of Life Sciences & School of Nutrition and Health Promotion.

1R01AG055444-01 (Reiman/Tariot/Lopera) 4/1/2017-3/31/2022
NIH/NIA \$1,654,577 Annual Direct Costs
Alzheimer's Prevention Initiative ADAD Colombia Trial

1R01AG055444-01 (Reiman/Tariot/Lopera) 4/1/17-3/31/22
NIH/NIA \$1,654,577 Annual Direct Costs
Alzheimer's Prevention Initiative ADAD Colombia Trial
Role: Co-Investigator

1R01AG056333-01 (Langbaum) NIH/NIA Alzheimer's Prevention Registry: The Science of Recruitment and Enrollment Role: Principal Investigator	7/1/17-6/30/22 \$499,606 Annual Direct Costs
1R01AG055444-01 (Reiman/Tariot/Lopera) NIH/NIA Alzheimer's Prevention Initiative ADAD Colombia Trial	4/1/17-3/31/22 \$1,654,577 Annual Direct Costs
2R01AG019610-17S1 (Reiman) NIH/NIA Arizona Alzheimer's Disease Core Center –Revision	7/1/17-6/30/18 \$819,270 Annual Direct Costs
NIH R21 via ASU (Walker) Is O-GlcNAcylation involved in Alzheimer's disease neuroinflammation?	9/1/16-8/31/18 \$5,453 Annual DC
Beach, Thomas NIH R01 via Harvard Medical School BIDMC (Gibbons) Skin Biopsy in the Central Alpha-Synucleinopathies	8/1/16-7/31/21 \$16,065 Annual DC
Beach, Thomas NIH R01 via ASU (Mastroeni) Profiling and predicting precursor cell fate through the actions of 5-hydroxymethylation	10/1/16-9/30/21 \$4,181 Annual DC
Beach, Thomas NIH R01 via Arizona State University (Oddo) Molecular interplay between A β , tau and mTOR: Mechanisms of neurodegeneration	7/1/16-6/30/21 \$10,382 Annual DC
Beach, Thomas NIH R01 via Arizona State University (Oddo)	7/1/16-6/30/21 \$10,382 Annual DC
Beach, Thomas NIH R01 via ASU (Walker) Neuronal-Microglial cross-regulation of inflammation	8/1/16-7/31/121 \$8,404 Annual DC
Beach, Thomas NIH R03 via University of Arizona (Su) Ultra-sensitive and label-free detection of Alzheimer's disease biomarkers	12/1/16-11/30/17 \$10,000 Annual DC
Beach, Thomas NIH R21 via Linda Loma University (Dashtipour) DNA Methylation patterns provide information regarding susceptibility to and protection against Parkinson's Disease	10/1/16-9/30/18 \$18,901 Annual DC

Beach, Thomas NIH R21 via UCSF (Dugger) Tau in human peripheral tissues	12/1/16-11/30/17 \$8,100 Annual DC
NIH R21 via ASU (Walker) Is O-GlcNAcylation involved in Alzheimer's disease neuroinflammation?	9/1/16-8/31/18 \$5,453 Annual DC
Serrano, Geidy NIH R01 via Harvard Medical School BIDMC (Gibbons) Skin Biopsy in the Central Alpha-Synucleinopathies	8/1/16-7/31/21 \$16,065 Annual DC
Serrano, Geidy NIH R01 via ASU (Mastroeni) Profiling and predicting precursor cell fate through the actions of 5-hydroxymethylation	10/1/16-9/30/21 \$4,181 Annual DC
Serrano, Geidy NIH R01 via Arizona State University (Oddo) Molecular interplay between A β , tau and mTOR: Mechanisms of neurodegeneration	7/1/16-6/30/21 \$10,382 Annual DC
Serrano, Geidy NIH R01 via Arizona State University (Oddo) Identifying novel therapeutic targets for Alzheimer's disease using post mortem human brains and animal models	7/1/16-6/30/21 \$10,382 Annual DC
Serrano, Geidy NIH R01 via ASU (Walker) Neuronal-Microglial cross-regulation of inflammation: Role of CD200R and TREM2?	8/1/16-7/31/21 \$8,404 Annual DC
Serrano, Geidy NIH R03 via University of Arizona (Su) Ultra-sensitive and label-free detection of Alzheimer's disease biomarkers	12/1/16-11/30/17 \$10,000 Annual DC
Serrano, Geidy NIH R21 via Linda Loma University (Dashtipour) DNA Methylation patterns provide information regarding susceptibility to and protection against Parkinson's Disease	10/1/16-9/30/18 \$18,901 Annual DC
Serrano, Geidy NIH R21 via UCSF (Dugger) Tau in human peripheral tissues	12/1/16-11/30/17 \$8,100 Annual DC
Baxter (Co-Investigator; Braden PI) Longitudinal Cognitive and Brain Aging in Autism Spectrum Disorder: Interactions with Gender	

B. Blair Braden (PI; Baxter, Co-PI)
Autism Science Foundation
Cognitive and Neural Correlates of Aging in Autism Spectrum Disorder: Interactions with Gender

Grant (Vargas) 07/01/2017 – 06/30/18
Mayo Clinic AZ \$44,238 direct costs
Mayo/ASU Team Science Grant 2016 Predicting Cancer Treatment Related Cardiac Damage
Employing Quantitative and Qualitative MRI Image Analysis and RNA Biomarker Profiling
Role: Co-Investigator

R01 (Kaczorowski) 04/01/2017 – 03/31/2022
NIH \$10,028
Systems Genetics of Cognitive Aging and Alzheimer's Disease
Role: Co-Investigator

R21 (Huentelman) 07/01/2017 – 06/30/2019
NIH \$140,477 direct costs
Multi omics analysis in middle temporal gyrus of Alzheimers Disease patients
Role: Principal Investigator

R01 (Myers/Huentelman) 07/01/2017 – 06/30/2022
NIH \$125,000/year directs costs
STEMBRAIN Toolkit: Autopsy confirmed cell lines as a rapid model system for neurologic
disease
Role: Multi-PI

R21 (Huentelman) 07/01/2017 – 06/30/2019
NIH \$64,981/year direct cots
A longitudinal molecular profiling approach to study relapse-remitting multiple sclerosis using
dried blood spots.
Role: Principal Investigator

R01 (Peter) 07/01/2017 - 06/30/2022
NIH \$51,155/year direct costs
Genetics of speech sound disorders
Role: Co-Investigator

R21 (Huentelman) 07/01/2017 - 06/30/2019
NIH \$128,303
Longitudinal dried blood spot profiling to predict migraine onset
Role: Principal Investigator

R01 (Huentelman) 07/01/2017 - 06/30/2022
NIH \$215,000

Genetic mechanisms of multisensory processing
Role: Principal Investigator

W81XWH-16-TSCRIP-IDA (Vinodh Narayanan) 12/01/2016 – 11/30/19
DoD \$150,000
TS160074:Phenotypic Variability in Tuberous Sclerosis Complex (TSC)
Role: Co-Investigator

Liang, Winnie
R01 (David Brafman) 02/01/2017 – 01/31/2018
NIH \$20,570
Investigating the mechanisms of a multi-state model of Wnt signaling
Role: Co-Investigator

Liang, Winnie
W81XWH-16-PRARP-TRPA (Mastroeni) 05/01/2017 – 04/30/2018
DoD \$39,519
Predicting Alzheimer's disease in at risk populations using novel blood test

Liang, Winnie
R01 (Mastroeni) 04/01/2017 – 03/31/22
NIH \$7,000
Can Species-, Age, and Disease state-appropriate 3D brain organoids recapitulate aging and Alzheimer's diseases in a dish
Role: Co-Investigator

Liang, Winnie
R01 (Diego Mastroeni) 09/01/2017 – 08/31/22
NIH \$13,700
The effect of intracellular oligomeric amyloid beta on the electron transport chain in Alzheimer's disease
Role: Co-Investigator

Liang, Winnie
Contract (Jonathan Keats) 02/01/2017 – 01/31/2018
Quantum Leap Health Care Collaborative \$420,000
Role: Co-Investigator

Liang, Winnie
P01 (Jonathan Keats) 07/01/2017 – 06/30/2022
NIH \$5,814
I SPY 2+: Evolving the ISPY 2 Trial to include MRI-directed, adaptive sequential treatment in the setting of non-response
Role: Co-Investigator

Liang, Winnie
 R01 (Muhammed Murtaza) 09/01/2017 – 08/31/2022
 NIH \$530,965
 Individualized monitoring of treatment response and resistance in patients with metastatic melanoma
 Role: Co-Investigator

Liang, Winnie
 R01 (Sampath Rangasamy) 09/01/2017 – 08/31/2022
 NIH \$184,687
 Novel Biomarkers and Genetics of Diabetic Retinopathy
 Role: Co-Investigator

SC160284 03/01/2016 -02/28/2017
 DOD CDMRP \$16,129
 Discovering biomarkers and therapeutic targets for Ischemic spinal cord injury using a canine model of TAAA repair
 Role: Co-Investigator (PI: Hamdy ElSayed-Awad)

Van Keuren-Jensen, Kendall
 R01 09/01/2018 -08/31/2020
 NIH \$30,000
 Mesenchymal stem cell-derived exosomes for the treatment of multiple sclerosis: a new paradigm for cell-free therapy
 Role: Co-Investigator (PI: Weian Zhao)

Van Keuren-Jensen, Kendall
 Grant 10/01/2018- 9/30/2019
 MS \$52,264
 Mesenchymal stem cell-derived exosomes for the treatment of multiple sclerosis
 Role: Co-Investigator (PI: Weian Zhao)

Van Keuren-Jensen, Kendall
 Michael J Fox Foundation for Parkinson's Research 01/01/2017 – 12/31/2018

Van Keuren-Jensen, Kendall \$90,568
 RNAseq and miRNAseq in PPMI whole blood samples (2nd)
 Planning Director for the RNASeq strategies for the PPMI and conduct pilot study.
 Role: PI (Van Keuren-Jensen)

Barnes, Carol A. (UA PIs: Alexander, Barnes Ryan) 07/01/17 – 06/30/21
 2 P30 AG019610 Supplement \$19,387 Annual DC
 Core G: Brain Imaging and Fluid Biomarkers Core

Barnes, Carol A. (PIs: Barnes/Ekstrom) 09/01/17 – 03/31/21
 NIH/ NIMH 1 R01 MH114231 \$167,807 Annual DC
 Hippocampal Low-Frequency Oscillations Across Different Scale and Species

Brinton, Robbie D. (PI) NIH Aging and Estrogenic Control of the Bioenergetic System in the Brain	04/01/17 – 03/31/22 \$633,057 Total DC	
Brinton, Robbie D. (PI) NIH Allopregnanolone as a Regenerative Therapeutic for Alzheimer's: Phase 2 Clinical Trial	07/01/17 – 06/30/22 \$3,839,893 Total DC	
Edgin, Jamie, O (PI) NIH – UCSD Subcontract Changing ASD Early Detection: Examining Impact of Community-Implemented and Technology-Enhanced Get SET Early Models in America's Poorest Regions Using a RCT Design	07/01/17 – 06/30/22 \$39,685 Total DC	
Edgin, Jamie, O (PI) NIH Predicting Daily Cognitive Fluctuations as a Function of Glucose and Sleep	07/01/17 – 06/30/22 \$3,282,507 Total DC	
Ryan, Lee (co-I) (PI: Chen; coI's Chou, Ryan, Trouard) NIH Mapping Functional Subregions of Hippocampus and amygdala Using High Spatial-Temporal-Resolution fMRI and Inherent Susceptibility Compensation	10/1/17 – 3/30/19 \$275,000 Total DC	
Su, Judith (PI) NSF 1708554 (Su) Precise positioning of photonic nanostructures on optical resonators for creating ultra-sensitive and portable biosensors	09/01/16-9/30/19 \$248,087 Total DC	
Trouard, Ted (co-I) NIH RO1 Development of High-Speed and Quantitative Neuro MRI Technologies for Challenging Patient Populations	07/01/17 – 06/30/21 \$507,585 Total DC	
Trouard, Ted (co-I) NIH RO1 Mapping Functional Subregions of Hippocampus and Amygdala Using High Spatial-Temporal-Resolution fMRI with Inherent Susceptibility Compensation	10/01/17 – 09/30/19 \$275,000 Total DC	
R21 HD (Lifshitz) NIH/NICHHD Effectiveness of brain injury rehabilitation depends on active engagement and immediate protein synthesis Role: PI	04/01/2017 – 03/31/2019 \$125,000	2.0 calendar
R21 DA041595-01A1 (Lifshitz)	06/01/2017 – 05/30/2019	2.0 calendar

NIH/NIDA Role: PI	\$150,000	
I01RX002472 (Lifshitz) VA/RR&D Role: PI	12/01/2016 – 11:30/2020 \$230,000	7.0 calendar
R01 PA-13-302 (Godbout) NIH/Ohio State University Subaward Acute and Long-Term Benefits of Methylene blue Intervention after TBI on Neuroinflammation, Glial Dysfunction, and Neuropsychiatric Complications Role: Co-I	12/01/2016 – 11/30/2021 \$21,678	1.2 calendar
R01 NR016950-01 (Morrison) NIH Microglia Phagocytic Function after Ischemic Stroke: Ascertaining Sex and Menopause Differences Role: Co-I	04/01/2017 – 03/31/2022 \$426,686	0.6 calendar
R01 NS100793-01 (Thomas) NIH Electrochemical assessment of behaviorally relevant circuit function after TBI	10/01/2017 – 09/30/2022 \$336,284	1.8 calendar
Co-PI: Jose Hernandez Funding agency: Morris Animal Foundation, Pilot Grant Elucidating the role of <i>Rhipicephalus sanguineus</i> (the Brown dog tick) as a vector for Rocky Mountain Spotted Fever (RMSF) transmission in Arizona.	08/01/17-08/31/17 \$10,800	
PI: Gadagkar, Sudhindra NIH-R25 Subcontract to TGen Graduate Training in Bioinformatics Applications of Big Data	09/01/17-08/31/19 \$274,271	
PI: Olsen, Mark American Kennel Association Evaluation of Aspartyl(Asparaginyl)-beta-Hydroxylase Expression in Canine Tumor and Blood Samples	10/01/16-09/30/17 \$15,000	



**Arizona Alzheimer's Consortium
19th Annual Scientific Conference
Thursday, May 18, 2017**

**Mayo Clinic (Host Institution)
Vaugh Auditorium
Phoenix, Arizona**

Poster Abstracts

Poster 1

ANTEMORTEM-POSTMORTEM CORRELATION OF FLORBETAPIR (18F) PET AMYLOID IMAGING WITH QUANTITATIVE BIOCHEMICAL MEASURES OF A β 40 AND A β 42. Beach TG, Maarouf CL, Intorcica A, Sue LI, Serrano GE, Roher AE. Banner Sun Health Research Institute; Avid Radiopharmaceuticals; Arizona Alzheimer's Consortium.

Background: Amyloid imaging effectively demonstrates the in vivo presence of amyloid-beta (A β) deposits in the aging human brain but it is still unknown which of the many structural forms and modifications of A β are detected. In Alzheimer's disease, most amyloid deposits are predominantly composed of A β ending at amino acid residues Val40 or Ala42. The relative abundance of these two major forms differs between individuals but in general it has been reported that A β 40 is largely restricted to neuritic plaques and larger blood vessels while A β 42 may be deposited in senile plaques and vascular amyloid of all types, and is often the sole component of diffuse plaques. The distinction is important as it is mainly the neuritic plaques that correlate with cognitive impairment while diffuse plaques may be the initial type of A β deposited. Whether PET amyloid ligands such as florbetapir-18F (Amyvid) are partially or wholly selective for brain deposits of A β 40 or A β 42 is currently unknown.

Methods: We compared the antemortem florbetapir PET cortical/cerebellar signal intensity (SUV_r) of 55 subjects with postmortem biochemical (ELISA) measurements employing specific antibodies against A β 1-40 and A β 1-42.

Results: Correlation analyses were significant for both A β 40 and A β 42, but were much stronger for A β 42. Despite this, SUV_r in these 55 individuals was not dependent on the A β 40/42 concentration ratio.

Conclusions: These results suggest that while florbetapir binds to both species in the human brain, the interaction with A β 42 dominates over that with A β 40. This may be in large part due to the generally higher A β 42 concentrations, but may also be partially due to preferential A β 42 binding, possibly due to its higher likelihood to be present in a β -pleated sheet tertiary structure, or to differences in β -pleated sheet tertiary or quaternary structure. ☺

Poster 2

STAGING ALZHEIMER'S DISEASE-LIKE PATHOLOGY IN 3XTG-AD MICE. Belfiore R, Ferreira ET, Velasquez R, Oddo S. Arizona State University; Arizona Alzheimer's Consortium.

Background: Animal models of Alzheimer's disease (AD) are widely used to obtain insights into the pathogenesis of the disease, and as tools to evaluate potential therapeutic compounds. Accumulation of amyloid- β (A β) and fibrillary tangles, as well as memory loss are hallmarks of AD. After almost 15 years from its generation, 3xTg-AD mice are still one of the most reliable transgenic model. These mice show age-dependent extracellular and intracellular A β accumulation, tau hyperphosphorylation, and accumulation of tangles. These changes are associated with selective neuroinflammation. Progressive cognitive impairments have also been widely reported. These mice are being used by over 100 investigators throughout the world, which has led to the generation of multiple independent colonies. Converging evidence indicates that the phenotype of 3xTg-AD mice has shifted over the years and contradicting reports about onset of pathology or cognitive deficits are apparent in the literature. Here, we sought to stage the current progression of AD-like pathology in 3xTg-AD mice. The data obtained will facilitate the design of preclinical studies in which these are used to test new therapeutic approaches.

Methods: We used 3-, 6-, 12-, 16- and 20-month-old female 3xTg-AD and non-transgenic (NonTg). Mice were assessed in a battery of cognitive and non-cognitive behavioral tests, including the open field, the novel object recognition, the rotarod, the contextual fear conditioning, and the Morris water maze (MWM). At the end of the behavioral test, mice were sacrificed and their brains extracted and cut midsagittally. The left hemibrains were used to dissect cortex, hippocampus and cerebellum. This tissue was used to assess A β and tau changes by sandwich ELISA and western blots. The left hemibrains were used for histological and stereological analyses.

Results: Our preliminary data indicate that the onset of cognitive deficits, as indicated by MWM performance, start at 6 months of age. As the mice age, these changes become progressively more apparent. We will also present data about all of the other behavioral tests and a detailed neuropathological assessment.

Conclusions: We will present a detailed cognitive and neuropathological assessment of the age-dependent AD-like pathology developed by 3xTg-AD mice. These data will aid the design of future pre-clinical studies.

Poster 3

DIFFERENTIAL REGIONAL ALTERATIONS OF WHITE MATTER INTEGRITY IN HEALTHY COGNITIVE AGING. Bharadwaj PK, Nguyen LA, Haws KA, Hishaw GA, Trouard TP, Alexander GE. University of Arizona; Arizona Alzheimer's Consortium.

Background: White matter (WM) microstructural integrity assessed with diffusion weighted imaging is diminished in the context of healthy aging. Such age effects have often shown an anterior-posterior gradient and may be influenced by vascular risk factors such as hypertension. The aim of this study was to investigate regional differences in WM integrity measured by fractional anisotropy (FA) and mean diffusivity (MD) in a sample of community dwelling neurologically healthy older adults ranging in age from 50 to 89 years.

Methods: The study sample comprised 81 healthy older adults divided into four age groups (50-59, 60-69, 70-79 and 80-89 years). Only neurologically healthy participants without a clinical diagnosis of diabetes or hypertension were included. T1-weighted and diffusion weighted images were acquired at 3T and processed using FreeSurfer (Fischl et al., 2004a) and TRACULA (Yendiki et al., 2011) to perform probabilistic tractography and compute regional values of FA and MD for 18 major WM tracts. After adjusting for head motion and signal quality measures, the diffusion metrics were tested using ANCOVA with age as the between group factor and total cortical WM volume as a covariate. Correction for multiple comparisons was performed using the false discovery rate (FDR) with $p < 0.05$.

Results: Results showed that FA decreased with age in the forceps major ($p = 0.019$), left inferior longitudinal fasciculus (ILF; $p = 0.027$), and left temporal branch of the superior longitudinal fasciculus (SLFT, $p = 0.019$). MD demonstrated age-related increases in the forceps major ($p = 0.005$), forceps minor ($p = 0.012$), bilaterally in the ATR ($1.9E-04 \leq p \leq 2.5E-04$), cingulum angular bundles ($2.5E-05 \leq p \leq 5.5E-04$), inferior longitudinal fasciculus ($p = 5.1E-05$), parietal branch of the superior longitudinal fasciculus (SLFP; $1.42E-02 \leq p \leq 1.75E-02$) uncinate fasciculus ($3.8E-04 \leq p \leq 5.2E-05$), and the SLFT ($8.9E-04 \leq p \leq 2.8E-03$). Follow up analyses of pairwise comparisons of FA and MD in these tracts showed the 80-89 year age group being the most affected and differing from other age groups.

Conclusions: In the absence of common health conditions of aging, rate of diffusion of water in WM may be more sensitive to age-related differences than its directional preference. The predominant pattern of pairwise comparisons observed across both measures showed the 80-89 year age group being the most affected and differing from other age groups. Together, these findings indicate a possible age-related threshold for the accumulation of deleterious WM differences in healthy aging. Further work with older adults without this pattern may help identify factors that can potentially mitigate age-related WM deterioration. Further research is needed to better understand how cardiovascular risk factors such as hypertension and diabetes impact the observed differences in WM across the older adult age spectrum and to evaluate the trajectory of WM changes over time in the context of healthy cognitive aging.

Poster 4

AGE-RELATED CORTICAL THICKNESS DIFFERENCES IN ADULTS WITH AUTISM SPECTRUM DISORDER. Braden BB, Riecken C. Arizona State University; Arizona Alzheimer's Consortium.

Background: Over the course of the last 30 years, autism spectrum disorder (ASD) diagnoses have grown exponentially, thus identifying a large group of aging individuals with ASD. Currently, little is known about how aging will affect these individuals on a neuroanatomical level, compared to a typically developing (TD) population. Brain aging in ASD is of concern due to the anatomical overlap of ASD-related pathology and age-related cortical thinning. Both phenomenon follow an anterior-to-posterior severity gradient, resulting in frontal lobe vulnerability and relative sparing of the occipital lobe.

Methods: To investigate whether adults with ASD experience exacerbated brain aging, compared to TD adults, two studies were performed using available data from the Autism Brain Imaging Data Exchange (ABIDE). The first study compared differences in cortical thickness via FreeSurfer between ASD (n=16) and TD (n=15) participants in two age groups: young adults (YA), between 18 and 25 years of age (n = 31) and middle-aged (MA), from 39 to 58 years of age (n = 31). There were no significant differences in IQ between ASD and TD participants within YA and MA groups. The second study compared correlations between cortical thickness and age in adult ASD (n = 150) and TD groups (n = 166), controlling for IQ and site, to investigate the interaction of cortical thickness and age between the two groups.

Results: Study 1 found significant interactions between diagnosis group (ASD and TD) and age group (YA and MA) for the frontal and parietal lobes, and the interaction approached significance for the temporal lobe. As predicted, no interaction was observed for the occipital lobe. Study 2 found significant differences between diagnosis groups in the relationship between age and cortical thickness for areas of the left anterior insula, middle, inferior, and fusiform temporal gyri, and superior parietal cortex. Both studies demonstrated greater age-related cortical thinning in adults with ASD, compared to TD.

Conclusions: As predicted, adults with ASD demonstrated exacerbated age-related brain differences, as measured by cortical thickness, compared to TD. These differences largely followed the anterior-to-posterior gradient, with relative sparing of the occipital lobe. Findings demonstrate significant age-related anatomical differences between ASD and TD adults from young adult into middle-age years. Future work is warranted to investigate whether differences in brain age trajectories will translate to unique behavioral needs in older adults with ASD.

Poster 5

AN FMRI INVESTIGATION OF WORKING MEMORY IN OLDER ADULTS WITH AUTISM SPECTRUM DISORDER: FRONTO-HIPPO-STRIATAL-THALAMIC NETWORK DIFFERENCES. Braden BB, Smith CJ, Thompson A, Glaspy TK, Wood E, Vatsa D, Baxter LC. Arizona State University; Southwest Autism Research & Resource Center; Barrow Neurological Institute; Arizona Alzheimer's Consortium.

Background: The effects of aging in adults with autism spectrum disorder (ASD) are understudied, but of increasing importance in order to anticipate unique needs of this growing group of individuals. Executive functioning is particularly vulnerable in both high-functioning ASD and in normal aging. Deficits are thought to arise from structural and functional connectivity disturbances between the frontal lobe and posterior brain regions. Recently, a cross-sectional study revealed sharper age-related declines of structural connectivity in adults with ASD, relative to typical adults (Koolschijn et al., 2016). However, differences in functional connectivity disturbances are still unknown; yet, emerging evidence suggests functional connectivity is the best neurobiological predictor of age-related cognitive decline. The current study investigated functional brain network recruitment during an fMRI executive function task in middle-aged men with ASD, compared to age- and IQ-matched typically developing (TD) men.

Methods: We evaluated 16 ASD and 17 matched TD men from ages 40 to 64 and of average to high intellectual functioning (IQ: 83-131). For participants with ASD, diagnosis was confirmed via the Autism Diagnostic Observation Schedule-2 and developmental history assessment. TD participants were screened for presence of ASD symptoms via the Social Responsiveness Scale-2. All participants were given the Kaufman Brief Intelligence Test-2. Participants performed the n-back fMRI task, a working memory task that is good indicator of executive function. Participants monitored a series of letters and identified targets that range from simply matching a target ("0-back") to identifying matches that are two letters apart ("2-back"). Functionally connected network activity was assessed via group independent component analysis. Working memory load comparisons were made by the 2 vs. 0 contrasts. Reaction time and accuracy were recorded. Participants performed the task twice.

Results: Both groups performed well on all conditions of the n-back task (over 85% accuracy). The ASD participants' reaction time was slower on the 2-back condition, but were similar for accuracy in all conditions. For the working memory load comparison (2 back vs. 0 back), both groups showed similar activation a classic cortical working memory network including the bilateral dorsolateral prefrontal cortex (dlPFC), parietal cortex, insula, and the anterior cingulate cortex, and deactivation the default mode network (DMN). However, only the TD group activated an additional network including the left inferior frontal lobe, and bilateral hippocampi, striatum, and thalamus. This working memory activation was significantly greater in TD older adults than older adults with ASD.

Conclusions: Results showed reduced engagement a fronto-hippo-striatal-thalamic neural network during working memory performance in older adults with ASD, compared to matched TD adults. Dysfunction within this network may underlie working memory and other executive function struggles in high functioning older adults with ASD, and could impact independence as aging ensues. Longitudinal evaluation of differences in age-related cognitive and brain trajectories between older adults with ASD and TD adults are in progress.

Poster 6

MECHANISMS OF NEURONAL LOSS IN ALZHEIMER'S DISEASE. Branca C, Caccamo A, Piras IS, Ferreira E, Huentelman MJ, Liang WS, Readhead B, Dudley JT, Spangenberg EE, Green KN, Belfiore R, Winslow W, Oddo S. Arizona State University; Translational Genomics Research Institute; Icahn School of Medicine at Mount Sinai; University of California, Irvine; Arizona Alzheimer's Consortium.

Background: While several key aspects of AD pathogenesis have been identified, the mechanisms that govern cell loss in AD remain elusive. Necroptosis, a programmed form of necrosis, is executed by the mixed lineage kinase domain-like (MLKL) protein, which is triggered by receptor-interactive protein kinases (RIPK) 1 and 3.

Methods: To dissect the mechanisms of neuronal loss in AD, we employed multidisciplinary approaches. Specifically, we have used multiple animal models, human tissue from multiple cohorts, and gene expression data from multiple laboratories.

Results: We will show that necroptosis is activated in human AD brains and its activation correlates with brain weight. We will also show that in two animal models of AD that necroptosis is activated only in the line characterized by marked neuronal loss. We further show that genetically increasing necroptosis in a mouse model of AD induces neuronal degeneration to a greater degree than in non-transgenic controls, indicating that mice with AD pathology are more prone to necroptosis-induced cell loss.

Conclusions: Our novel and exciting data expected to fill a critical gap in knowledge; we provide the first direct evidence that necroptosis is activated in human AD brains, as well as in a mouse model of AD that develops neuronal loss. Our studies open new venues of research and interventions for this insidious disorder, which affects more than 40 million people worldwide

Poster 7

ALLOPREGNANOLONE AS A REGENERATIVE THERAPEUTIC FOR ALZHEIMER'S DISEASE: PHASE 1B/2A UPDATE. Brinton RD, Schneider LS, Law M, Rodgers K, Shi Y, Irwin R, Rogawski M. University of Arizona; University of Southern California; University of California Davis; Arizona Alzheimer's Consortium.

Background: Allopregnanolone (Allo) is a regenerative therapeutic that promotes neurogenesis, regeneration of human neural stem cells, restores cognitive function in a preclinical AD model and wild type aged mice and reduces AD pathology. Allo, a neurosteroid, has abundant existing safety data in animals and humans. Its mechanisms of neural stem cell proliferation, restoration of cognitive function and AD pathology reduction are well characterized and unlikely to induce amyloid related imaging abnormalities (ARIA).

Methods: Phase 1b/ 2a clinical trial to establish safety and maximally tolerated Dose: Allopregnanolone for Mild Cognitive Impairment Due to Alzheimer's Disease or Mild AD. ClinicalTrials.gov Identifier: NCT02221622. Secondary objectives are to: assess potential short-term effects of Allo dosing on cognition and MRI indicators of AD; inform subsequent phase 2 proof of concept trial with MRI-based biomarkers of regenerative efficacy.

Results: Allopregnanolone at two doses, 2mg and 4mg, was intravenously infused once per week for 12 weeks to 8 participants / dose cohort (6 allopregnanolone + 2 placebo / dose cohort). Within 15 minutes of start of infusion, peak plasma level was reached $C_{max} = 46.34 \pm 23$ nanomolar. No sedation was observed in any participant during or after infusion indicating a tolerable dose. The C_{max} closely correlated ($R=0.77$) with Allo delivered in mg/kg dose. Twelve-week exposure to 2mg or 4mg of Allopregnanolone once per week had no detectable adverse effects. Dose cohort 3 is underway. Primary safety outcomes and secondary exploratory outcomes of MRI based biomarkers, metabolomics, cognition and iPSC derived neural stem cell response to Allo will be presented.

Conclusions: Allopregnanolone is a first in class regenerative therapeutic for MCI and AD that targets endogenous neural stem cells and disease modifying mechanisms. Trial outcomes will provide: 1) an estimated safe and well-tolerated dose of Allo; 2) parameter estimates for MRI based markers of regeneration, cognitive efficacy and iPSC / neural stem cell based indicator of responders and foundation to advance to a Phase 2 proof of concept trial of Allo in persons diagnosed with early AD. Supported by NIA U01AG03111 & U01AG047222 to RDB; UF1AG046148 to RDB & LS; ADDF to RDB

Poster 8

P62 IMPROVES AD-LIKE PATHOLOGY BY INCREASING AUTOPHAGY. Caccamo A, Ferreira E, Branca C, Oddo S. Arizona State University; Arizona Alzheimer's Consortium.

Background: The multifunctional protein p62 is associated with neuropathological inclusions in several neurodegenerative disorders, including frontotemporal lobar degeneration, amyotrophic lateral sclerosis, and Alzheimer's disease (AD). Strong evidence shows that in AD, p62 immunoreactivity is associated with neurofibrillary tangles and is involved in tau degradation. However, it remains to be determined whether p62 also plays a role in regulating amyloid- β aggregation and degradation.

Methods: We used a gene therapy approach to increase the levels of p62 in the brain of APP/PS1 mice, a widely used animal model of Alzheimer's disease.

Results: We show that increasing brain p62 expression rescues cognitive deficits in the APP/PS1 mice. The cognitive improvement was associated with a decrease in amyloid- β levels and plaque load. Using complementary genetic and pharmacologic approaches, we found that the p62-mediated changes in A β were due to an increase in autophagy. To this end, we showed that removing the LIR domain of p62, which facilitates p62-mediated selective autophagy, or blocking autophagy with a pharmacological inhibitor, was sufficient to prevent the decrease in A β .

Conclusions: Overall, these data provide the first direct in vivo evidence showing that p62 regulates A β turnover.

Poster 9

PROGRESSION FROM PRECLINICAL AD TO MCI OVER A DECADE: COGNITIVE AND BRAIN IMAGING TRAJECTORIES. Caselli RJ, Chen K, Chen Y, Thiyyagura P, Kuang X, Bauer III R, Stonnington CM, Reiman EM. Mayo Clinic Scottsdale; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: To correlate the cognitive, structural, and cerebral metabolic changes that characterize the progression from preclinical Alzheimer's disease (AD) to incident mild cognitive impairment (MCI).

Methods: Cognitively normal residents of Maricopa County who were at least 50 years old at entry and who reported a first degree relative with dementia underwent apolipoprotein E (APOE) genotyping. APOE e4 homozygotes were matched by age, education, and sex to one e4 heterozygote and two e4 noncarriers who were evaluated every two years with neuropsychological testing (that included the Mini-Mental Status Exam [MMSE], Auditory Verbal Learning Test [AVLT], Boston Naming Test [BNT], Judgment of Line Orientation (JLO), and Controlled Oral Word Association [COWA] tests), structural MRI, and fluorodeoxyglucose (FDG)-PET measurement of the cerebral metabolic rate for glucose (CMRgl). Over a mean interval of 13.9 years (range 10-21), 14 (11 APOE e4 carriers and 3 noncarriers) progressed to incident MCI and were compared to 32 nonprogressors (25 e4 carriers and 7 noncarriers) matched for e4 gene dose, age, followup duration, sex and education using R-package MatchControl followed over a mean interval of 19 years (range 6-21).

Results: There were no baseline differences between progressors and nonprogressors in mean age (57.9+/-4.6 years), education (15.7+/-2.0 years), sex (51.6% women), e4 carrier status (78.3% carriers), or on any neuropsychological measure (MMSE 29.7+/-0.6, AVLT recall 8.8+/-3.0, BNT 56.8+/-3.4, COWA 47.8+/-10), but annualized declines in MCI progressor scores were significantly greater ($p < .0001$) on the MMSE (-0.17 vs -0.01), AVLT (-0.50 vs -0.11), BNT (-0.46 vs -0.02), and COWA (-0.55 vs +0.24). With small volume correction (SVC) at $p = 0.05$ for multiple comparisons in prespecified regions, there was significantly accelerated atrophy on MRI bilaterally in the hippocampus ($p = 7.72e-05$), medial and lateral temporal neocortices ($p = 1.76e-04$), and parahippocampal gyri ($p = 1.46e-04$). Significantly accelerated CMRgl declines were observed bilaterally in posterior cingulate cortices ($p = 8.20e-05$) and hippocampi ($p = 9.95e-03$) as well as in the right precuneus ($p = 9.65e-03$), all SVC corrected for multiple comparisons.

Conclusions: Preclinical decline in cognition, cerebral structure and metabolism in individuals progressing to MCI mirrors that seen in the clinical stages of Alzheimer's disease.

Poster 10

CAVEATS WHEN SUBTRACTING TWO SERIAL MEASUREMENTS TO ESTIMATE THE NUMBER OF PARTICIPANTS NEEDED FOR CLINICAL TRIALS THAT ARE LONGER OR SHORTER THAN THE OBSERVED MEASUREMENT INTERVAL.

Chen K, Xiong C, Harvey D, Guo X, Weiner M, Jagust WJ, Reiman RM. Banner Alzheimer's Institute; University of Arizona; Arizona State University; Arizona Alzheimer's Consortium; Knight Alzheimer's Disease Research Center; Washington University in St. Louis School of Medicine; University of California, Davis; Beijing Normal University; San Francisco Veterans Administration Medical Center; University of California, San Francisco; Lawrence Berkeley National Laboratory; University of California Berkeley; Translational Genomics Research Institute.

Background: One approach to estimate the number of participants needed to detect a treatment effect in Alzheimer's disease randomized clinical trials (RCTs) involves the subtraction of measurements acquired at two times over an observed time interval (ΔT_o). While this approach may be suitable for RCT in which the proposed treatment time interval (ΔT_t) is equal to ΔT_o , its use need some cautions when a) ΔT_t is longer or shorter than ΔT_o or b) there are substantial individual variations in ΔT_o .

Methods: We considered the case in which each individual j had two observed serial measurements, x_{j1} and x_{j2} , before and after time interval ΔT_{oj} for total of N subjects in an existing data set. The subtraction method multiplies the annualized subject group's mean change $\mu = (1/N)(\sum(x_{j1} - x_{j2})/\Delta T_{oj})$ and its standard deviation σ by ΔT_t to form $\Delta T_t \times \mu$ and $\Delta T_t \times \sigma$ and use them to estimate the number of subjects needed to detect a particular treatment effect with a pre-defined statistical power and type-I error. As is known, the sample size estimate would then depend entirely on the std/mean ratio, with no additional consideration of the impact of longer or shorter ΔT_t rather than serving as a common multiplier.

Results: 1), Using the simple subtraction method, sample size estimates would be the same regardless of RCT duration due to the common std/mean ratio and without additional models or assumptions. 2), The reason for this limitation is the misuse of the linear assumption (common multiplier) and the overlook of the measurement errors in forming the subtraction. 3), The simple subtraction approach works if one of several conditions holds. For example, a) the measurement error is ignorable or linearly related to trial duration; b) the proposed RCT duration ΔT_t and the individualized ΔT_{oj} satisfy $\sum_{j=1}^N (1/\Delta T_{oj}^2) = N/\Delta T_t^2$; or c) if the between-subject variability of the annualized change is low, the std/mean ratio will be proportional to $1/\Delta T_t$ and the corresponding sample size decreases for longer ΔT_t duration.

Conclusions: The subtraction procedure should be used with caution. Importantly, this approach would require assumptions about the impact of treatment interval or use of an alternative (e.g., mixed model) approach.

Poster 11

DIFFERENTIAL PATTERN OF ALTERED GENE EXPRESSION AMONG BRAIN REGIONS IN AGING AND ALZHEIMER'S DISEASE. Coleman PD, Mastroeni D, Delvaux E, Nolz J, Berchtold N, Cotman C. Arizona State University; Banner Sun Health Research Institute; University of California, Irvine; Arizona Alzheimer's Consortium.

Background: The relationship between “normal” aging and Alzheimer's disease has long been a subject for speculation. The fact that age is the major risk factor for sporadic Alzheimer's disease has led some to speculate that if we lived long enough we would all get Alzheimer's disease. On the other hand, examples of centenarians who remain cognitively intact suggest otherwise. At a molecular level, multiple studies have indicated correlated changes of gene expression in aging and AD. However these studies have, for the most part, been conducted in brain regions, such as association cortex, that are affected by both age and AD. We examined expression of synapse-related genes as a function of age and Alzheimer's in multiple brain regions, including one (primary somato-sensory neocortex of the post central gyrus) that is essentially unaffected in Alzheimer's disease.

Methods: 400 brain samples were obtained from 7 Alzheimer Disease Research Centers (ADRC or ADCC). Cases ranged in age from 20 to 100 years old. A subset of the older cases had been diagnosed with neuropathologically confirmed Alzheimer's disease. Four brain regions were sampled: hippocampus, entorhinal cortex, frontal association cortex and post central gyrus. The resulting samples were homogenized, RNA extracted and checked for quality. RNA samples were processed and hybridized to ~400 Affymetrix Hg-U133 plus 2.0 arrays at the University of California, Irvine. Expression values were determined from CEL files using GC-RMA and statistical analysis was conducted using GeneSpring 7.3.1. This resulted in a list of 662 probe sets which was reduced to 562 probe sets by the elimination of probe sets that were absent on more than 50% of the chips. These probe sets represented 340 synaptic genes. Significant probe sets were determined as previously described (Berchtold et al., 2008). Regression analyses were used to examine relationships between the effects of age and of AD on the expression of synapse-related genes among the 4 brain regions studied.

Results: Expression of synaptic genes in the postcentral gyrus was unchanged in AD. However, this brain region exhibited major decreases of gene expression as a function of age. The correlation between expression changes in the aged postcentral gyrus with Alzheimer-related expression changes in the Alzheimer hippocampus was +0.89. Since the hippocampus is a source of both age and disease related changes we removed the age effect from the hippocampus data by means of partial regression analysis, which reduced the correlation to +0.82.

Conclusions: These data are consistent with the concept that cells have a limited repertoire of responses to stress, in this case stresses related to age or to Alzheimer's disease. However, the present findings that similar changes in gene expression are found in two different brain regions as a function of either aging or AD convincingly indicates that although age and AD may lead to similar expression profiles they do so in different brain regions. A conclusion that brain regions are differentially affected by age and by Alzheimer's disease provides persuasive evidence that these two are not the same. This conclusion is consistent with the conclusion reached in an unbiased stereological study of cell numbers in the sub regions of the hippocampus in the aging and Alzheimer's human brain showing that CA1 loses neurons in AD but not in aging (West et al., 1994). Acknowledgments: Supported by R01 to C Cotman and 1R01AG036400 to PDC

Poster 12

INTERVENTION DEVELOPMENT FOR CAREGIVERS OF PEOPLE WITH ADRD AND DOWN SYNDROME/ID. Coon DW, Carll P, Goldman J, Montague R, Stotler K. Arizona State University; Arizona Alzheimer's Consortium.

Background: An underserved and understudied group of family caregivers impacted by Alzheimer's Disease and related dementias (ADRD) are those who assist adults with Down syndrome or another Intellectual Disability (ID) who develop ADRD as they age. Individuals with Down syndrome have a genetic propensity to develop early onset ADRD. Recent research estimates that between 50% and 70% will be affected after age 60. Lifelong caregiving, amplified by ADRD, may create "double jeopardy" for their informal caregivers. Moreover, no evidence-based interventions have been identified to help these caregivers manage changes in care, caregiving associated stressors, and caregiver-related distress.

Methods: This project gather preliminary data in two ways. 1) Descriptive data was collected anonymously with the Alzheimer's Association at a conference focused on Alzheimer's disease and those impacted by Down syndrome or another ID. Participants provided demographic information and feedback on the value of various strategies in reducing stress and distress for caregivers of people with Down syndrome or other IDs and ADRD. 2) Family caregivers and professionals served as informants by offering their opinions on ways to tailor empirically supported psychoeducational skill-building caregiver intervention strategies for ADRD caregivers. This project data is helping to develop intervention components for a pilot intervention for family caregivers of people with ADRD and Down syndrome or another ID.

Results: A majority of conference participants described themselves as non-Hispanic White (78.6%) in comparison to Hispanic/Latino (10.7%), African American/Black (7.1%), or another ethnic/racial minority (3.6%). They varied in their roles with regard to caregiving including: 1) professionals working directly with people with Down Syndrome or another ID and/or their families (42.6%); 2) professionals helping develop or manage programs to serve these individuals (28.6%); 3) family or friend caregivers (60.7%); or 4) people in another professional role (10.7%). One-third (32.1%) reported that they managed two or three roles simultaneously. A majority of participants rated each of the following strategies as very useful in helping reduce the stress and distress of caregivers helping people with ADRD and Down syndrome or another ID: skills training to improve communication with care recipients (88.9%); techniques to manage behavior changes (89.3%); skills training to improve communication with providers (85.7%); care values and care task clarification for future care planning (78.6%); strategies to help caregivers manage unhelpful thinking (78.6%); communication skills to improve interactions with family and friends (78.6%); pleasant events scheduling for care recipients (74%); and pleasant event scheduling for caregivers (71.4%). Informants identified other critical issues for the project team to consider: the need for interventions for individuals in group home/group living situations; the need for interventions for staff caregivers; the need for sessions in group homes that recognize staffing and cost challenges; and the potential for a combination of in-person and online or telephone group based interventions.

Conclusions: Feedback from family members and professionals has helped to shape psychoeducational skill-building intervention components for piloting with people caring for individuals with ADRD and Down Syndrome or another ID.

Poster 13

UPDATES FROM AN INNOVATIVE, COMMUNITY-LEVEL, MUSIC-BASED INTERVENTION FOR PEOPLE WITH ADRD. Coon D, Cortés M, McCarthy M, Rio R, Todd M, Bontrager V, Montague R, Rosas V, Carbajal L, Glinka A, Burlson M. Arizona State University; The Phoenix Symphony; Arizona Alzheimer's Consortium.

Background: Little research examines interdisciplinary efforts (nursing, music therapy, music performance, behavioral science) to provide community-level music-based interventions that affect the quality of life of people with dementia in long-term care settings. A recent (Fall 2016) research-practice-music performance collaboration advances these efforts by combining nurse ratings of mood and behavior with biomarkers of stress (salivary alpha amylase and salivary cortisol) across two distinct long-term care communities. The second iteration of this pilot study was conducted in a long-term care facility focused on serving a low-income, underserved population. It expands upon our initial findings (changes in quality of life indicators) from an innovative community-level, music-based intervention with policy implications for people with dementia residing in long-term care communities.

Methods: Professional symphony musicians and music therapists delivered interdisciplinary (music, music therapy, nursing, behavioral science) intervention through 7 music-based events across 6 weeks. Nurse raters evaluated resident affect before/after each event while symphony musicians, facility staff, and family caregivers completed similar self-ratings. Participants provided saliva samples to investigate the impact on biomarkers of stress (salivary alpha-amylase, cortisol). The second pilot incorporated new measures to evaluate the environment of the units in terms of resident mood and behavior on evenings w/ and w/o morning music events.

Results: Findings from both mood and behavior ratings and saliva samples provided evidence of the intervention's impact in terms of significant changes in participant mood and in behavioral activation, as measured by salivary alpha-amylase. In addition, a sub-study measuring salivary cortisol indicated that morning music events may have enabled residents to better regulate their stress responses around an afternoon stressor (bathing). On evenings when morning music events occurred, versus those without music events, nurse ratings of the resident environment showed a) significantly more positive increases in the overall mood of the unit and resident cooperation during evening care, as well as b) significant decreases in levels of verbal/physical disruption at change of shift and dinnertime and in the number of critical events including falls, acute illness, deaths, staff shortages. Participants rated perceived benefit and acceptability of the intervention very highly across the board. Results support our conceptual model that integrates three key parameters (receptive to active; observation to relationship; and planned to improvisation), thereby bridging science and practice and emphasizing the importance of community-level music-based interventions in long-term care.

Conclusions: This project extends original pilot work into a long-term care setting focused on lower-income, underserved residents. In contrast to other music interventions, it also extends the scientific and practice literatures by measuring changes in mood and behavior immediately before and after music-based events, providing substantive evidence of the intervention's impact, feasibility, and perceived benefit. Moreover, these findings were bolstered by evening environmental ratings that showed the sustained impact of the intervention on the overall milieu. Finally, these results may have both cost (reduction in staff time, patient critical events, staff turnover) and policy implications for the treatment of residents with dementia residing in long-term care.

Poster 14

BREAKING BARRIERS TO LATINO PARTICIPATION IN DEMENTIA-RELATED RESEARCH & SERVICES. Cortés M, Carbajal B, Rosas V, Coon DW. Arizona State University; Arizona Alzheimer's Consortium.

Background: The National Plan to Address Alzheimer's Disease states that identifying Alzheimer's Disease and related dementias (ARD) in the early stages creates advantages for early-stage people (EPs) and their care partners (CPs). A recent review of ethnic/racial differences in ARD treatment, care, and research supports consistent evidence that minority groups, including Latinos, access dementia diagnostic services later than non-Hispanic whites due to later presentation to clinical research and dementia-related services. Past research suggests that Latino family caregivers are critical "cultural brokers" in accessing treatment, care, services and research opportunities for people with dementia, as are networks of Latino professionals and community health workers (*promotores*). However, researchers and service providers continue to encounter ongoing challenges in recruiting Latinos earlier in the ARD disease process.

Methods: This presentation combines data from 16 focus groups across 4 different projects designed in part to uncover ways to recruit Latinos into dementia-related research and services earlier. Bilingual professionals conducted 1.5 to 2-hour semi-structured focus groups in English and Spanish, after which trained research staff transcribed the audio recordings. Data were analyzed using an approach that encompasses both constant comparative and content analyses.

Results: Self-identified Latinos (N=132) were recruited including 59 family caregivers participating in 8 different groups; 44 professionals and staff working with Latino older adults and family caregivers in 5 different groups; and 29 *promotores* in 3 different groups. Family caregiver average age ranged from 50 to 57 across groups and was higher than the average age range of professionals, staff, and *promotores* (41 to 48). The majority of participants identified ethnicity similarly across the focus groups with at least 85% of family caregivers and 73% of professionals, staff, and community health workers self-selecting Mexican or Mexican American. Key themes were shared between family and provider groups: the need for bilingual/bicultural staff skilled in the delivery of culturally responsive services and research; lack of understanding about dementia and cultural stigma of dementia within the Latino community; challenges in language translation that foster misunderstandings associated with dementia and research; the importance of recruitment via Latino media outlets; and concerns about their loved ones. Family caregivers emphasized the desire to meet others with similar challenges and they cited barriers, such as multi-generational care responsibilities, transportation issues, and the inability to miss work. Providers noted the importance of trained *promotores* in the community; the need for sustainable culturally responsive services; and the view of research as a necessary sacrifice for better services.

Conclusions: Findings highlight the need for effective approaches to bridge gaps in dementia-related education, services, and research in the Latino community. Ongoing concerns included the need to address beliefs about dementia, language barriers, logistical issues, and the lack of access to well-trained and culturally responsive providers. New findings emphasize issues of mistrust associated with the language of research and service provision that can imply a sense of being "investigated" and create the perception of putting the family at risk. Well-trained and equipped *promotores* could serve a particularly critical role in filling the gap between the community and dementia-related services and research.

Poster 15

SENSORY VS. COGNITIVE COMPONENTS OF OLFACTION: TARGET ODOR DETECTION TRAINING WITH VARIABLE BACKGROUND ODORS ENHANCES TARGET DETECTION AGAINST NEW BACKGROUND ODORS. Daniels CW, Sanabria F, Smith BH. Arizona State University; Arizona Alzheimer's Consortium.

Background: Neurodegenerative diseases such as Parkinson's and Alzheimer's can be characterized by a dramatic loss in the sense of smell. However, the diseases differ in whether this loss is manifested as sensory or more cognitive in origin. In order to augment more traditional smell tests (e.g. UPSIT), we have developed a fairly easy to implement task on which sensory and cognitive components can be manipulated via switching odor backgrounds against which a target odor is evaluated.

Methods: Mice were trained in a simultaneous 2-alternative forced choice target + background vs. background alone olfactory discrimination task. Mice were either exposed to a single background, which was present on every trial, or to multiple backgrounds, which were randomly sampled from a list of 4 on every trial. Once performance stabilized (>90% correct), a transfer test was conducted with novel single or multiple backgrounds.

Results: Mice trained on a single background and tested on multiple backgrounds took longer than other mice in learning the new backgrounds. Baseline training was reinstated and a second transfer test was conducted—mice previously tested on a single background were now tested in multiple backgrounds, and vice-versa. All mice performed similarly in the second test. These results suggest that training with, or possibly mere exposure to, multiple backgrounds enhances the detection of a trained target on novel multiple backgrounds.

Conclusions: The task we have developed is analogous to more complicated 'real world' olfactory tasks that all animals need to perform. Our planned future studies now include evaluating genetic lines of mice that have been developed as models of human neurodegenerative disorders. In addition, we plan to evaluate how modulation of the olfactory bulb and cortical regions enable performance on this task.

Poster 16

MULTIVARIATE ANALYSIS OF GENE EXPRESSION OF PERIPHERAL BLOOD LEUKOCYTES DIFFERENTIATES PERSONS AT RISK FOR ALZHEIMER'S DISEASE FROM PERSONS NOT AT RISK. Delvaux E, Mastroeni D, Nolz J, Marshall F, Coleman PD. Arizona State University; Banner Sun Health Research Institute; University of Rochester Medical Center; Arizona Alzheimer's Consortium.

Background: Multiple imaging, neuropathological, cognitive and molecular studies have established that Alzheimer's disease has a very long "preclinical" phase during which the disease is damaging nerve cells. The lack of success of current therapeutic interventions in clinically diagnosed persons has led to a shift in conceptual framework toward treating the disease prior to the point at which disease has become serious enough for clinical diagnosis. Although imaging and other methods are able to detect early stages of disease their expense, time consuming or intrusive nature preclude their utility for detecting disease in the general population. An inexpensive and minimally invasive method of detecting early Alzheimer's disease is needed.

Methods: Longitudinal blood samples were obtained from 253 persons enrolled in the University of Rochester arm of the ADAPT (Alzheimer's Disease Anti-Inflammatory Prevention Trial) Study. At entry, all persons were clinically determined to be cognitively intact. Inclusion criteria were age 70 or greater and having at least one first degree relative who had been diagnosed with Alzheimer's disease or "dementia". 2/7 of study participants were on naproxen, 2/7 on Celebrex and 3/7 on placebo. Five years after entry into the study xx persons had phenoconverted to Alzheimer's disease and YYY of these had been on placebo. We formed three groups of age and gender matched cases: (1) Those at risk who had phenoconverted to AD, (2) those at risk who had not phenoconverted to AD and (3) a matched group not at risk for AD. This third group was formed because of evidence indicating that cognitively intact persons who had a first degree relative diagnosed with AD showed changes detected by imaging and other studies. Blood was collected into PaxGene tubes, RNA extracted and expression of selected genes quantified by qPCR. Multivariate analysis of expression of a selected set of transcripts was used to quantify differences among groups in patterns of gene expression. Analysis of data was limited to persons who were on placebo.

Results: Multivariate analysis of gene expression by peripheral blood leukocytes was not convincingly able to distinguish persons at risk who had phenoconverted to AD from persons at risk who had not phenoconverted to AD. We assert that this is because these persons were well along the path toward a clinical diagnosis of AD. However, the distinction of persons not at risk from those at risk was 100% accurate. Thus, we were able to distinguish risk on the basis of having a first degree relative diagnosed with AD or dementia. This finding suggests, but does not formally prove, that the procedure we have developed may be able to determine risk of a future diagnosis of AD that is not based on having a first degree relative that has been diagnosed with AD. Other data demonstrate the ability of this procedure to distinguish Alzheimer's from Parkinson's disease, thus indicating the specificity of the procedure we have developed.

Conclusions: The present data establish RNA extracted from peripheral blood leukocytes as a promising biomarker of Alzheimer's disease. Although the numbers of cases in the present study is relatively small, the data clearly indicate the potential for development of a minimally invasive, potentially inexpensive blood test for detection of persons at risk for a future diagnosis of Alzheimer's disease. Acknowledgments: Thanks to the ADAPT study, led by John C Breitner. Supported by AG R21AG030429 to PDC and by an Anonymous Donor.

Poster 17

STRATEGIC MEMORY ALZHEIMERS REHABILITATION TRAINING (SMART): COGNITIVE PROTECTION AND INTERVENTION FOR AMNESTIC-TYPE MILD COGNITIVE IMPAIRMENT (MCI). DenBoer J, Valla J. SMART Brain Aging, Inc.; Grand Canyon University.

Background: Dementia is a world-wide phenomenon, impacting more than 6 million people in the United States. Despite its projected prevalence, this is a significantly under-represented phenomena, with underestimate ranges from 15-35%. The combined effects of the aging of the population (caused by the shift of the baby boomer generation into dementia) and significant increase in life expectancy has combined to put dementia into the range of our largest medical, if not societal, problems.

Methods: The SMART Memory Program (DenBoer, 2008) is a cognitive intervention designed to promote the reduction of early-stage dementia. Although it has been found useful in all forms of dementia, it is particularly useful in amnesic-type MCI/VCI.

Results: The current longitudinal study examined 536 non-paid clients (all with amnesic type MCI, mean baseline MoCA = 20) across a two year span of this program, finding an average (although temporary) improvement of 4.25 MoCA points at the conclusion of this 8-week program. More sustaining Improvements in quality of life (QOL) and mood were also observed as a result of this program.

Conclusions: While the SMART program may show promise, future research in this area is certainly needed.

Poster 18

INVESTIGATING THE DIFFERENCES BETWEEN APE AND HUMAN GFAP PROTEINS INVOLVED IN NEURODEGENERATION. Eisemann R, Bae NS, Swanson MJ. Midwestern University; Arizona Alzheimer's Consortium.

Advances in medicine have led to increased average life expectancy in humans and an aging society, resulting in a growing importance of age-related diseases, such as Alzheimer's disease (AD). Unlike many other diseases, the number of deaths due to AD is on the rise primarily due to a lack of understanding of how the disease is caused at the cellular level. The greatest known risk factor for AD is advancing age. It has been reported that non-human primates are not prone to AD. Here, we propose that the interaction between a protein that protects the telomeres and a protein expressed exclusively in astrocytes modulates the accumulation of amyloid deposits, a characteristic of the AD brain. The shelterin protein complex is responsible for maintaining genomic stability by preventing DNA damage to the telomeres. RAP1 is the subunit of the shelterin complex that protects telomeres from illegitimate fusion results in genomic instability and cancer. Our lab has recently identified a novel interaction between RAP1 and GFAP δ , a protein associated with neurodegenerative diseases. The GFAP δ isoform also interacts with PS1, the protease responsible for creating the proteins that aggregate into senile plaques in AD. GFAP δ has several naturally occurring variants in humans. Interestingly, such variation does not occur in non-human primates. We hypothesize that the amino acid sequence differences in the three human variant proteins will correspond to differences in interactions among RAP1, PS1 and GFAP δ , as well as alterations in AB production. The rationale for this project is to determine if mutations associated with AD affect the RAP1 – PS1- GFAP δ interactions and whether specific mutations mimicking primate sequences are protective.

Poster 19

IDENTIFICATION OF PROTEIN NETWORKS AFFECTED BY RIBOSOMAL S6 KINASE ACTIVITY IN THE HIPPOCAMPI OF 3XTG-AD, A MOUSE MODEL OF ALZHEIMER'S DISEASE. Ferreira E, Piras IS, Huentelman M, Dave N, Oddo S. Arizona State University; Translational Genomics Research Institute; Arizona Alzheimer's Consortium.

Background: The two neuropathological hallmarks of Alzheimer's Disease (AD) are amyloid plaques and neurofibrillary tangles (NFTs), whereas memory loss is its most profound clinical manifestation. 3xTg-AD mice, a widely used animal model of AD, display a phenotype consistent with these hallmarks. This AD-like phenotype is driven in part by increased activity of the mammalian target of rapamycin (mTOR), a serine/threonine kinase of which ribosomal S6 Kinase1 (S6K1) is a key downstream effector. Reducing S6K1 levels using a hemizygous S6K1 knockout in 3xTg-AD mice significantly mitigates neuropathology as well as cognitive deficits. Here we seek to identify how this modulation of S6K1 affects the mouse hippocampal proteome and to identify specific protein interaction networks that are most affected.

Methods: Using isobaric tags (iTRAQ) and proteomic methods, here we measured hippocampal protein levels of 3xTg-AD mice (n=4), 3xTg-AD mice with a hemizygous knockout of S6K1 (3xTg-AD/p70S6K1^{+/-}, n=4) and also non-transgenic (NonTg, n=4) control mice. Relative protein level ratios were used to identify proteins for which hippocampal levels were both significantly altered in 3xTg-AD mice and also reverted to baseline control levels observed in the NonTg hippocampal proteome. For proteins meeting these criteria, we used protein interaction and gene ontology databases to identify protein networks and overrepresented cell pathways. In addition, these proteins were cross-referenced to human RNA array data measuring mRNA transcript levels in medial frontal cortex from post-mortem AD patients (n=97) and age-matched controls (n=98) to identify proteins that were altered similarly between human AD versus controls and between 3xTg-AD and NonTg mice.

Results: We found that, of 3,182 proteins detected by iTRAQ, 77 proteins were both significantly altered in 3xTg-AD compared to controls and also measured at baseline levels in 3xTg-AD/p70S6K1^{+/-}. Pathway enrichment analysis yielded 77 proteins significantly overrepresented in the annotated pathways for metabolism, focal adhesion, ribosomal assembly, and Alzheimer's pathogenesis. One of the 77 proteins identified is brain selective kinase 2 (Brsk2), a serine threonine kinase shown to phosphorylate tau and interact with VCP/p97, a critical chaperone protein. Additionally, the decrease in Brsk2 levels in 3xTg-AD mice compared to control NonTg (log₂ fold change = -0.361037, adj p-value= 0.0089) compared similarly to human RNA array data comparing medial temporal gyrus of AD to control ND (log₂ fold change = -0.345, adj p-value= 2.9E-06).

Conclusions: Proteins altered in mouse hippocampus due to decreased levels S6K1 are disproportionately involved in metabolism, ribosomal assembly, focal adhesion, and AD cellular pathways. Brsk2 protein levels showed a similar decrease in 3xTg-AD mice as Brsk2 transcript levels in human AD subjects. Though known to phosphorylate tau, as well as play a role in autophagy and protein chaperone during ER stress, the role of Brsk2 in AD is largely unknown. However, these mouse proteomics and human RNA array data suggest that Brsk2 may play an important role in AD pathogenesis. Ongoing experiments seek to determine correlation between Brsk2 levels and AD pathology and cognitive impairment.

Poster 20

GENDER DIFFERENCES IN ALZHEIMER'S DISEASE: BRAIN ATROPHY, HISTOPATHOLOGY BURDEN AND COGNITION. Filon JR, Intorcia AJ, Sue LI, Vazquez Arreola E, Wilson J, Davis KJ, Sabbagh MN, Belden CM, Caselli RJ, Adler CH, Woodruff BK, Rapscak SZ, Ahern GL, Burke AD, Jacobson S, Shill H, Driver-Dunckley E, Chen K, Reiman EM, Beach TG, Serrano GE. Banner Sun Health Research Institute; Arizona State University; Barrow Neurological Institute; Mayo Clinic Arizona; University of Arizona; Banner Alzheimer Institute; Arizona Alzheimer's Consortium.

Background: Multiple studies have suggested that females are affected by Alzheimer's disease (AD) more severely and more frequently than males. Other studies have failed to confirm this and there has been no clear resolution of the issue. Difficulties include differences in study methods and male versus female life expectancy. Another element of uncertainty is that the majority of studies lack neuropathological confirmation of diagnosis.

Methods: We compared clinical and pathological AD severity in more than 1000 deceased subjects with full neuropathological examinations.

Results: Age of dementia onset did not differ by gender but females were more likely to proceed to very severe disease, both clinically and pathologically, with significantly higher proportions of females having a Mini Mental State Examination score of 5 or less and Braak stage VI neurofibrillary degeneration. Median neuritic plaque densities were similar in AD females and males but females had significantly greater tangle density scores. In addition, we found that AD-control brain weight differences were significantly greater for females, even after adjustment for age, disease duration and comorbid conditions.

Conclusions: These suggest that, when affected by AD, females progress more often to severe cognitive dysfunction, due to more severe neurofibrillary degeneration and greater loss of brain parenchyma.

Poster 21

TELOMERE PROTEIN RAP1 LEVELS ARE AFFECTED BY CELLULAR AGING AND OXIDATIVE STRESS. Gallas G, Chia J, Bentz G, Swanson MJ, Bae NS. Midwestern University; Mercer University School of Medicine; Arizona Alzheimer's Consortium.

Background: Telomeres are important for maintaining the integrity of the genome through the action of the shelterin complex. Previous studies indicated that the length of the telomere did not have an effect on the amount of the shelterin subunits, but those experiments were done using immortalized cells with stable telomere lengths. Our interest was to see how decreasing telomere lengths over successive generations would affect the shelterin subunits. As neonatal human dermal fibroblasts aged and their telomeres became shorter, the levels of the telomere-binding protein TRF2 decreased sharply. In contrast, the levels of one of its binding partners, RAP1, decreased to a lesser extent than would be expected from the decrease in TRF2. Other subunits, TIN2 and POT1, remained stable. The decrease in RAP1 in the older cells occurred in both the nuclear and cytoplasmic fractions. Hydrogen peroxide stress was used as an artificial means of aging the cells, and this resulted in RAP1 levels decreasing, but the effect was only seen in the nuclear portion. Similar results were obtained using U251 glioblastoma cells treated with hydrogen peroxide or grown in serum-depleted medium. Leptomycin B treatment of the cells show that nuclear RAP1 is blocked from shuttling to cytoplasm. Fluorescent microscopy data shows the translocation of RAP1 into cytoplasm upon oxidative cellular stress.

Methods: The human glioblastoma cell line U251 was grown in DMEM with 10% FBS. Cells were treated with varying concentrations of hydrogen peroxide (H₂O₂), and cell extracts or fractions were compared to untreated control cells using immunoblotting to detect proteins of interest. For fluorescence microscopy, cells were grown and treated as describe above, then fixed with paraformaldehyde prior to antibody staining. To determine whether RAP1 was translocating from the nucleus, leptomycin B was added to block nuclear export before H₂O₂ treatment. For nutrient starvation, cells were washed once with serum-free medium, then were incubated with fresh serum-free medium for 24-48 hours.

Results: As the cells responded to the H₂O₂ and serum deprivation stresses, RAP1 levels increased in the cytoplasm while TRF2 remained exclusively nuclear.

Conclusions: The relocation of RAP1 suggests a new, non-telomeric role of RAP1 in the response to oxidative stress, which might involve interactions with proteins specific to neuronal cells.

Poster 22

THE SAFE AND EFFECTIVE APPLICATIONS OF ESSENTIAL OILS IN ALZHEIMER'S DEMENTIA. Geiger JL. Banner Desert Medical Center; Arizona Alzheimer's Consortium.

The diagnosis and treatment of Alzheimer's dementia (AD) has gained much attention due to the current and predicted prevalence of the disease. The biomarkers of neural inflammation, oxidative stress, genetics and the multiple medical etiologies of AD coupled with the poly-pharmacy of comorbid conditions and diseases associated with AD are complex. The medical diagnosis and treatments of AD need more reliability and integrative health regimens, such as supplementation with generally regarded as safe (GRAS) essential oils that could be implemented clinically to lessen the global economic impact to societies, and disruptions to families.

Treatment has focused on the management of AD based on the cholinergic theory attempting to prevent cognitive decline while preserving short-term memory with prescription medications exhibiting acetylcholinesterase inhibition (ACHEI) derived from natural phyto-preparations to manage behavioral and psychiatric issues. Advancing age and anesthesia increase the risk of post-operative cognitive decline (POCD) associated with common procedures and surgeries that AD patients commonly undergo. Globally, plant material preparations such as extracts and essential oils are analyzed for anti-inflammatory, anti-infectious and anti-anxiety, anti-depressive, analgesic and ACHEI activity.

The potential for use of GRAS essential oils as supplements in the prevention and treatment of the cognitive decline and behavioral disruptions of aging should be studied to determine the safe and effective methods of inhalational, topical and ingestion aromatherapy techniques. The landmark literature reviewed here explores the mechanisms and synergy of the aromatic sciences that can provide the basis for designing and implementing protocols for the integration of GRAS essential oils into the wide variety of clinical situations encountered in the aging population, with and without AD.

Keywords: Alzheimer's Dementia Memory Anxiety Aromatherapy Acetylcholinesterase Anesthesia Essential oils Lavender Ginger

Copyright James L. Geiger MD All rights reserved 2017

Poster 23

IMPROVED DIAGNOSIS OF PARKINSON'S DISEASE FROM A DETAILED OLFACTORY PHENOTYPE. Gerkin RC, Adler CH, Hentz JG, Shill HA, Driver-Dunckley E, Mehta SH, Sabbagh MN, Caviness JN, Dugger B, Serrano G, Belden C, Smith BH, Sue LI, Davis KJ, Zamrini E, Beach TG. Arizona State University; Mayo Clinic Scottsdale; Barrow Neurological Institute; Banner Sun Health Research Institute; University of California, San Francisco; Arizona Alzheimer's Consortium.

Background: Olfactory decline is associated with the earliest stages of both PD and AD. Consequently, olfactory ability has been proposed as a biomarker for early diagnosis. Such ability has typically been assessed with either self-report or total score on an UPSIT test. We investigated whether the predictive potential of this test could be enhanced by using the complete response pattern instead of simply the total score.

Methods: We analyzed a large dataset from the Arizona Study of Aging and Neurodegenerative Disorders (AZSAND), a longitudinal clinicopathological study of health and disease in elderly volunteers.

Using both the complete pattern of responses to all 40 items in each subject's UPSIT, we built predictive models of neurodegenerative disease, and we validated these models out-of-sample by comparing model predictions against post-mortem pathological diagnosis.

Results: Consistent with anatomical considerations, we found that the specific UPSIT response pattern had additional predictive power compared with a conventional measure--total UPSIT score--in Parkinson's disease (PD) but not Alzheimer's disease (AD).

We also identified specific UPSIT questions that carry the greatest predictive power for disease diagnosis.

Conclusions: A more accurate clinical diagnosis can be made using the pattern of responses to all the test questions, and validated this against the "gold standard" of pathological diagnosis.

Information in the response pattern also suggests specific modifications to the UPSIT that may optimize predictive power under the typical clinical constraint of limited time.

We recommend that future studies retain the individual item responses for each subject, and not just the total score, both to enable more accurate diagnosis and to enable additional future insights.

Poster 24

SSRI USE ASSOCIATED WITH REDUCED AMYLOID BURDEN IN PERSONS WITH COMBAT-RELATED PTSD: PRELIMINARY FINDINGS FROM ADNI-DOD. Goradia DD, Chen K, Chen Y, Snyder N, Harvey D, Landau SM, Jagust WJ, Weiner M, Reiman EM. Banner Alzheimer's Institute; Arizona Alzheimer's Consortium; University of Arizona; Arizona State University; University of California, Davis; University of California Berkeley; Lawrence Berkeley National Laboratory; University of California, San Francisco; San Francisco Veterans Administration Medical Center; Translational Genomics Research Institute.

Background: Sheline et al. (2014) has suggested that selective serotonin reuptake inhibitors (SSRI) may be associated with reduced amyloid plaque burden. SSRIs are commonly used in the treatment of posttraumatic stress disorder (PTSD). Using data from the ADNI-DOD study, we previously demonstrated that, compared to normal controls, older adults with combat-related PTSD had lower amyloid plaque burden, computed as the cerebral-to-cerebellar standard uptake value ratio (SUVR), and a smaller proportion of amyloid positive cases based on florbetapir PET measurements, suggesting that the reduction in amyloid burden was partly attributable to their use of SSRI and other antidepressants. In this study, we directly evaluated the impact of SSRI use on amyloid burden in PTSD participants from ADNI-DOD. As an omnibus approach, we used the Monte Carlo simulation procedure to compute the global type-I error that the number of voxels with lower SUVR in the SSRI treated PTSD participants is higher than the number of voxels in the opposite direction (lower SUVR in the non-treated PTSD participants).

Methods: An automated brain mapping algorithm was used to generate a brain map of mean gray matter SUVR differences between 16 PTSD participants treated with SSRI (68.6 ± 4.5 years) and 27 PTSD participants who were not treated with SSRI (67.7 ± 3.7 years). We computed the number of cerebral gray matter voxels associated with lower versus higher mean SUVRs in the SSRI treated versus non-treated groups, controlled for effects of age, education, APOE4 carrier status, MMSE scores and depression ratings, and used 1000 Monte Carlo simulations to assess the type-I error of finding the higher proportion of voxels with lower than higher SUVRs in the SSRI groups.

Results: In comparison with non-users, SSRI users had 55 times more cerebral voxels with lower than higher mean SUVR (13536 versus 245 voxels, $P_{mc} < 0.001$). Consistently, the chi-square test showed that the proportion of lower than higher mean SUVR in SSRI treated group was significant ($p < 0.01$).

Conclusions: This study provides additional support for the possibility that SSRI use is associated with reduced amyloid plaque burden. Additional studies are needed to confirm our findings and further clarify their generalizability to other populations.

Poster 25

ALZHEIMER'S PREVENTION REGISTRY: LESSONS LEARNED IN DEVELOPING A SHARED RESOURCE TO THE SCIENTIFIC COMMUNITY. High N, Nichols J, Gordon D, Walsh T, Aggarwal R, Aisen PS, Albert MS, Comer M, Cummings JL, Manly JJ, Petersen RC, Sperling RA, Strobel G, Weiner MW, Reiman EM, Tariot PN, Langbaum JB. Banner Alzheimer's Institute; Provo; University of Southern California; Johns Hopkins University School of Medicine; Geoffrey Beene Foundation Alzheimer's Initiative; Cleveland Clinic Lou Ruvo Center for Brain Health; Columbia University; Mayo Clinic Rochester; Harvard Medical School; Alzforum; University of California San Francisco; Arizona Alzheimer's Consortium.

Background: Recruitment and enrollment into clinical trials is a major obstacle faced by researchers and study sponsors. It has been estimated that fewer than 10% of Americans participate in clinical trials, mostly due to lack of awareness about study opportunities, resulting in approximately 80% of research studies failing to meet their enrollment goals in the stated timeframes. Given the growing number of preclinical and symptomatic treatment trials being conducted or in the planning stages, in 2012 we developed a web-based Alzheimer's Prevention Registry (APR) to help studies make enrollment more efficient and timely. Serving as a shared resource to the Alzheimer's scientific community, the APR has been designed to complement and enhance local recruitment efforts.

Methods: Interested adults age 18+, with and without memory and thinking problems, are eligible to join at www.endALZnow.org. Based on lessons learned from the Arizona Alzheimer's Research Registry and modeled after other web-based research registries, this Registry was purposely designed to have a low threshold of commitment at entry. At enrollment, individuals are asked to provide their name, email address, zip/postal code and year of birth; after enrollment they can complete additional contact and demographic information at their discretion. APR members receive regular email communication to keep them apprised of the latest news in Alzheimer's prevention research. In addition, enrollees receive email notifications when study opportunities become available in their communities, with information on next steps to explore the possibility of their participation.

Results: As of February 2017, over 260,000 individuals have joined the APR. APR members are predominantly women (78%), report a family history of dementia (70%) and have no diagnosis of cognitive impairment (95%). 36% of members are between the ages of 46-60; 41% are between the ages of 61-75.

Conclusions: The APR is an engaged community of individuals who want to stay abreast of the latest in Alzheimer's news and scientific advances, and to be connected to research studies taking place in their communities. The APR has been well-received and enrollment continues to increase; results from A/B testing and the impact that website modifications had on enrollment will be discussed. Efforts are underway to significantly increase the APR study listing portfolio to allow more opportunities for participation. The planned Researcher Portal will enable us to report the success of APR in facilitating enrollment into studies. We continue to explore novel approaches for increasing enrollment and engagement of enrollees, as well as collaborating with researchers to help promote relevant studies taking place in their catchment areas.

Poster 26

A SURVEY OF MICROBIAL DNA PRESENT IN THE BRAIN TISSUE OF INDIVIDUALS WITH AD AND MCI COMPARED TO NON-DEMENTED HIGH PATHOLOGY AND NORMAL CONTROLS. Jentarra G, Chu P, Chavira B, Tullot T, Kaufman J, Jones B, Vallejo J, Jones D, Potter P. Midwestern University; Arizona Alzheimer's Consortium.

Background: The potential involvement of microorganisms in the development of Alzheimer's disease (AD) pathology and symptoms has been previously proposed by many investigators. A variety of microorganisms (*C. pneumoniae*, *C. albicans*, *B. burgdorferi*, HSV-1, etc.) have been reported to be more commonly present in the brain tissue of AD patients than in non-demented controls. However, investigators have failed to definitively show that one specific microbe is present in all cases of AD. We therefore hypothesized that if AD has an infectious trigger, the pathology and symptoms of AD may be a non-specific response to any invading or chronically present microbe. We undertook a survey of potentially involved microbes starting with HSV-1, as it is very commonly implicated in AD. The analysis is being continued with an unbiased (i.e. not targeting a specific microbe) survey of possible bacterial or fungal microorganisms.

Methods: Brain tissue samples from four subject groups (superior frontal gyrus) were obtained from the Banner Sun Health Research Institute. These groups included: AD patients, patients with mild cognitive impairment (MCI), normal controls, and high pathology controls. For all analyses, DNA was extracted from tissue samples using the MoBio Powerlyzer kit with a modified extraction procedure. Nested PCR amplification of the HSV-1 UL27 gene was performed to test samples for the presence of HSV-1 DNA. Analysis for the presence of bacterial DNA (based on the V4 region of the 16S rRNA gene) is being performed on the extracted DNA by the ASU Microbiome Analysis Laboratory. Fungal organisms are being surveyed by nested PCR amplification of the ITS-1 and ITS-2 regions of the 18S rRNA gene in our own laboratory. The amplicons are being sequenced for identification of the source microbe.

Results: In 12 normal control subjects, no positive results for the presence of HSV-1 DNA were found. The normal control individuals are non-demented and do not meet the pathological criteria for a diagnosis of AD. One of the 12 high pathology control subjects was positive for HSV-1 DNA. These subjects meet the pathological criteria (plaques and tangles) for AD but do not display dementia. Of the 12 subjects diagnosed with mild cognitive impairment, one subject was positive for HSV-1 DNA. One of 12 subjects diagnosed with AD was also positive. Results of analysis for the presence of bacterial and fungal DNA are ongoing and will be presented.

Conclusions: A small number of subjects with either AD or MCI as well as subjects with AD-type pathology but no dementia, were found to have HSV-1 DNA in tissue from the superior frontal gyrus. However, none of 12 normal control subjects had detectable HSV-1 DNA. This data will be combined with data from studies of the presence of bacterial and fungal DNA to assess the full microbial load in brain tissue from each group of subjects.

Poster 27

EFFECTS OF CANDIDA ALBICANS INFECTION IN 3X-TG-AD MICE. Jones TB, Vallejo J, Gonzalez F, Kaufman J, Jentarra G, Kerry-Gnazzo A, Potter P, Tullot T, Jones D. Midwestern University; Arizona Alzheimer's Consortium.

Background: Alzheimer disease (AD) is a chronic neurodegenerative disorder characterized by the presence of amyloid plaques (mainly comprised of the A β (1-40 or 1-42) peptides) and neurofibrillary tangles (aggregated hyperphosphorylated tau protein) in the brain. AD is associated with enhanced inflammatory signaling, which suggests ongoing stimulation of immune responses. Many reports have identified the presence of a wide range of microorganisms in brain tissue in association with AD. Reports include spirochete-type bacteria, herpes simplex virus 1 (HSV-1), and fungal organisms, including various species of *Candida albicans*. The chronic presence of any of these microorganisms in the brain may be sufficient to produce inflammation in AD patients. The presence of fungal organisms (i.e., yeast and/or fungal hyphae) in the brain may activate resident microglia which would promote the recruitment of inflammatory cells from the periphery; many of the cytokines produced in response to fungal infections (e.g., IL-6, TNF- α) have been identified in AD brains. The goal of this project was to assess the ability of 3xTg-AD mice to clear *C. albicans* infection from the brain and various peripheral tissues. The 3xTg mouse is a triple-transgenic (B6;129-Psen1tmlMpmTg(APP^{Swe},tauP301L)1Lfa/Mmjax) animal model of AD. We hypothesized that 3xTg mice would exhibit impaired clearance of the candida and as a consequence, would exhibit a greater fungal load compared to age-matched controls (C57/BL6).

Methods: *C. albicans* was cultured on Sabouraud dextrose agar at 30°C. Prior to inoculation of mice, the concentration of yeast cells was adjusted to 1.0 x 10⁶/mL. Female and male mice (6-7 months old) were then inoculated with 0.1 mL (1.0 X 10⁵) of the fungal suspension via the lateral tail vein. This protocol has been shown to produce widespread dissemination of *Candida*, including CNS invasion in C57/BL6 mice. Infected mice were sacrificed at 1, 3, 7, and 14 days post-inoculation (dpi) and the spleen, kidney, liver, and brain were aseptically removed, weighed, and then bisected for analyses. To test fungal burden and clearance, tissue was homogenized, plated on agar and fungal growth was determined.

Results: The mortality rate for inoculated 3xTg mice sacrificed 14 dpi was 25% (3/12) compared to 0% for C57 control mice. At both 7 and 14 days post-inoculation, there was a significant increase in the mean splenic weight in 3xTg mice compared to control mice at the same time post-inoculation ($p < 0.0001$). Interestingly, spleen weights in male 3xTg mice were 1.5-2-fold greater than observed in female 3xTg mice while spleen weight was comparable between male and female control mice. The effects of *C. albicans* infection on the liver were similar to those observed in the spleen, although were generally less pronounced. For the liver, there was a significant effect of group, indicating that the livers of 3xTg mice weighed significantly more than those of control mice at 7 and 14 days dpi ($p = 0.009$). There was also a significant effect of time post-inoculation which indicated that the liver weights at 14 days dpi were significantly greater in the 3xTg mice than at 7 days dpi. This was not observed in control mice. There was no difference in the weight of kidney or brain across groups, or across time post-inoculation.

Conclusions: Splenomegaly and hepatomegaly observed in the 3xTg-AD mice compared to C57 controls suggest that the transgenic mice respond differently to fungal infection and exhibit chronic inflammation following *C. albicans* inoculation.

Poster 28

HISTOLOGICAL TOOLS FOR DETECTING INFECTIOUS AGENTS IN NEURAL TISSUE. Kaufman JA, Castro MJ, Jones DC, Jones TB, Potter PE, Tullo T, Vallejo J, Jentarra GM. Midwestern University, Arizona Alzheimer's Consortium.

Background: The etiology of sporadic Alzheimer's Disease (AD) remains unknown. The cholinergic hypothesis, the amyloid or amyloid cascade hypothesis, and the tau hypothesis have all been subjects of intense investigation. While these hypotheses are not mutually exclusive, and are based on known neuropathological findings associated with AD, the underlying causal factors leading to Alzheimer's neurodegeneration are still elusive and a definitive treatment has not yet been identified.

One hypothesis that does posit an underlying causal factor is the infection hypothesis. A variety of microorganisms have been reported in brain tissue in association with AD, including fungi, HSV-1, and spirochete bacteria. A low-level chronic infection that crosses the blood-brain-barrier may produce inflammation. Moreover, A β appears to have antimicrobial properties and has been induced by the presence of microbes. In order for the infection hypothesis to be critically investigated, it is necessary to employ histological probes effective at detecting microbes in neural tissues.

Methods: We investigated several histological methods for identifying infectious microorganisms in brain tissue. Traditional pathology stains included acid-fast bacteria, periodic acid-Schiff, Grocott's methenamine silver, Warthin-Starry, and calcofluor white. Immunohistochemical approaches included antibodies against peptidoglycan (a component of the bacterial cell wall), as well as specific microorganisms such as Candida, Borrelia, Mycobacterium, and HSV-1.

Results: Traditional pathology stains such as acid-fast bacteria and Warthin-Starry are problematic because they stain normal tissue components, making it very difficult to identify microorganisms. Immunohistochemistry yielded better visibility of microbes with less background and/or non-specific staining. However, "pan-bacterial" antibodies such as those raised against peptidoglycan did not perform well. Instead, antibodies raised against specific pathogens, such as Candida and Borrelia, offered the highest quality immunostaining. These antibodies do exhibit certain amounts of cross-reactivity. For example, the anti-Candida antibody stains other types of fungi, and the anti-Borrelia antibody reacts with other spirochete bacteria.

Conclusions: Clinical pathology stains, such as periodic acid-Schiff and Warthin-Starry, stain excessive amounts of normal tissue, limiting their use for investigating the infection hypothesis. Instead, immunohistochemistry offers better promise. While pan-bacterial antibodies - such as those raised against peptidoglycan - are difficult to optimize, antibodies raised against specific microbes provide a good approach for investigating the infection hypothesis in AD. Since many antibodies are cross-reactive to other microbes within a family or genus, the potential for detecting the presence of infectious agents is greater. At the same time, identification of microorganisms to the genus and/or species level may require genetic sampling of tissue homogenates in conjunction with histology.

Poster 29

NUCLEAR, BUT NOT MITOCHONDRIAL ENCODED OXPHOS GENES ARE ALTERED IN AGING, MILD COGNITIVE IMPAIRMENT, AND ALZHEIMER'S DISEASE. Khdour OM, Delvaux E, Nolz J, Olsen G, Berchtold N, Cotman C, Hecht SM, Coleman PD, Mastroeni D. Arizona State University; Banner Sun Health Research Institute; University of California, Irvine; Arizona Alzheimer's Consortium.

Background: We have comprehensively described the expression profiles of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) gene that encode subunits of the respiratory oxidative phosphorylation (OXPHOS) complexes (I-V) in the hippocampus from young controls, age match, mild cognitively impaired (MCI), and autopsy confirmed AD subjects.

Methods: Frozen unfixed tissue was obtained from 44 non-AD controls (NC), 10 amnesic mild cognitive impaired (MCI) cases and 18 Alzheimer's disease (AD) cases. Total RNA was extracted from hippocampus and hybridized to arrays. Guanine-cytosine robust multi-assay analysis expression values were calculated, and expression differences were analyzed by comparative analysis between individuals, groups and disease status.

Results: The microarray data revealed significant down regulation in many of the respiratory chain complexes in AD, particularly those which were nuclear encoded. In contrast, there was up regulation of the same gene(s) in MCI subjects compared to AD and ND cases. Interestingly, no significant differences were observed in mtDNA genes between AD, ND and MCI subjects. Our results show that complex I and II seem to be about equally affected by age and AD, complex III more by age than AD, and complex IV and V more by AD than age.

Conclusions: Our findings suggest that restoration of mitochondrial function in aging could be an impending strategy in blunting AD. Moreover, we have previously proposed a model of Alzheimer pathophysiology in which oligomeric abeta disrupts exchange of molecules between the cell nucleus and the cytoplasm. The present data showing effect of AD on expression of nuclear encoded but not mitochondrial encoded OXPHOS genes complements this model. Furthermore, a detailed analysis of compensatory mechanisms as shown here might be a starting point for further treatment and diagnostic strategies to combat AD.

Acknowledgements: The authors declare no competing financial or conflict of interests. We are grateful to the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona for the provision of human brain samples. The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson's Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson's Disease Consortium) and the Michael J. Fox Foundation for Parkinson's Research .” This work was supported by NIRG-14-321390 and ADHS14-080000 FY2015 to D.M. Supported by RO1 to C Cotman and 1R01AGO36400 to PDC.

Poster 30

FDG-PET, NEUROPSYCHIATRIC SYMPTOMS AND THE RISK OF INCIDENT MILD COGNITIVE IMPAIRMENT. Krell-Roesch J, Lowe VJ, Pink A, Stokin GB, Roberts RO, Mielke MM, Knopman DS, Christianson TJ, Machulda MM, Jack CR, Petersen RC, Geda YE. Mayo Clinic Scottsdale; Mayo Clinic Rochester; International Clinical Research Center, Brno, Czech Republic; Arizona Alzheimer's Consortium.

Background: Little is known about the association between neuropsychiatric symptoms (NPS) and biomarker changes among cognitively normal (CN) elderly persons. We previously reported a cross-sectional association between an abnormal FDG-PET and NPS. However, such findings need to be confirmed by a prospective cohort study. We therefore investigated the interaction between an abnormal FDG-PET and NPS in elevating the risk of incident mild cognitive impairment (MCI) among CN persons.

Methods: We conducted a prospective cohort study derived from the population-based Mayo Clinic Study of Aging in Olmsted County, MN. We followed 1251 CN participants aged ≥ 50 years for a median of 32 months to the outcome of incident MCI or censoring variables. Participants underwent FDG-PET scans and NPS assessment using Beck Depression Inventory (BDI-II), Beck Anxiety Inventory (BAI) and Neuropsychiatric Inventory Questionnaire (NPI-Q). An abnormal FDG-PET (FDG-PET+) was defined as SUVR < 1.56 . We used Cox Proportional Hazards model to calculate hazard ratios (HR) and 95% confidence intervals (CI) after adjusting for age (time scale), sex and education.

Results: FDG-PET+ participants with depression (BDI-II ≥ 13) had a more than 3-fold increased risk of incident MCI (HR [95% CI], 3.76 [1.87, 7.54]) as compared to the reference group (FDG-PET-, not depressed). Similarly, risk of incident MCI was elevated (6.06 [2.85, 12.9]) for participants who were FDG-PET+ and had anxiety (BAI ≥ 10). Additionally, we observed a dose-response pattern: Being FDG-PET+ and having 1 NPS increased the risk by more than three times (3.82 [2.17, 6.71]), having ≥ 2 NPS by more than four times (4.73 [2.44, 9.17]), and having ≥ 3 NPS by more than five times (5.58 [2.60, 12.0]).

Conclusions: NPS interact with an abnormal FDG-PET in further elevating the risk of incident MCI. NPS may therefore be an important additional tool to the biomarker-based investigation of pre-symptomatic Alzheimer's disease.

Poster 31

COMPARISON OF NEUROPSYCHOLOGICAL TEST SCORES BEFORE AND AFTER TRANSITION TO MILD COGNITIVE IMPAIRMENT: A GRAPHICAL APPROACH.

Langlais BT, Caselli RJ, Dueck AC. Mayo Clinic Arizona; Arizona Alzheimer's Consortium.

Background: Previous analysis showed significant differences in changes over time in scores on the NEO Personality Inventory (NEO-PI-R) between MCI converters and non-converters. A similar analysis can investigate changes over time in other scores in the neuropsychological battery.

Methods: Members of the Arizona APOE Cohort above age 50 were selected into two overlapping cohorts; 1) those having two or more comprehensive neuropsychology battery administrations (26 test scores across four functional domains were evaluated) and 2) those with two or more NEO-PI-R administrations (five factors each comprised of six facets). MCI converters were identified as those with a transition to MCI between baseline and ending administration. Baseline scores were compared between MCI converter and non-converter groups using t-tests, change scores were evaluated by comparing ending scores while adjusting for baseline scores and the time between baseline and ending visits using analysis of covariance (ANCOVA), and frequencies compared by chi-square tests. $P < .05$ was considered as statistically significant unless specified otherwise. To supplement primary univariate and multivariate statistical testing, novel graphical approaches were investigated including multicolored, two-way grouped heat maps of standardized change scores and a new multi-sectional taproot plot displaying domain-, subdomain-, and individual-specific effect sizes.

Results: Neuropsychology Battery: There were 57 (13%) MCI converters and 385 (87%) non-converters having all 26 tests administered at least twice. Converters were older (mean years: 65 vs 60; $p < .001$) and more predominantly male (47% vs 30%; $p = .02$). Baseline test scores showed no difference between groups in the Language domain, minimal difference in Executive and Visuospatial domains, and complete differences were seen in the Memory domain. Mean change scores differed among all 26 test scores from baseline to ending administration (ANCOVA $p < .01$), as expected.

NEO-PI-R: There were 16 (6%) converters and 237 (94%) non-converters having NEO-PI-R administrations at least twice. MCI groups did not differ in age or sex (mean years: 63; 30% male) or baseline factor scores. Mean change scores among converters relatively increased in Neuroticism Factor (+3.3 vs 0.0; ANCOVA $p = .03$) and relatively decreased in Openness Factor (-4.1 vs -1.0; ANCOVA $p = .02$). Facets within these significant NEO-PI-R factors also trended in the same direction.

Heat mapping of changes in test scores from the neuropsychology battery and NEO-PI-R provide visual representation of overall change between MCI groups by individual patient while maintaining domain categorization. Multi-sectional taproot plots show +/- mean change scores similarly in aggregate.

Conclusions: MCI is associated with specific changes in neuropsychological test scores overtime. Utilizing multivariate heat mapping and sectional taproot plots may be useful in identifying patterns over a large number of grouped covariates.

Poster 32

INTEGRATIVE GENOMICS ANALYSES UNVEIL DOWNSTREAM BIOLOGICAL EFFECTORS OF ALZHEIMER'S DISEASE POLYMORPHISMS BURIED IN INTERGENIC / NON-CODING REGIONS. Li H†, Achour I†, Bastarache L†, Berghout J, Gardeux V, Li J, Lee Y, Pesce L, Yang X, Ramos KS, Foster I, Denny JC, Moore JH, Lussier YA. University of Arizona; University of Illinois at Chicago; University of Chicago; Vanderbilt University; Argonne National Laboratory; Dartmouth College; University of Pennsylvania; Arizona Alzheimer's Consortium.

† These authors contributed equally to the work.

This work was conducted in part at the University of Chicago and the University of Illinois.

Background: Functionally altered biological mechanisms arising from disease-associated polymorphisms, remain difficult to characterize when those variants are intergenic, or, fall between genes. We sought to identify shared downstream mechanisms by which inter- and intragenic single nucleotide polymorphisms (SNPs) contribute to a specific physiopathology.

Methods: Using computational modeling of 2 million pairs of disease-associated SNPs drawn from genome wide association studies (GWAS), integrated with expression Quantitative Trait Loci (eQTL) and Gene Ontology functional annotations, we predicted 3,870 inter-intra and inter-intra SNP pairs with convergent biological mechanisms (FDR<0.05). We conducted two types of evaluations: identifying if the SNP-Pairs predicted to share a mechanism for Alzheimer's disease were more likely to be in genetic synergy or antagonism in an independent GWAS, and whether these predicted SNP-Pairs on different chromosomes were more likely to be observed in chromatin interactions reported by ENCODE.

Results: These prioritized SNP pairs with overlapping mRNA targets or similar functional annotations were more likely to be associated with the same disease than unrelated pathologies (OR>12). We additionally confirmed synergistic and antagonistic genetic interactions for a subset of prioritized SNP pairs in independent studies of Alzheimer's disease (entropy $p=0.046$), bladder cancer (entropy $p=0.039$), and rheumatoid arthritis (PheWAS case-control $p<10^{-4}$). We discovered one unreported synergistic and one reported antagonistic SNP-Pair in AD. Using ENCODE datasets, we further statistically validated that the biological mechanisms shared within prioritized SNP pairs are frequently governed by matching transcription factor binding sites and long-range chromatin interactions.

Conclusions: These results provide a "roadmap" of disease mechanisms emerging from GWAS and further identify candidate therapeutic targets among downstream effectors of intergenic SNPs.

Poster 33

AGE-RELATED CHANGES OF STRUCTURAL BRAIN NETWORK ACROSS THE ADULT LIFESPAN. Liu K, Yao S, Chen K, Zhang J, Yao L, Guo X. Beijing Normal University; Banner Alzheimer's Institute; Arizona State University; Arizona Alzheimer's Consortium.

Background: A number of brain magnetic resonance imaging (MRI) studies have investigated age-related structural network changes in normal adults. However, most of them focused primarily on network changes in different age groups. The trajectories of the brain structural networks across the adult lifespan need to be investigated further.

Methods: In this study, all structural MRI data were obtained from a large public database of the Information eXtraction from Images (IXI) (<http://brain-development.org/ixi-dataset/>). Five hundred and thirty-six healthy subjects (Females/Males = 273/263, age range 20-86 years) were included. We performed a multivariate independent component analysis (ICA) to identify structural brain networks based on covariant gray matter volume and then linear, quadratic and cubic regression analyses were performed separately between each column of the ICA weights (dependent variables) and age (independent variable) to explore the trajectories of networks throughout the adult lifespan. Bayesian Information Criterion (BIC) was used to determine the optimal regression model with the smallest BIC value. A single-sample T-test was performed on the regression coefficients of the highest-order age item with the statistical significance threshold set at $p < 0.05$ with Bonferroni correction for each optimal regression model.

Results: Twenty independent components (ICs) were extracted in the ICA. The BIC and T-test revealed sixteen ICs associated with age. Most of the trajectories of ICA weights demonstrated significant linear decline tendencies, and the corresponding structural networks primarily included the anterior and posterior dorsal attention networks, the ventral and posterior default mode networks, the auditory network, five cerebellum networks and the hippocampus-related network with the most significant decreasing tendency among all ICs (p of age = $1.11E-77$). Only the temporal lobe-related network showed a significant quadratic tendency with age (p of age² = $5.66E-06$).

Conclusions: Sixteen structural networks, with the exception of the temporal lobe-related network, showed a linear decline trajectory with age from 20 to 86 years. Our findings not only provide insight into the mechanism of age-related structural changes based on the network in the human brain, but also provide a foundation for understanding abnormal aging.

Poster 34

STEPS TO H.O.P.E.: BUILDING HEALTH, OPTIMISM, PURPOSE AND ENDURANCE IN PALLIATIVE CARE FOR FAMILY CAREGIVERS OF PERSONS WITH DEMENTIA. Long CO, Favaro S, Malek-Ahmadi M, Dougherty J. Capstone Healthcare; Palliative Care Essentials; Arizona State University; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: Currently, over 5 million Americans are living with Alzheimer's disease and related dementias. Family caregivers may experience loss and grief, leading to ambiguity in their caregiving role. Interventions are necessary to help family members cope with the progressive and unsurmountable grief experiences with these terminal conditions. The Steps to H.O.P.E. program was created to offer guidance, education, and support for family caregivers in understanding and managing ambiguous loss while promoting individual self-care. It is hypothesized that caregivers who participate in the Steps to H.O.P.E. program and pilot study will experience a reduction in ambiguous loss, stress and burden, and report self-care measures to improve overall health and well-being.

Methods: Boundary Ambiguity and Ambiguous Loss Theories are the guiding theoretical frameworks which postulate that ambiguous loss, which happens when a person with dementia is physically present but psychologically absent, may contribute to caregiver stress, anxiety, compromised coping and the inability to initiate self-care strategies necessary for successful caregiving. Four pilot groups of 8 to 11 caregivers of persons with dementia at Banner Alzheimer's Institute received focused discussion and guidance regarding ambiguous loss and grief over a four-week period and subsequent booster sessions. Program evaluation and caregiver outcomes for this convenience sample consisted of descriptive analyses, paired t-tests, and repeated measures ANOVA.

Results: Thirty-seven (37) caregivers participated in the research study. Repeated measures ANOVA revealed that there was a statistically significant reduction in caregiver stress and burden on the Zarit Burden Interview Short Form ($F=26.88$, $p<0.001$), a reduction in ambiguity on the Boundary Ambiguity Scale ($F=44.82$, $p<0.001$), and a group interaction effect for The Dependence Scale ($F=3.16$, $p=.037$) meaning that the caregiver's perception of a reduction in the person with dementia's dependency upon others was related to their group affiliation. Numerous self-care activities were reported.

Conclusions: Through education and problem-solving activities in managing loss and grief, family caregivers may begin to initiate self-care strategies, reduce ambiguity and promote inner resilience. Understanding the relationship between the Steps to H.O.P.E. program interventions and outcomes and the caregiver perception of decreased dependency and decline in the person with dementia requires further investigation.

Poster 35

SYNAPTOSOMAL LEVELS OF PHOSPHORYLATED ALPHA SYNUCLEIN ARE CORRELATED WITH, AND ARE SYNERGISTIC WITH QEEG IN PARKINSON'S DISEASE. Lue L, Walker DG, Beach TG, Caviness JN. Arizona State University; Banner Sun Health Research Institute; Mayo Clinic Scottsdale; Arizona Alzheimer's Consortium.

Background: Evidence now points to the synapse as a major site affected by synucleinopathy and a therapeutic target in Parkinson's disease (PD). Current work from our laboratories with postmortem brain tissues has found that the levels of phosphorylated α -synuclein at serine 129 (p- α -syn) correlate with abnormal slow waves in the electroencephalogram (EEG) as well as with synaptosomal-associated protein 25 (SNAP25), an important core protein of the SNARE complex of pre-synapses. We are the first group to characterize the relationship between p- α -syn expression and EEG patterns in matching cases. As changes in EEG measures reflect changes in synaptic function, in this project, we determined the relationship between synaptosomal p- α -syn, amyloid beta and QEEG measures in PD.

Methods: Cingulate gyrus frozen cortical tissues were obtained from deceased donors previously enrolled in the Arizona Study of Aging and Neurodegenerative Disorders (AZSAND), a program overseen at Banner Sun Health Research Institute. All PD subjects also undergo premortem biennial digital EEG recording. Upon death, subjects were autopsied in a rapid autopsy program, the Brain and Body Donation Program. We processed tissues for synaptosomal fractionation from 44 cases. In brief, cortical gray matter was Dounce homogenized in 1 ml Syn-PER/100 mg of tissue, followed by centrifugation at 1000 g for 10 minutes. The supernatant was transferred to a new tube and centrifuged at 15,000 g for 20 minutes. The resulting pellet, the synaptic fraction, was resuspended in Syn-PER reagent, and recentrifuged at 12,000 g to remove any contaminants (primarily mitochondria). The levels of α -syn and p- α -syn in monomer or oligomeric forms, as well as amyloid beta, were measured by western blotting.

Results: Major findings: (1) We detected significant correlations between monomeric p- α -syn levels in synaptosomal fraction and values of QEEG: alpha, beta, delta, and theta band power. (2) No correlation was detected between soluble A β and QEEG band powers, in groups with or without a comorbid AD neuropathological diagnosis. (3) We detected significant positive correlations between synaptosomal A β and p- α -syn levels in subjects from both groups.

Conclusions: Increased p- α -syn association with synapses is correlated with abnormal QEEG measures in PD. The results also suggest a synergy between synaptosomal A β and p- α -syn in their effects on QEEG.

Poster 36

INTERACTION OF COGNITIVE RESERVE PROXY MEASURES WITH ALZHEIMER'S DISEASE NEUROPATHOLOGY IMPACTS EPISODIC MEMORY AND EXECUTIVE FUNCTION. Malek-Ahmadi M, Lu S, Chan Y, Perez SE, Chen K, Mufson EJ. Banner Alzheimer's Institute; Barrow Neurological Institute; Arizona Alzheimer's Consortium.

Background: Years of education are the most common proxy for measuring cognitive reserve (CR) when assessing the relationship between Alzheimer's disease (AD) pathology and cognition. Alternatively, intellectual function may be a better measure of CR. The present study determined whether tests of vocabulary and reading ability were better proxies of CR than years of education in terms of their impact on episodic memory and executive function.

Methods: The study sample consisted of 249 older deceased and autopsied persons who died with a premortem clinical diagnosis of No Cognitive Impairment (NCI; n = 123), Mild Cognitive Impairment (MCI; n = 79), and AD (n = 47). CERAD neuropathological criteria determined pathology severity. Regression analyses using a CR by CERAD interaction term determined whether vocabulary scores, reading test scores, or years of education were associated with cognition. All models adjusted for clinical diagnosis, age at death, gender, APOE e4 carrier status, and Braak stage.

Results: For episodic memory, the CR by CERAD interaction term was statistically significant for the reading test and vocabulary, but not for education: Education (Beta = -0.009, SE = 0.01, p = 0.38); Reading Test (Beta = -0.02, SE = 0.01, p = 0.04); Vocabulary (Beta = -0.05, SE = 0.01, p < 0.001). For executive function, only the Vocabulary test by CERAD interaction showed a significant effect: Education (Beta = -0.007, SE = 0.01, p = 0.55); Reading Test (Beta = -0.02, SE = 0.01, p = 0.15); Vocabulary (Beta = -0.04, SE = 0.01, p = 0.008). For Braak stage, only the interaction for Braak by Vocabulary within episodic memory was significant (Beta = 0.04, SE = 0.01, p < 0.001). Among the four CERAD classifications, there was a significant association between vocabulary performance and cognition. However, respective regression slopes for each classification declined with increased plaque pathology. CR by clinical diagnosis interactions did not reveal a significant association with the cognitive outcomes.

Conclusions: Intellectual function, which evolves over a lifetime, may be a better proxy of CR compared to years of education, which is a static measure of CR. Moreover, the interaction of intellectual function and pathology severity significantly affects cognitive test scores.

Poster 37

INCREASED 5-HYDROXYMETHYLATION LEVELS IN THE SUB VENTRICULAR ZONE OF THE ALZHEIMER'S BRAIN. Mastroeni D, Chouliaras L, Van den Hove DL, Rutten BPF, Nolz J, Delvaux E, Coleman PD. Arizona State University; Banner Sun Health Research Institute; Maastricht University Medical Centre, Maastricht, The Netherlands; University of Oxford; Arizona Alzheimer's Consortium.

Background: The subventricular zone (SVZ) is a site of neurogenesis in the aging brain, and epigenetic mechanisms have been implicated in regulating the "normal" distribution of new nerve cells into the existing cellular milieu. Although the existence and biological functions of active methylation (generally mediating gene repression) and de-methylation (generally inducing gene expression) are still in its infancy, 5hmC has been implicated in active DNA de-methylation; particularly in multipotent genes.

Methods: We examined precursor cells from the SVZ in autopsy confirmed AD and ND human subject, both in vivo and in vitro. In order to determine 5hmeC levels we used a wide array of techniques including immunohistochemistry, immunocytochemistry, and slot blots. WST-1 assays were performed in culture to determine proliferation potential in AD and ND subjects.

Results: In a case control study of human primary SVZ cultures and fixed tissue from the same individuals, we have found significant increases in DNA hydroxymethylation levels in the SVZ of Alzheimer's disease (AD) patients compared to Non-diseased (ND) control subjects. We show that this increase in 5-hydroxymethylation directly correlates to an increase in cellular proliferation in AD precursor cells; which implicates the hydroxymethylation tag to a higher degree of cellular proliferation.

Conclusions: Although an increase in the number proliferating cells has been reported in AD glial, vascular and neuronal precursor cells none have identified a causal mechanism. It has been previously reported that 5hmeC levels are linked to a higher degree of multipotency and cellular proliferation, and here we provide evidence that the increase in proliferation in AD may be directly linked to the increase in 5hmeC levels.

Acknowledgements: We are grateful to the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona for the provision of human brain samples. The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson's Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson's Disease Consortium) and the Michael J. Fox Foundation for Parkinson's Research." This work was supported by NIRG-14-321390 and ADHS14-080000 FY2015 to D.M.

Poster 38

A PARADIGM SHIFT IN MICROGLIAL EXPRESSION PROFILES IN HUMAN BRAIN. Mastroeni D, Sekar S, Delvaux E, Nolz J, Liang WS, Coleman PD. Arizona State University; Banner Sun Health Research Institute; Translational Genomics Institute; Arizona Alzheimer's Consortium.

Background: Conventional wisdom holds that brain microglia are similar regardless of brain region. Array expression data from many labs, including our own show large changes in expression of many glial-specific genes in Alzheimer's disease (AD) compared to normal controls (NC). The problem is however; homogenates are often used to obtain these data which introduces un-wanted complication because of the number of cell types analyzed. Although selected glial-specific changes are distinguishable, there are thousands of genes that are not cell class-specific but play major roles in cell function.

Methods: In order to investigate disease and regional effects on gene expression we isolated microglial cells by laser captured micro-dissection from CA1 of hippocampus and substantia nigra (SN) of AD, NC and Parkinson's disease (PD) cases, followed by RNA sequencing.

Results: Laser captured cells allowed more precise definition of relationships between microglia and their expression profiles based on disease and location. In AD CA1 366 significant ($p < .01$) differentially expressed transcripts were observed and 409 in PD CA1. Of those genes which were differentially expressed, less than 5% overlap was observed between AD and PD; implying that different neurodegenerative diseases affect microglia differently in the same brain region. Expanding the analysis to brain regions (e.g. CA1 vs SN) we show over two-thousand differentially expressed genes. These data show that the expression profile within microglial sub-populations are the equally significant among brain regions.

Conclusions: It has been known for more than a decade that microglia have the ability to release neurotoxic inflammatory factors. These pro-inflammatory factors or cytokines, have prompted hundreds of studies and clinical trials to suppress their function, but none have been successful to date. Although there are several explanations listed in the literature on why these clinical studies failed to recapitulate in vitro findings, we hypothesize that these studies failed due to the inability to address probable heterogeneity among glial cells as a function of brain region. These findings lay the foundation for future development of therapeutic targets, aid in the pursuit of new research leads, and answer fundamental biological questions regarding the interplay between glial types and their function based on location, and disease.

Acknowledgements: The authors declare no competing financial or conflict of interests. We are grateful to the Banner Sun Health Research Institute Brain and Body Donation Program of Sun City, Arizona for the provision of human brain samples. The Brain and Body Donation Program is supported by the National Institute of Neurological Disorders and Stroke (U24 NS072026 National Brain and Tissue Resource for Parkinson's Disease and Related Disorders), the National Institute on Aging (P30 AG19610 Arizona Alzheimer's Disease Core Center), the Arizona Department of Health Services (contract 211002, Arizona Alzheimer's Research Center), the Arizona Biomedical Research Commission (contracts 4001, 0011, 05-901 and 1001 to the Arizona Parkinson's Disease Consortium) and the Michael J. Fox Foundation for Parkinson's Research ." This work was supported by the Arizona Alzheimer's Research Center NIRG-14-321390 and ADHS14-080000 FY2015 to D.M.

Poster 39

LONG-TERM CYCLIC + TONIC ESTRADIOL IMPROVES, AND CYCLIC ESTRADIOL ALONE IMPAIRS, SPATIAL WORKING MEMORY IN OVARECTOMIZED MIDDLE-AGED FEMALE RATS. Nishimura K, Koebele SV, Kemmou S, Ortiz JB, Judd JM, Bimonte-Nelson H, Conrad CD. Arizona State University; Arizona Alzheimer's Consortium.

Background: There has been much research investigating the influence of estrogens on cognition in female rats. Reports demonstrate that many factors can impact outcomes, including, but not limited to, age of the subject, estrogen duration, mode of estrogen administration, estrogen type, stress history, and presence of progestogens. Age of the subject is particularly important translationally given that women are living longer with nearly a third of their life in a post-menopausal state. Consequently, understanding the factors by which estrogens could contribute to healthy cognitive aging is of great importance. Previous research from our lab has demonstrated that three months of estrogen treatment via tonic pellet implant or cyclic injections can enhance learning and memory in 12-15 month old female rats (Bimonte-Nelson et al., 2006). It is unknown whether a longer-term, further extended exposure to estrogens would benefit cognitive abilities and whether the mode of estrogen administration would influence these outcomes.

Methods: Here, we examined the effects of seven months of 17 β -estradiol (E2) exposure using a regimen of cyclic exposure (bimonthly s.c. injection of 10 μ g E2), or cyclic+tonic exposure (bimonthly s.c. injection of 10 μ g E2 + silastic capsules) on a battery of learning and memory tasks in middle-aged, ovariectomized female Fischer-344-CDF rats. The treatment regimen continued for the duration of the seven-month study. At the end of month six, when rats were 14-15 months old, all groups were tested on a battery of cognitive tests that included the water radial arm maze (WRAM), Morris Water Maze (MWM), visible platform, open field, object placement, and object recognition. WRAM was used to assess spatial working and reference memory and is considered to be a taxing memory task requiring greater cognitive flexibility as trials increase.

Results: On the WRAM and MWM, all groups showed a learning curve and were able to successfully locate the platform(s) by the end of testing. Interestingly, on the WRAM, rats administered a cyclic+tonic dose showed improved working memory performance on trial four of days 10-12 compared to cyclic administration alone. This improvement persisted even after a delay period was implemented on the last day of WRAM testing, suggesting that cyclic+tonic administration facilitated spatial working memory during the highest memory load. Assessment of spatial memory on object placement and object recognition was not possible due to insufficient exploration of objects; however, analysis revealed that cyclic+tonic administration increased total object exploration compared to vehicle-treated animals.

Conclusions: A tonic background of 17 β -estradiol with bi-weekly cyclic 17 β -estradiol administration enhanced memory performance compared to intermittent, bi-weekly 17 β -estradiol administration alone. This suggests that chronic circulating 17 β -estradiol levels are important for spatial learning and memory processes. Pinpointing the parameters of estrogen administration that optimally enhance cognition will provide a critical step towards understanding necessary requirements for matching cognitive and brain health in women across the lifespan.

Poster 40

THE MTOR/P70S6K PATHWAY PLAYS A KEY ROLE IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE. Oddo S, Caccamo A, Branca C, Ferreira E. Arizona State University; Arizona Alzheimer's Consortium.

Background: Aging is the major risk factor for Alzheimer's disease (AD); however, little is known as to how the aging process facilitates the development of AD. Reducing the activity of the mammalian target of rapamycin (mTOR), and its downstream target p70S6K, increases lifespan and health-span in several genetically different species. The goal of this work is to assess the role of the mTOR/p70S6K pathways in cognitive aging and in the pathogenesis of AD.

Methods: We used complementary approaches to modify mTOR signaling in the brains of different animal models of AD. Readout measures were cognitive function and neuropathological assessment.

Results: We will show that genetic and pharmacologic reduction of mTOR and p70S6K signaling reduced amyloid- β and tau pathology and rescued memory deficits. Mechanistically, the reduction in mTOR signaling led to an increase in autophagy induction and restored the hippocampal gene expression signature of the Tg2576 mice to wild type levels.

Conclusions: The data presented here have profound clinical implications for aging and Alzheimer's disease and provide the molecular basis for how aging may contribute to AD pathology.

Poster 41

EFFECTS OF A COMBINED TRANSCRANIAL MAGNETIC STIMULATION (TMS) AND COGNITIVE TRAINING IN ALZHEIMER PATIENTS: RESULTS OF MEDICAL DEVICE PIVOTAL MULTI-CENTER STUDY. Pascual-Leone A, Sadowsky C, Tousi B, Agronin ME, Alva G, Armon C, Bernick C, Keegan AP, Karantzoulis S. Barrow Neurological Institute; Arizona Alzheimer's Consortium.

Background: Noninvasive brain stimulation with trains of repetitive Transcranial Magnetic Stimulation (TMS) can modulate activity in specific cortical brain regions and networks, and thus affect cognitive function. The neuroAD™ Therapy System delivers brain MRI neuro-navigated, focal TMS concurrently with Cognitive Training exercises. The Cognitive Training tasks are designed to engage the cognitive functions of the brain networks targeted by a preceding train of TMS.

Methods: This pivotal, aimed to gain regulatory clearance, randomized, double-blind, sham-controlled clinical trial was designed to evaluate the efficacy and safety of a 6 week course of daily neuroAD™ Therapy in the treatment of cognitive impairment in subjects with mild to moderate Alzheimer's disease (NIA-AA criteria). In a multi-center study at 10 sites, 131 subjects were consented, enrolled and randomized to neuroAD™ Therapy or Sham treatments (placebo). Participants were 60-90 years old, with MMSE scores between 18-26, CDR scores of 1 or 2, and either unmedicated for AD or on stable doses of an acetylcholinesterase inhibitor and / or memantine. Enrolled subjects were randomized to either Active or Sham treatments involving hour long sessions five days / week over six weeks, for a total of 30 sessions. The ADAS-Cog and CGI-C were administered at baseline, at the end of treatment (week 7) and at week 12.

Results: The per patient protocol analysis in the prespecified mild subjects (ADAS<30) showed a significant benefit of 1.8 points favoring treatment over placebo at 12 weeks. When an even milder sub-group was evaluated (ADAS <20), the effect was even larger at 2.3 points. The CGIC favored treatment over placebo (delta=0.45, p=0.07). The AE rate was low.

Conclusions: In this phase III device based randomized treatment trial of rTMS combined with cognitive training (the NeuroAD treatment) we find that the treatment demonstrated an excellent safety profile. In a pre-specified manner, we find efficacy demonstrated for ~85% of study population, specifically patients with milder AD symptoms (Baseline ADAS-Cog \leq 30). Statistically significant benefit on the ADAS favoring treatment over sham was demonstrated at week 12 with an improvement of 1.8 points on the ADAS-cog and -0.45 on the CGIC. Patients continue to improve after treatment sessions completed. The treatment was well tolerated with an extremely high adherence rate and low AEs. These data suggests that TMS combined with cognitive training is effective as a symptomatic treatment for AD with acute to subacute effects demonstrated in relatively short periods of time

Poster 42

INCREASED EXPRESSION OF AZGP1 IN THE MIDDLE TEMPORAL GYRUS OF ALZHEIMER'S DISEASE PATIENTS. Piras IS, Krate J, Brokaw D, Delvaux E, Nolz J, De Both MD, Mastroeni D, Beach TG, Huentelman MJ, Coleman PD. Translational Genomics Research Institute; Arizona State University; Banner Sun Health Research Institute; University of Arizona; Arizona Alzheimer's Consortium.

Background: In order to explore the RNA expression changes occurring in the middle temporal gyrus (MTG) of Alzheimer's Disease patients (AD), we sequenced the whole transcriptome of 8 AD and 8 Non-Demented (ND) controls.

Methods: RNA samples were sequenced using Illumina HiSeq2000, and reads were aligned to the reference genome using STAR. The differential expression analysis was assessed using R-package DESeq2, correcting the p-values with the False Discovery Rate (FDR) method. The data were validated in a larger cohort characterized with microarrays including the 16 samples sequenced (AD = 97; ND = 98), and in a public dataset from the same brain region (GSE5281; AD = 12; ND = 6). Finally, we looked for genetic associations of Single Nucleotide Polymorphisms (SNPs) located in the candidate genes, using the summary statistics from the International Genomics of Alzheimer's Project (IGAP), including 17,002 AD and 37,154 ND.

Results: We detected a total of 119 differentially expressed genes ($\text{adj-p} < 0.05$). After filtering for Fold Change (FC) $> |1.00|$, we obtained 8 overexpressed genes in AD. They included: 5 protein coding (SLC5A11, SERPINA5, HSD11B2, AZGP1, RGR and AEBP1) and 2 lincRNAs (RP3-406A7.7 and RP11-552D4.1) genes. HSD11B2, AZGP1 and AEBP1 showed concordant dysregulation and significance at the genome-wide level in the other two microarray expression datasets, whereas the lincRNAs were not included in the microarray design. Moreover, AEBP1 showed a significant positive correlation with Braak Staging in AD ($p = 2.8E-05$), when considering the entire microarray sample (AD = 97; ND = 98). Finally, in the IGAP cohort we detected 6 nominal significant SNPs located in AZGP1, and 1 nominal SNP in AEBP1 (all $p < 0.05$).

Conclusions: Our results support the association of AZGP1 (Alpha-2-Glycoprotein 1, Zinc-Binding) with AD. Recently, the expression of the same protein was detected for the first time downregulated in the serum of Chinese AD patients.

AEBP1 was also recently associated in the hippocampus of AD patients. The finding for HSD11B2 is concordant with the genetic association detected for the functionally related gene HSD11B1. Finally, we reported for the first time the overexpression in AD of the two lincRNAs RP3-406A7.7 and RP11-552D4.1.

Poster 43

REGION- AND AGE-SPECIFIC EFFECTS OF APOE4 ON MURINE MICROGLIAL PHENOTYPE. *Potter RM, *Jones TB, Jentarra G, Vallejo J. Midwestern University; Arizona Alzheimer's Consortium.

*Indicates shared first authorship.

Background: The apolipoprotein E ϵ 4 (APOE4) allele is the most significant risk factor for late-onset Alzheimer's disease (AD). ApoE is known to influence microglial activation state, and the APOE4 allele is associated with promotion of a neurotoxic phenotype in microglia and may contribute to the development of an inflamed CNS environment observed in AD. Activated microglia present diverse functional states ranging from neurotoxic (i.e., M1, pro-inflammatory) to neuroprotective (i.e., M2, reparative); however, the microglial functional profile during AD has not been characterized. Recent work by our group demonstrated that APOE4 genotype modifies inflammatory signaling at the mRNA level in a distinct manner in the mouse cortex vs the hippocampus and that the effects of APOE4 on microglial phenotype (M1 vs M2) are more pronounced in the hippocampus as mice age. However, it has been difficult to dissect macrophage phenotypes in vivo due to the low or poor-selective capacity of the canonical protein markers for M1 and M2 macrophages (Nos-2 and Arginase-1, respectively). CD38 and early growth response protein 2 (Egr2) have recently been identified as novel and improved M1- and M2-exclusive markers, respectively. We evaluated the effects of APOE4 expression on microglial functional profile in APOE3 (E3) and APOE4 (E4) targeted replacement (TR) mice by quantification of the relative protein expression of canonical and novel M1 and M2 protein markers within the mouse cortex and hippocampus at different ages (young, adult, and old age). We hypothesized that the cortex and hippocampus of E4 mice exhibit a pro-inflammatory bias that would increase as the mice age consistent with progression of inflammation in AD brain.

Methods: Brains from E4 and E3 TR mice were removed at young age (4-5 months) adult age (6-9 months), and old age (10-12 months). The cortex and hippocampus were dissected and snap frozen for Western blot (WB) analyses. Data were analyzed using the Odyssey imaging system and supportive software.

Results: In the cortex of young E4 mice, the canonical M1 marker iNOS was lower than in the cortex of young E3 mice. As the mice aged, cortical iNOS remained unchanged in E3 mice, while levels in E4 mice increased with age to become equivalent to E3. Interestingly, hippocampal iNOS decreased with age in E3 mice, while it remained unchanged in E4 mice. The expression pattern of the novel M1 marker CD38 differed slightly in the E3 cortex decreasing over time and E4 not changing. In the hippocampus, CD38 levels decreased slightly in E3 mice with overall lower levels in E4 mice regardless of age. The canonical M2 marker Arg1 seemed to increase in E4 mice in the hippocampus as they age with no other changes observed. The novel M2 marker EGR2 appeared much more stable and generally showed no effects of age with similar expression levels across genotypes.

Conclusions: We previously demonstrated that the APOE4 genotype modifies inflammatory signaling at the mRNA level in a distinct manner in the cortex vs the hippocampus and that the effects of APOE4 on microglial phenotype are more pronounced in the hippocampus as the mice age. Here, we demonstrate that the corresponding protein changes are much more variable than we observed for gene changes and that CD38 and EGR2 data are in general agreement with canonical M1/M2 markers thus serving as suitable markers for evaluating changes in microglial phenotype.

Poster 44

CHEMICAL AND NEUROPATHOLOGICAL ANALYSES OF AN ALZHEIMER'S DISEASE PATIENT TREATED WITH SOLANEZUMAB. Roher AE, Maarouf CL, Kokjohn TA, Belden C, Serrano G, Sabbagh MS, Beach TG. Midwestern University; Barrow Neurological Institute; Banner Sun Health Research Institute; Arizona Alzheimer's Consortium.

Background: Based on the amyloid cascade hypothesis of Alzheimer's disease (AD) pathogenesis, a series of clinical trials involving immunotherapies have been undertaken including infusion with the IgG1 monoclonal anti-A β antibody solanezumab directed against the middle of the soluble A β peptide. In this report, we give an account of the clinical history, psychometric testing, gross and microscopic neuropathology as well as immunochemical quantitation of soluble and insoluble A β peptides and other proteins of interest related to AD pathophysiology in a patient treated with solanezumab.

Methods: The solanezumab-treated AD case (SOLA-AD) was compared to non-demented control (NDC, n = 5) and non-immunized AD (NI-AD, n = 5) subjects. Brain sections were stained with H&E, Thioflavine-S, Campbell-Switzer and Gallyas methods. ELISA and Western blots were used for quantification of proteins of interest.

Results: The SOLA-AD subject's neuropathology and biochemistry differed sharply from the NDC and NI-AD groups. The SOLA-AD case had copious numbers of amyloid laden blood vessels in all areas of the cerebral cortex, from leptomeningeal perforating arteries to arteriolar deposits which attained the cerebral amyloid angiopathy (CAA) maximum score of 12. In contrast, the maximum CAA for the NI-AD cases averaged a total of 3.6, while the NDC cases only reached 0.75. The SOLA-AD subject had 4.4-fold more soluble A β 40 and 5.6-fold more insoluble A β 40 in the frontal lobe compared to NI-AD cases. In the temporal lobe of the SOLA-AD case, the soluble A β 40 was 80-fold increased, and the insoluble A β 40 was 13-fold more abundant compared to the non-immunized AD cases. Both soluble and insoluble A β 42 levels were not dramatically different between the SOLA-AD and NI-AD cohort.

Conclusions: Solanezumab immunotherapy provided no apparent relief in the clinical evolution of dementia in this particular AD patient, since there was a continuous cognitive deterioration and full expression of amyloid deposition and neuropathology.

Poster 45

AGING WITH TRAUMATIC BRAIN INJURY: AGE-AT-INJURY EFFECTS ON BEHAVIORAL MORBIDITIES AND UNDERLYING NEUROVASCULAR PATHOLOGY. Rowe RK, Ziebell JM, Morrison H, Vickers J, Lifshitz J. University of Arizona College of Medicine-Phoenix; Barrow Neurological Institute at Phoenix Children's Hospital; Phoenix VA Healthcare System; University of Tasmania; University of Arizona; Arizona Alzheimer's Consortium.

Background: Traumatic brain injury (TBI) is more than a singular event, such that a disease state ensues with functional morbidities developing and persisting over an extended timeframe following the initial insult. Recovery from injury and the extent of morbidities may depend on many parameters, including the biological age at the time of injury. A vulnerable population of TBI survivors includes children and adolescents whose on-going developmental processes are disrupted by TBI pathophysiology. Throughout “normal” aging there is an increase in the inflammatory status of the brain and homeostatic processes become unregulated. A similar activated inflammatory status is evident following TBI and this sustained inflammatory state results in disruption to neurovascular units, including microglia, that contribute to injury-induced behavioral morbidities. Given the similarities between the inflammatory profile in the aged brain and the pro-inflammatory environment initiated by TBI, we hypothesize that TBI accelerates pathological hallmarks of aging in the brain.

Methods: A single cohort of male Sprague-Dawley rats (n=69) was received at post-natal day (PND)10 and subgroups of this cohort (n=11-12/group) were subjected to a single moderate midline fluid percussion injury (mFPI) at age PND17, PND35, 2mo, 4mo, or 6mo or were naïve. Rats were assessed for motor function, anxiety-like behavior, cognitive performance and depression-like behavior across their lifespan. Tissue was collected at 10mo for histopathological analyses. A skeleton analysis method was used to quantify microglia morphologies in Iba-1 stained cortical tissue.

Results: TBI resulted in vestibulomotor deficits on a beam walk. Brain-injured rats committed significantly more foot-faults compared to naïve at 1.5, 3mo, and 5mo. TBI resulted in age-at-injury dependent anxiety at 8mo assessed by the open field task. Rats injured at 2mo, 4mo, and 6mo spent significantly less time in the center of the arena compared to naïve. TBI also resulted in age-at-injury dependent spatial memory deficits assessed using a novel location task. There was an overall effect on the average duration of visits to the novel location at 8mo and 9mo and rats injured at PND17 and PND35 had significantly shorter visits to the novel location compared to naïve. Lastly, TBI did not result in depressive-like behavior at 9mo assessed by forced swim task measured as the amount of time spent trying to escape or total number of escape attempts. Microglia were analyzed for changes in ramified morphologies. There was no overall difference in number of microglia endpoints per cell or process length per cell among treatment groups at 10 mo of age.

Conclusions: Overall, these data support TBI can negatively impact neurological function during discrete stages of development and aging. The interplay of age-at-injury and aging with an injury are translationally important factors that influence behavioral performance as a quality of life metric. More complete understanding of these factors can direct rehabilitative efforts and personalized medicine for TBI survivors.

Poster 46

EXPECTATION OF LARGE REWARDS ELICITS BURSTS OF BETA-BAND OSCILLATIONS IN THE AGED RAT AMYGDALA. Samson RD, Duarte L, Barnes CA.
University of Arizona; Arizona Alzheimer's Consortium.

Background: With aging, older adults tend to use strategies that differ from those used by young adults to solve decision making tasks. This is often accompanied by the recruitment of larger brain areas, inter-hemispheric bilateralization or added brain structures, which can be interpreted as compensatory mechanisms for less effective brain networks. It has been suggested that this process is facilitated through synchronized oscillations that occur between distant brain areas, presumably enabling connections that allow more optimal performance. Because the aging process is known to alter circuit properties that may impact brain oscillations, the present study examined how network changes in the basolateral complex of the amygdala (BLA), known to support reward-based decision making, may be altered in aged rats.

Methods: To examine this problem, we trained young and old rats to perform three different versions of a decision making task. Two of the tasks were versions of discrimination problems in which either the reward magnitude (reward magnitude discrimination) or the probability of receiving a reward (probability discrimination) was manipulated. The third task version was a probability discounting task in which rats had a choice between a small/certain reward and a large/uncertain reward (probability discounting).

Results: In the BLA of old, but not young rats, we found task-specific increased oscillatory power in the beta range (15-30Hz) after lever presses as the animals reached the goal location. Periods of high-power beta were minimal at first, but developed over training days in the aged rats. Within a daily session, the incidence of beta epochs was greater for the early trials and less evident by the end of the session. Both the incidence and power of beta epochs were affected by tasks that involved differing reward magnitudes. Indeed, beta power was significantly greater after pressing for the large reward option.

Conclusions: Thus, our results suggest that aging impacts BLA networks in a way that promotes the emergence of beta band activity when learning or deciding between differently sized rewards. Furthermore, we found a correlation between beta incidence and how often the small/certain reward was selected in a session, for both the reward magnitude discrimination and probability discounting tasks. Thus, increased beta oscillations in the BLA of aged rats may reflect compensatory mechanisms which promote a more exploratory type strategy to solve certain reward-based decision making tasks.

Poster 47

USING CLINICAL NEUROPSYCHOLOGICAL ASSESSMENTS TO PREDICT MOTOR REHABILITATIVE RESPONSIVENESS IN NON-DEMENTED OLDER ADULTS. Schaefer SY, VanGilder JL, Hengge CR, Duff K. Arizona State University; Creighton University; University of Utah; Arizona Alzheimer's Consortium.

Background: Nearly 50% of physical rehabilitation patients in the United States are over age 65. While older adults are commonly seen, they appear to be less responsive to rehabilitative treatments designed to improve motor function compared to younger patients with the same diagnosis. Our working hypothesis is that this reduced responsiveness to motor rehabilitation may, in part, be linked to underlying cognitive impairments due to aging. We have recently shown that the extent to which patients diagnosed with amnesic Mild Cognitive Impairment exhibit practice effects on a functional motor task is related to their performance on a battery of visuospatial tests. The purpose of this study was to test whether these findings generalized to a sample of age-matched older adults with no memory impairment or amnesic symptoms.

Methods: Twenty-five adults age 65 years and older with no known neurological or psychological diagnoses participated in this study. All participants were assessed with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), from which we extracted age-adjusted scores for five cognitive domains. Participants' baseline performance on a motor task designed to improve upper extremity motor function was also measured prior to a short (9-trial) practice session; motor performance was re-assessed one week later. Motor performance was quantified as the time to complete a given trial of the task, with faster times indicating better performance. These protocols are identical to those from our previous study in amnesic Mild Cognitive Impairment (Schaefer and Duff, 2016).

Results: Stepwise regression indicated that the visuospatial/constructional index of the RBANS was the only cognitive domain that significantly predicted one-week follow-up motor performance ($p=0.04$) once baseline performance was accounted for. The visuospatial/constructional index is comprised of a judgement of line orientation test and a complex figure copying test to yield a single age-adjusted index score. Further regression analysis indicated that in this small sample, raw unadjusted line orientation scores were more related to the amount of improvement at one week ($p=0.02$) than figure copy scores were ($p=0.12$).

Conclusions: These findings are consistent with our previous work showing that visuospatial tests may be used prognostically to identify older patients' responsiveness to motor rehabilitation, irrespective of any other underlying cognitive impairments. We are now currently exploring the underlying neural mechanism of why visuospatial tests appear to uniquely capture the aging brain's motor learning capacity.

Poster 48

SENSITIVITY AND SPECIFICITY OF THE MAYO SLEEP QUESTIONNAIRE IN PREDICTING ALPHA-SYNUCLEIN PATHOLOGY. Shprecher DR, Zhang N, Hentz JG, Dugger BN, Adler CH, Shill HA, Caviness JN, Driver-Dunckley E, Mehta SH, Sabbagh MN, Belden CM, Savica R, Serrano GE, Zamrini E, Sue LI, Beach TG. Banner Sun Health Research Institute; Mayo Clinic College of Medicine; University of California San Francisco; Barrow Neurological Institute; Arizona Alzheimer's Consortium.

Background: Idiopathic REM sleep behavior disorder (iRBD), a condition characterized by violent dream enactment behavior, is associated with an 81-91% lifetime risk for subsequent development of neurodegenerative disease, primarily alpha-synucleinopathy (AS.) The Mayo Sleep Questionnaire (MSQ) has been reported to provide 100% sensitivity and 95% specificity to determine the presence of polysomnogram-confirmed RBD, when completed with input from an informant (such a spouse.) The aim of this study was to determine the sensitivity and specificity of MSQ-ascertained probable RBD (pRBD) in predicting the histological presence of AS.

Methods: Since 2007, 244 subjects in the Arizona Study of Aging and Neurodegenerative Disorders had an informant available to complete the MSQ, completed cognitive and movement examinations, and have come to autopsy. Final clinicopathological diagnoses were assigned. Multiple diagnoses [for example Parkinson disease (PD) and Alzheimer disease (AD)] were allowed.

Results: Mean age at death was 84 years (SD 8). Histological evidence of alpha-synucleinopathy (AS) was found in 54/70 (77%) cases who had pRBD and 70/174 (40%) without pRBD ($p < 0.001$); sensitivity for predicting alpha-synucleinopathy by pRBD was 43.6%% and specificity 86.7%.

The MSQ indicated that pRBD was present in the following overlapping groups: 2/42 healthy controls, 7/43 (16.3%) of AD without Lewy body (LB) pathology, 24/58 (41.4%) of AD with PD, DLB or any other AS, 8/22 (36.4%) DLB, 37/55 (67.3%) PD, 8/27 (26.6%) cases with LB pathology not otherwise specified, 0/19 incidental Lewy body disease, 3/22 (13.6%) vascular dementia, 6/17 (35.3%) progressive supranuclear palsy, and 1/3 (33.3%) corticobasal degeneration, and 1/1 MSA case.

Conclusions: Informant completed MSQ appears to be useful for predicting alpha-synucleinopathy. However, MSQ reported pRBD may be relatively common in other neurodegenerative disorders, such as AD and PSP without co-existing alpha-synuclein pathology. A prospective population-based study of polysomnogram--confirmed idiopathic RBD cases followed to autopsy is needed to generalize these findings beyond a research center setting.

Poster 49

DEVELOPMENT OF ADVANCED MULTIPARAMETRIC MRI SIGNATURES OF ALZHEIMER'S DISEASE. Stokes AM, Baxter LC, McGee S, Sabbagh MN, Caselli RJ, Quarles CC. Barrow Neurological Institute; Mayo Clinic Scottsdale; Arizona Alzheimer's Consortium.

Background: The goal of this project is to establish advanced MR imaging signatures that are phenotypical of each stage of AD, from no cognitive impairment to MCI to clinically diagnosed AD. These advanced imaging methods will allow us to non-invasively investigate the underlying neurobiological changes that precede the cognitive impairments characteristic of later stages of AD. While structural MRI is known to change with later disease progression, advanced MR imaging of blood volume and flow, molecular species, and iron deposition may provide specific signatures of disease progression. Each metric was chosen for its sensitivity to a known abnormality related to AD. We hypothesize that the corresponding vascular or molecular changes could be an early indicator of incipient MCI or AD, prior to morphological changes.

Methods: Three subject groups are being recruited for this study (no cognitive impairment, MCI, and AD; n = 12 per group). All subjects undergo cognitive testing using the Montreal Cognitive Assessment (MoCA), functional assessment staging (FAST), and clock draw immediately prior to the MRI. MRI data is acquired at the Keller Center for Imaging Innovation (BNI, 3T Philips MRI). Structural MRI data is obtained using ADNI (Alzheimer's Disease Neuroimaging Initiative) protocols, and structural parcellation is performed using FreeSurfer software. Advanced MRI methods are used to measure microvascular perfusion fraction (using intravoxel incoherent diffusion), blood flow (using arterial spin labeling (ASL)), molecular species (using chemical exchange saturation transfer (CEST)), and iron deposition (using susceptibility-weighted imaging (SWI)). Regional metrics will be compared using an unpaired t-test.

Results: Preliminary imaging results show excellent image quality and advanced metrics within the expected physiological limits. Data collection is ongoing, and current study results will be presented at the meeting.

Conclusions: This study lays the framework for the development of advanced multi-parametric MRI to characterize the neuropathological changes that occur in Alzheimer's disease. By characterizing early imaging signatures, we hope to develop imaging signatures that are early indicators of incipient MCI or AD.

Poster 50

DEVELOPMENT OF PRECLINICAL MRI BIOMARKERS IN MOUSE MODELS OF ALZHEIMER'S DISEASE. Stokes AM, Velazquez R, Oddo S, Quarles CC. Barrow Neurological Institute; Arizona State University; Arizona Alzheimer's Consortium.

Background: The overall goal of this project is to identify structural, metabolic and molecular neuroimaging signatures of Alzheimer's Disease (AD) in mouse models that recapitulate human pathophysiology. Our multi-parametric approach has the potential to shed new insights into AD progression, inform clinical interpretation of biomarker analysis, and establishes the foundation for the use of multiparametric MRI biomarkers in pre-clinical drug development.

Methods: Four groups of mice (wild type (n = 4), S6K1+/- (n = 6), 3xTg-AD/S6K1+/- (n = 6), and 3xTg-AD (n = 5)) were imaged using multiparametric MRI (Bruker, 7T). High-resolution MR images were acquired to quantify volumetric changes in the brain, including the whole brain, hippocampus, cortex, total ventricles, and caudate putamen. Cerebral blood flow (CBF) was quantified using an arterial spin labeling method, while molecular and metabolic species were probed using a chemical exchange saturation transfer (CEST) method. Diffusion tensor imaging (DTI) data were acquired to quantify microstructural white matter changes. Within 1 week of imaging, the mice were sacrificed and brains extracted for genetic and histological analysis.

Results: Volumetric analysis showed significant regional differences between the groups, particularly for hippocampus, caudate-putamen, and cortex. The 3xTg-AD group had significantly lower cortical volume compared to the 3xTg-AD/S6K1+/- group, which supports the improved cognition previously observed in the latter group. CBF, a marker of vascularity, was significantly reduced in the corpus callosum for the 3xTg-AD group compared to the 3xTg-AD/S6K1+/- group, which may indicate that upregulation of S6K1 leads to hyperperfusion in the pathogenesis of AD. Myo-inositol, indicative of glial proliferation, was probed using CEST and showed significant differences in the hippocampus between the 3xTg-AD and 3xTg-AD/S6K1+/- groups. Compared to the wild-type group, DTI showed significantly reduced fractional anisotropy in the hippocampus and caudate-putamen for the 3xTg-AD group, while axial diffusivity was significantly reduced in the hippocampus, corpus callosum, and caudate-putamen for the 3xTg-AD group.

Conclusions: The pathophysiological phenotype in these mouse models of AD corresponds to brain volume atrophy, metabolic changes, decreased axial diffusivity, and hyperperfusion using multiparametric MRI. These results suggest that multiparametric MRI can increase sensitivity to early AD-induced changes in the 3xTg-AD/S6K1+/- and 3xTg-AD mouse models. This work represents the first in vivo imaging characterization of these novel mouse models of AD.

Poster 51

LORAZEPAM CHALLENGE FOR INDIVIDUALS AT VARYING GENETIC RISK FOR ALZHEIMER'S DISEASE. Stonnington CM, Harel B, Locke DEC, Hentz JG, Zhang N, Maruff P, Caselli RJ. Mayo Clinic Arizona; Cogstate, Ltd.; Arizona Alzheimer's Consortium.

Background: This study set out to clarify the differential acute cognitive impact of lorazepam based on varying genetic risk for Alzheimer's disease.

Methods: Fifty-seven cognitively unimpaired individuals aged 51 to 88, genotyped according to apolipoprotein E (APOE) and translocase of outer mitochondrial membrane (TOMM40) poly-T lengths, completed cognitive testing before, 2.5 and 5 hours after receiving a 1 mg dose of lorazepam.

Results: Post-lorazepam, there were significant ($P < 0.05$) declines from baseline in memory, psychomotor processing speed, and executive function. At 2.5 hours, the magnitude of this lorazepam-induced cognitive change was significantly greater in the APOE3/4 group than in the APOE3/3 group for tests of working memory and visuospatial memory/executive function. At 5 hours post-challenge, verbal memory and working memory deficits persisted in the APOE3/4 group compared to the APOE3/3 group. At 5 hours after lorazepam challenge, as compared to the VL/VL group, the S/S group performed slightly worse on a test of working memory ($P < 0.05$), but no other differences were observed among TOMM40 poly-T variant groups.

Conclusions: The lorazepam challenge may be unmasking pre-symptomatic cognitive dysfunction associated with APOE4 carriage.

Poster 52

ELUCIDATING TAU'S INVOLVEMENT IN LEARNING AND MEMORY DURING ADULTHOOD USING AN INDUCIBLE TAU SHRNA. Velazquez R, Tran A, Oddo S. Arizona State University; Arizona Alzheimer's Consortium.

Background: Misfolded and hyperphosphorylated tau accumulates in several neurodegenerative disorders, which are collectively known as tauopathies. These disorders include, among others, Alzheimer's disease (AD), frontotemporal dementia with Parkinsonism, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Down syndrome, and Pick's disease. Growing evidence indicates that tau-mediated neuropathology may occur by both toxic gain-of-function and by loss-of-function. Additionally, anti-tau therapies are quickly approaching clinical trials. While a wealth of data is available on the role of tau in the formation of toxic inclusions, less is known about its function in the adult brain. To this end, knockout models of Tau show minimal behavioral deficits and a developmental compensation by the microtubule-associated protein 1A (MAP1A), which can complicate interpretations on Tau's role in adulthood. In order to fully understand Tau's involvement in learning and memory in the adult brain, manipulations need to be done solely in adult mice to remove any developmental compensations. Here, we validated an adeno associated virus (AAV) expressing inducible TauShRNA and will leverage it to determine Tau's involvement in learning and memory.

Methods: We have generated an AAV expressing CamKII-TetON-Tet/U6-Tau-ShRNA (TauShRNA). To confirm the TauShRNA can reduce Tau levels in vivo, we designed a series of preliminary experiments where sixteen C57Bl6 mice were stereotaxically injected with the TauShRNA into one hemisphere (CA1 of the hippocampus) while the other hemisphere served as an internal control. Twelve mice were put on Doxycycline (Doxy), thus inducing expression of the TauShRNA, seven days after the surgery. Mice were then sacrificed at different time points following Doxy induction. The remaining 4 mice were never put on Doxy to control for leakage of the TauShRNA. To determine the effects of reducing tau on learning and memory, we injected 8-month-old C57Bl6 mice bilaterally with either TauShRNA or a Tau Scramble (TauScrm; served as a control), into the CA1 region of the hippocampus. All animals were given a seven day recovery period, then put on Doxy to induce expression of the shRNA. Mice were tested in a battery of cognitive and non-cognitive tests, including the open field, Rota rod, and Morris water maze.

Results: Our data show that the TauShRNA reduces Tau levels by ~50% at seven, 14 and 30 days post induction, when compared to the control hemisphere. Additionally, mice injected with the TauShRNA that were not put on Doxy did not show reductions of Tau illustrating no leakage of the construct. We will also show the effects of reducing tau on learning and memory.

Conclusions: Our results show that the TauShRNA reduces Tau levels in vivo. The results of our one-month Tau reduction study will provide insight into the role of Tau in learning and memory. This system has the potential to make a major impact on AD and other tauopathies as, for the first time, we will be able to directly address important questions regarding the role of tau in adult brains.

Poster 53

CHARACTERIZATION OF RNA ISOLATED FROM EIGHTEEN DIFFERENT HUMAN TISSUES: RESULTS FROM A RAPID HUMAN AUTOPSY PROGRAM.

Walker DG, Whetzel AM, Serrano G, Sue LI, Lue LF, Beach TG. Arizona State University; Banner Sun Health Research Institute; Arizona Alzheimer's Consortium.

Background: Many factors affect the integrity of messenger RNA from human autopsy tissues including postmortem interval (PMI) between death and tissue preservation and the pre-mortem agonal and disease states. In this communication, we describe RNA isolation and characterization of 389 samples from 18 different tissues from elderly donors who were participants in a rapid whole-body autopsy program located in Sun City, Arizona.

Methods: Most tissues in the program are collected within a PMI of 2-6 h (median 3.15 h; N = 455), but for this study, tissue from cases with longer PMIs (1.25-29.25 h) were included. RNA quality was assessed by RNA integrity number (RIN) and total yield (ng RNA/mg tissue).

Results: RIN correlated with PMI for heart ($r = -0.531$, $p = 0.009$) and liver ($r = -0.558$, $p = 0.0017$), while RNA yield correlated with PMI for colon ($r = -0.485$, $p = 0.016$) and skin ($r = -0.460$, $p = 0.031$). RNAs with the lowest integrity were from skin and cervix where 22.7 and 31.4 % of samples respectively failed to produce intact RNA; by contrast all samples from esophagus, lymph node, jejunum, lung, stomach, submandibular gland and kidney produced RNA with measurable RINs.

Expression levels in heart RNA of 4 common housekeeping normalization genes showed significant correlations of Ct values with RIN, but only one gene, glyceraldehyde-3 phosphate dehydrogenase, showed a correlation of Ct with PMI. There were no correlations between RIN values obtained for liver, adrenal, cervix, esophagus and lymph node and those obtained from corresponding brain samples.

Conclusions: We show that high quality RNA can be produced from most human autopsy tissues, though with significant differences between tissues and donors. The RNA stability and yield did not depend solely on PMI; other undetermined factors are involved, but these do not include the age of the donor.

Poster 54

IMPACT OF APOE GENOTYPE ON THE SEX-DIFFERENTIATED BIOENERGETIC TRAJECTORIES AND AD RISKS IN AGING MOUSE BRAINS. Yin F, Wang Y, Mishra A, Mao J, Brinton RD. University of Arizona; University of Southern California; Arizona Alzheimer's Consortium.

Background: Age, APOE4 genotype, and female sex are top risk factors for AD. Our previous studies demonstrated that the bioenergetic shifts occurring in the perimenopausal female brain could contribute to an increased AD risk in women. We also observed substantial sex disparities in brain bioenergetic trajectories in normal aging mice. The goal of this study is to determine the sex differences in the impact of APOE genotype on AD at-risk phenotypes during brain aging.

Methods: Female and male, APOE3- and APOE4 targeted replacement (TR) mice were assessed for: (a) bioenergetic-, inflammatory-, and AD pathology-related gene expression in hippocampus followed by bioinformatic analysis, and (b) key metabolic parameters in the periphery. Functional assessments are undergoing, including: (a) FDG-microPET for cerebral glucose metabolism, (b) brain mitochondrial function, and (c) glucose tolerance test for peripheral metabolism.

Results: Gene expression analyses revealed that female APOE4 mice at pre-menopause age (6-month old) exhibited a significantly different bioenergetic profile relative to APOE3 controls: APOE4 mice showed a higher expression of genes involved in mitochondrial oxidative phosphorylation, mitochondrial membrane transport, and mitochondrial fusion. These changes in female APOE4 brains could represent an adaptive response to deficits in brain glucose availability and a shift in energy fuels from glucose to ketone bodies and glucose. This hypothesis is further supported by: (a) APOE4 mice had lower levels of plasma glucose in both sexes while the higher levels of ketone bodies and triglycerides were only seen in the females; (b) both APOE3 and APOE4 females had lower peripheral glucose levels and higher ketone body levels than their genotype-matched male counterparts, indicating a potential earlier shift in bioenergetic fuel usage in females.

Conclusions: These findings suggest that APOE4 genotype interacts with the age-related decline in glucose metabolism and the bioenergetic shift to alternative fuels, particularly in females. Outcomes of this study will provide mechanistic details of the APOE4 genetic burden on the sex-differentiated bioenergetic fluctuation during aging and its contribution to higher AD risks in women. This work was supported by NIA 5P01AG026572 to RDB and Alzheimer's Association SAGA-17-419459 to RDB.

Poster 55

AMYLOID-BETA INCREASES TOTAL TAU BY MEDIATING SIRTUIN 3 IN ALZHEIMER'S DISEASE. Yin J, Han P, Beach TG, Serrano GE, Song M, Nielsen M, Liang WS, Caselli RJ, Shi J. Barrow Neurological Institute; St. Joseph Hospital and Medical Center; Banner Sun Health Research Institute; University of Pennsylvania; Translational Genomics Research Institute; Mayo Clinic Arizona; Tianjin Medical University General Hospital; Arizona Alzheimer's Consortium.

Background: Increasing evidence indicates that Sirtuin 3 (Sirt3) has neuroprotective effects in regulating oxidative stress and energy metabolism, both of which are involved in the pathogenesis of Alzheimer's disease (AD). However, it is unclear whether Sirt3 is associated with cognitive performance and pathological changes in AD.

Methods: We conducted a case-control study of the brains of late-onset AD, mild cognitive impairment and age matched cognitively normal (CN) subjects. We investigated Sirt3 expression, its association with cognitive performance and AD pathology.

Results: Sirt3 levels were reduced in the entorhinal cortex, the middle temporal gyrus and the superior frontal gyrus of AD subjects compared to CN. This reduction was associated with poorer cognitive test scores and the severity of tau pathology. Further study revealed that amyloid-beta increased total Tau protein expression via regulating Sirt3.

Conclusions: These data suggest that down-regulation of Sirt3 is critically involved in pathogenesis of AD.

Poster 56

PATCH-BASED SPARSE CODING AND MULTIVARIATE SURFACE MORPHOMETRY FOR PREDICTING PROGRESSION TO CLINICAL STAGES OF ALZHEIMER'S DISEASE. Zhang J, Wang Y, Li Q, Shi J, Bauer III RJ, Reiman EM, Caselli RJ, Chen K, Stonnington CM. Arizona State University; Banner Alzheimer's Institute; Mayo Clinic Arizona; Arizona Alzheimer's Consortium.

Background: While brain imaging measurements can help characterize the preclinical stages of Alzheimer's disease (AD), their prognostic value remains to be clarified. When combined with effective preclinical treatment, an accurate brain imaging based AD prognosis system could have enormous public health benefits. In our recent work, we developed surface multivariate tensor-based morphometry (mTBM) and grey matter morphology signatures to study important structural MRI AD biomarkers, including hippocampal morphology, lateral ventricular morphology and cortical thickness. Most recently, we developed patch-based sparse coding algorithms to successfully extract most discriminating features from MR images and achieved promising results to predict cognitive decline in initially cognitively unimpaired individuals longitudinally followed in the ADNI study. We also previously reported similar results from the Arizona longitudinal study of aging, but we did not account for APOE gene dose in either of the prior studies.

Methods: From the Arizona APOE cohort, a longitudinal study of cognitively unimpaired persons with two, one or no copies of the apolipoprotein E (APOE4) allele with a reported first degree family history of possible AD dementia, we examined volumetric MRI data from 18 adults who progressed to the clinical diagnosis of amnesic Mild Cognitive Impairment (aMCI) or dementia due to probable AD within 1.8 ± 0.8 years, compared to 20 adults who remained cognitively unimpaired for at least 4 years. The progressor and non-progressor groups were 68 ± 4 years-old at time of baseline scan. Groups were matched for their age, sex, education, and APOE4 gene dose. We segmented each baseline MRI scan with FSL (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki>), parameterized the hippocampal and ventricle surfaces as described previously, and generated the surface multivariate morphometry statistics (MMS) consisting of mTBM and radial distance (RD). We constructed a collection of overlapping patches on the surface as the initial sparse coding dictionary. Stochastic Coordinate Coding was then applied to learn a dictionary and sparse codes. We used the max-pooling algorithm on the newly learned high-dimensional features to obtain a final set of low-dimensional features. Finally, an AdaBoost classifier was applied to categorize aMCI and cognitively unimpaired individuals with 5-fold leave-one-out cross validation adopted to evaluate classification accuracy, sensitivity, specificity, positive and negative predictive values.

Results: The prediction result of aMCI was achieved with hippocampal surface MMS features with 100% accuracy, 100% sensitivity, 100% specificity, 100% positive and 100% negative predictive values.

Conclusions: While our findings should be considered preliminary, sparse coding together with the surface multivariate morphometry may be applied to individual volumetric MRIs to predict imminent progression from the preclinical to clinical stages of AD with great accuracy.

STUDENT POSTER PRESENTATIONS

Poster 57

AGE-RELATED ATTENTIONAL CONTROL AND SET SHIFTING IMPAIRMENTS ARISE INDEPENDENTLY IN MACAQUE MONKEYS. Andersh KM, Gray DT, Smith AC, Burke SN, Gazzaley A, Barnes CA. University of Arizona; University of Florida; University of California, San Francisco; Arizona Alzheimer's Consortium.

Background: Goal-directed behaviors provide the behavioral flexibility necessary for selecting appropriate responses when similar stimuli are encountered in different contexts. The cognitive processes that provide this flexibility have been collectively described under the category of executive functions. Executive function can be segregated into at least 3 separate components: inhibition of prepotent responses, set shifting, and attentional control and monitoring. In humans, partially non-overlapping neural networks in the prefrontal cortex underlie the different components of the executive function network. For example, orbitofrontal and striatal networks have been shown to underlie set shifting, while dorsolateral prefrontal and medial prefrontal networks have been shown to underlie attentional control processes. At the behavioral level, age-related impairments in inhibition, set shifting, and attentional control arise independently of one another, suggesting that the separate neural networks that underlie these behaviors are also altered independently with age.

Methods: To test whether different executive functions are similarly affected independently in the macaque, young (n = 6) and aged (n = 7) monkeys were tested on a set shifting and attentional control task in a Wisconsin general testing apparatus.

Results: The results show that aged monkeys were deficient on both tasks, but the impairment scores between the two paradigms did not correlate, suggesting that set shifting-impaired animals were not necessarily impaired on the attentional control task and vice versa.

Conclusions: These results suggest that, like in humans, different components of executive function in aged monkeys are impacted by normative aging independently. Furthermore, these data argue against the suggestion that the age-related deficits in attentional control seen in aged humans arise due to differences in exposure to technology, relative to young, which may negatively impact their ability to perform computerized tasks. All monkeys in the current study were exposed equally to all aspects of the task environment, suggesting that the detrimental effects seen in the aged individuals are in fact due to differences in attentional control processes.

Poster 58

IMPROVED ASSOCIATIONS BETWEEN REDUCED CEREBRAL GLUCOSE METABOLISM AND ELEVATED AB DEPOSITS WITH THE USE OF A CEREBRAL WHITE MATTER REFERENCE REGION. Ausdemore J, Bi R, Kramer H, Jing N, Chen Y, Kuang X, Luo J, Cary Savage, Reiman EM, Chen K. Desert Mountain High School; Brophy College Preparatory; University of California Berkeley; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Introduction: To reduce the observed variability in florbetapir PET measured longitudinal changes of fibrillary beta-amyloid ($A\beta$) quantified as cortical standard uptake value ratio (SUVR) with cerebellar reference region ($SUVR_{cereb}$), we and others recently introduced cerebral core white matter as an alternative reference region for cortical SUVR ($SUVR_{cwm}$). To characterize the reduction of cerebral metabolic rate for glucose (CMRgl) cross-sectionally and longitudinally, we developed separately the hypometabolic convergence index (HCI) and statistical region of interest (sROI) approaches. While HCI measures the degree of similarity of reduced CMRgl in an individual compared to a typical AD patient, sROI tracks CMRgl alterations in brain regions associated with longitudinal change. Based on the previous finding that decline in cerebral glucose metabolism and increases in beta-amyloid load are associated in patients with Alzheimer's disease (AD), we examined the same association in patients with mild cognitive impairment (MCI) and in cognitively unimpaired normal controls (NCs). Furthermore, we compared the associations for progressors within the MCI and NC groups separately. We hypothesized a stronger association of FDG-PET HCI or sROI with $SUVR_{cwm}$ than with $SUVR_{cereb}$ cross-sectionally and longitudinally.

Methods: We included FDG PET scans and florbetapir PET scans from 340 patients with MCI and 309 NCs from the Alzheimer's Disease Neuroimaging Initiative (ADNI). Of the MCI subjects, 87 progressed to AD and 253 remained stable (average follow-up time 1.43 ± 0.97 years) while in normal controls, 40 progressed to MCI and 269 remained stable (average follow-up time 1.25 ± 0.98 years). Using Pearson's product-moment correlation testing, we determined the strength of the cross-sectional and longitudinal associations of HCI or sROI with $SUVR_{cereb}$ or with $SUVR_{cwm}$. We used Steiger tests for dependent correlations to determine whether the correlations we found were significantly different.

Results: For cross-sectional baseline data, our results show that the correlation of either HCI or sROI with $SUVR_{cwm}$ is significantly stronger than that with $SUVR_{cereb}$ for the MCI group (overall $p < 0.001$) and for the NC group (overall $p < 0.01$). For longitudinal data, our results similarly show that the correlation of either HCI or sROI with $SUVR_{cwm}$ is significantly stronger than that with $SUVR_{cereb}$ for MCI patients (overall $p < 0.001$). However, for the NC group, $SUVR_{cwm}$ only had a significantly stronger correlation than $SUVR_{cereb}$ with sROI (overall $p = 0.023$) but not with HCI (overall $p = 0.29$).

Conclusions: As shown in our results, the use of white matter as a reference region for SUVR is significantly more correlated with HCI or sROI than the use of a cerebellar reference region. The use of white matter as a reference region for florbetapir PET can potentially better characterize the negative impact of fibrillary $A\beta$ on neuronal activity and provide a better, more stable biomarker of $A\beta$ load.

Poster 59

AN EVALUATION OF THE COGNITIVE EFFECTS OF CLINICALLY USED COMBINATION HORMONE THERAPY. Berns-Leone C, Prakapenka A, Hiroi R, Pena V, Northup-Smith S, Melikian R, Patel S, Ladwig D, Croft C, Bimonte-Nelson H. Arizona State University; Banner Neurological Institute; Arizona Alzheimer's Consortium.

Background: Estradiol (E2) and Levonorgestrel (Levo) are two hormones commonly used in hormone therapy (HT) to decrease symptoms associated with menopause. Both of these hormones have been shown to have beneficial effects on cognition when given alone in a rodent model of menopause. However, it is unknown whether these hormones, when taken in combination, are beneficial or harmful to cognition. This is a critically important question given that these hormones are often given in combination. Thus, the overarching goal of the two studies was to examine the cognitive effects of E2 and Levo using a rat model of surgical menopause.

Methods: In both studies, middle-aged female rats were ovariectomized and then given subcutaneous injections of either vehicle or exogenous hormone treatment. The water-radial arm maze (WRAM) was used to assess spatial working and reference memory and Morris water maze was used to assess spatial reference memory. Study 1 assessed how the dose of E2 treatment in rats impacted cognitive performance, and found that low dose E2 enhanced working memory performance. Next, based on the results from Study 1, Study 2 used low dose E2 in combination with different doses of Levo to examine the cognitive effects of several E2 to Levo ratio combinations.

Results: The results from Study 2 demonstrated that the combination of low dose E2 with a high dose of Levo at a 1:2 E2 to Levo ratio impaired performance on the WRAM, and that the ratio currently used in HT, 3:1 E2 to Levo, may also negatively impact spatial memory performance. Indeed, there was a dose response effect indicating that spatial working and spatial reference memory performance was incrementally impaired as Levo dose increased when combined with the low E2 dose.

Conclusions: The findings from these two studies suggest that the E2 plus Levo combination is likely not neutral for cognitive function, and prompts further research in preclinical models as well as in menopausal women, in order to optimize HT.

Poster 60

ASSOCIATIONS BETWEEN GLOBAL AND REGIONAL CEREBRAL GLUCOSE METABOLISM MEASUREMENTS IN NORMAL AGING AND MCI. Bi T, Ausdemore J, Jing N, Kramer H, Chen Y, Kuang X, Luo J, Cary Savage, Reiman EM, Chen K. Desert Mountain High School; University of California Berkeley; Brophy College Preparatory; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Introduction: To quantify global cerebral metabolic rates for glucose (CMRgl), we introduced the hypometabolic convergence index (HCI) and statistical region of interest (sROI) approaches. While HCI measures the degree of similarity of reduced CMRgl in an individual compared to a typical AD patient, sROI tracks CMRgl alterations in brain regions associated with longitudinal change. It is important to understand the relationships between global and regional CMRgl in brain regions affected by AD, such as precuneus and posterior cingulate. This study, therefore, investigated correlations between HCI and sROI with CMRgl in the left and right precuneus (CMRgl_{PreCL}, CMRgl_{PreCR}) and the posterior cingulate cortex (CMRgl_{PC}), both cross-sectionally and longitudinally in the early phase of the disease.

Methods: Our study included FDG PET scans from 340 patients with mild cognitive impairment (MCI), 87 who progressed to AD and 253 who remained stable (average follow-up time 1.43 ± 0.97 years), and 309 normal controls, 40 who progressed to MCI and 269 who remained stable (average follow-up time 1.25 ± 0.98 years), all from the Alzheimer's Disease Neuroimaging Initiative (ADNI). By using Pearson's product-moment correlation and Steiger tests for dependent correlations, we determined the strength of the cross-sectional and longitudinal associations between HCI or sROI and CMRgl_{PreCL}, CMRgl_{PreCR} and CMRgl_{PC}.

Results: For cross-sectional MCI and cognitively normal combined baseline results, there was a significantly stronger HCI correlations with regional CMRgl in each of the three regions than the corresponding sROI correlations (CMRgl_{PreCL}: $p=6.6e-36$, CMRgl_{PreCR}: $p=1.4e-37$, CMRgl_{PC}: $p=0.0011$). For longitudinal MCI and NC combined results, HCI also demonstrated significantly stronger correlations with regional CMRgl in all three regions than sROI (CMRgl_{PreCL}: $p=1.6e-09$, CMRgl_{PreCR}: $p=4.1e-07$, CMRgl_{PC}: $p=7.0e-10$).

Conclusions: Regional glucose uptake measures in the left and right precuneus and posterior cingulate cortex were more strongly correlated with HCI than with sROI. The use of HCI as a global index can potentially be more representative of regional CMRgl in brain areas affected by AD than the use of sROI.

Poster 61

NORCLOZAPINE REDUCES BETA AMYLOID1-42 LEVELS AND INCREASES CHAT LEVELS IN 3XTG-AD FEMALE MICE. Burkart A, Potter P, Jones D. Midwestern University; Arizona Alzheimer's Consortium.

Background: Alzheimer's disease (AD) is a neurodegenerative disease characterized by synaptic dysfunction and cholinergic cell death. Major pathological hallmarks include aggregations of beta-amyloid ($A\beta$) proteins, intracellular neurofibrillary tau tangles, and a loss of cholinergic neurons that innervate the hippocampus and cortex. The accumulation of neurotoxic $A\beta$ fragments in AD is caused by the improper processing of amyloid precursor protein (APP). Previous evidence suggests that insoluble $A\beta$ 1-42 oligomers induce synaptic loss and decreases choline acetyltransferase (ChAT) levels in AD brains. In addition, activation of cholinergic receptors, specifically muscarinic M1 receptors, has been shown to reduce the inappropriate and neurotoxic processing of APP. The goal of the current study was to evaluate the effects of M1 stimulation on $A\beta$ levels and cholinergic dysfunction in an animal model of AD, the 3xTg-AD mouse.

Methods: 3xTg-AD female mice (~6 mos old) were randomly divided into 4 experimental groups and administered (i.p.) either saline (control), an M1 agonist (norclozapine) or an M1 antagonist (scopolamine or tolterodine) for 30 days. On day 31, mice were sacrificed and the brain harvested and prepared for ELISA and IHC analysis. EILSA and IHC assays were carried out using antibodies against ChAT or $A\beta$ 1-42.

Results: ELISA results using whole brain homogenates indicated that norclozapine (8 mg/kg) significantly decreased $A\beta$ 1-42 protein levels whereas scopolamine (2 mg/kg) and tolterodine (2 mg/kg) had no significant effect on $A\beta$ 1-42 levels. Analysis of ChAT proteins in brain homogenates of treated 3xTg-AD mice found that norclozapine significantly increased ChAT levels while neither of the antagonists had an effect. Finally, immunohistochemical (IHC) analysis of sectioned brain tissue using an antibody against $A\beta$ 1-42 revealed decreased $A\beta$ immunoreactivity in the hippocampus and cortex of norclozapine treated animals.

Conclusions: The present results have characterized the effects of M1 stimulation on amyloid levels and cholinergic function in a common animal model of AD. These findings also suggest that (1) the M1 agonistic properties of norclozapine may be effective in reducing the accumulation of $A\beta$ associated with AD and (2) M1 receptor stimulation may be a valuable mechanism for the development of new drugs aimed to inhibit the progression of AD.

Poster 62

IMPACT OF A β POSITIVITY ON WHOLE BRAIN ATROPHY IN COGNITIVELY UNIMPAIRED ϵ 4 HETEROZYGOTES AND RELATED SAMPLE SIZE ESTIMATION FOR CLINICAL TRIALS. Chan Y, Lu S, Luo J, Malek-Ahmadi M, Cary Savage, Reiman EM, Chen K. Arizona State University; Williams College; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: Apolipoprotein ϵ 4 carriers are at increased risk for late-onset of Alzheimer's disease (AD) and have been shown to have increased beta amyloid (A β) load detected with positron emission tomography (PET). We have also found greater whole-brain atrophy (WBA) in cognitively unimpaired ϵ 4 carriers as measured by magnetic resonance imaging (MRI) and an iterative principal component analysis (IPCA) algorithm. In this study, we examine the impact of A β positivity on the IPCA-measured WBA in cognitively unimpaired ϵ 4 heterozygotes (HT) and estimate required sample sizes using WBA as an outcome measure to support our efforts to prepare for a prevention trial in A β + HTs.

Methods: 83 HTs were identified from the larger sample of 340 cognitively normal participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) study. All participants had volumetric MRI, florbetapir PET scans and were also tested for cognitive function at baseline and follow-up, with an average time interval of 2.01 ± 0.27 years. Baseline florbetapir PET was used to define A β + using a pre-defined threshold. WBA was estimated using IPCA on baseline and follow-up MRI scans. Cognitive decline was determined by difference scores between baseline and follow-up from a neuropsychological battery, including the Mini Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog), and short-term, long-term and total scores from the Auditory Verbal Learning Test (AVLT). Two-sample t-tests were conducted to examine WBA and cognitive test score change differences between A β + and A β - HTs. Additionally, we estimated required A β + HT sample sizes for each measure based on a 25% treatment effect with 80% power and two-tailed $p < 0.05$.

Results: A β + HTs had significantly higher WBA indices than their A β - counterparts ($p < 0.001$). However, no difference was found in MMSE ($p = 0.23$), ADAS-Cog ($p = 0.72$), or AVLT (Short term memory: $p = 0.10$, Long term memory: $p = 0.20$, Total: $p = 0.26$) scores. For IPCA-based WBA, we estimated that 125 subjects per arm would be needed in a randomized clinical trial with 80% power and two-tailed $p = 0.05$ and 25% treatment effects. In contrast, MMSE, ADAS-Cog, and all three AVLT measures were estimated to require over 10,000 subjects to detect an effect with 80% power.

Conclusions: Our results suggest that A β positivity is associated with greater whole brain atrophy, measured by IPCA in cognitively unimpaired ϵ 4 HT individuals. Furthermore, IPCA measured WBA required a smaller number of individuals for a hypothetical clinical trial. Overall, IPCA may be a viable approach to detecting AD-related WBA in future AD prevention trials with superior statistical power over measures of cognitive functioning.

Poster 63

A NOVEL DROSOPHILA MODEL OF DEMENTIA BASED ON TAR DNA BINDING PROTEIN-43 (TDP-43). Chaung M, Mathieson D, Muñoz E, Kraft R, Zarnescu DC. University of Arizona, Arizona Alzheimer's Consortium.

Background: Recent discoveries suggest that amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) belong to a spectrum of disorders characterized by shared genetic and pathological features. Notably, the RNA binding protein TDP-43 is present in aggregates, in 97% of ALS and 25-30% of dementia patients. We have previously developed a Drosophila (fruit fly) model of ALS based on TDP-43 that recapitulates key features of the disease including cytoplasmic aggregates, locomotor dysfunction and reduced lifespan. Using genetic and drug screening approaches we uncovered several targets and small molecules with therapeutic potential for ALS. Given these recent successes, we set out to develop a fruit fly model of dementia based on TDP-43.

Methods: Using the GAL4-Upstream Activating Sequence (GAL4-UAS) expression system we expressed TDP-43 protein (wild-type or disease associated mutant, TDPG298S) in mushroom body (mb) neurons, the center for learning and memory in the Drosophila brain. To evaluate learning and memory in this model, we employed a well-established behavioral paradigm based on courtship.

Results: Using this approach, we found that the assay is sensitive enough to quantify learning and memory and our "FTD" flies exhibit learning and memory comparable to control flies when young. We are currently investigating their learning and memory during aging to determine whether TDP-43 causes an age dependent cognitive decline similar to FTD in humans. In addition, we are testing the hypothesis that disease may "spread" through a prion like mechanism in the brain.

Conclusions: Based on our preliminary results, TDP-43 expression does not cause cognitive deficits in young animals. This is consistent with possible age dependent cognitive decline, which we are currently testing. In addition, preliminary imaging suggests that TDP-43 may "spread" in the brain, which can open up the possibility for genome wide genetic screens and drug screens to identify therapeutic targets for FTD.

Poster 64

EFFECTS OF SHORT-TERM OXIDATIVE STRESS ON RAP1 EXPRESSION IN HUMAN GLIOBLASTOMA (U251) CELLS. Chia J, Gallas G, Bae NS. Midwestern University; Arizona Alzheimer's Consortium.

Introduction: Telomeres are the nucleoprotein structures at the ends of linear chromosomes. In mammalian cells, a six-member protein complex, called shelterin, protects telomeres from being recognized as DNA double-strand breaks and from nucleolytic degradation. Of these six proteins, RAP1 (for Repressor/Activator Protein 1) is a key protein that represses homology-directed repair and protect telomeres from non-homologous end joining, thus maintaining genome stability. Previous cell fractionation studies demonstrated that RAP1 is present in both the cytoplasm and nucleus and that the amount of RAP1 changes as cells age. Using fluorescent microscopy, the interest of the present study was confirming what changes occur to RAP1 expression when cells are placed under oxidative stress and whether these changes could be attributed to translocation of RAP1 from the nucleus to the cytoplasm.

Methods: Human glioblastoma (U251) cells were plated overnight before being transfected with either GFP or RAP1-GFP constructs using Lipofectamine 2000 and allowed to incubate for an additional day. After transfection was confirmed via immunofluorescence, cells were treated with either 0mM, 1mM or 2mM of H₂O₂ for up to 4 hours before being fixed with a 3% PFA, 2% sucrose solution. Cells were then photographed using an Olympus IX73 inverted fluorescent microscope and the images analyzed for differences in nuclear or cytoplasmic brightness with ImageJ.

Results: Cells transfected with RAP1-GFP and treated with either 1mM or 2mM H₂O₂ had an average of 60% greater brightness ($p < 0.05$) of RAP1 in the cytoplasm compared to cells not treated with H₂O₂. In comparison, the amount of RAP1 expression in the nucleus did not change significantly between samples. This led to a significant decrease in the nuclear to cytoplasmic ratio of RAP1 in H₂O₂ treated cells compared to untreated, falling from 2.38 to 1.57.

Conclusion: These data support the hypothesis that RAP1 migrates from the nucleus to the cytoplasm when cells are under oxidative stress.

Poster 65

ACTIVATION OF NEURONAL POPULATIONS IN YOUNG AND AGED RAT LATERAL ENTORHINAL CORTEX DURING TRACK-RUNNING BEHAVIOR WITH ODORS. Comrie A, Lister JP, Chawla MK, Barnes CA. University of Arizona; University of California Los Angeles; Arizona Alzheimer's Consortium.

Background: The hippocampus is known to show biological changes with age that are related to changes in memory processes. For example, in aged rats, the CA1 region of the hippocampus fails to show accurate map retrieval upon revisiting a familiar environment (Barnes et al., 1997). The distal part of CA1 receives major inputs from Lateral Entorhinal Cortex (LEC) layer III. In contrast to the well-studied Medial Entorhinal Cortex (MEC), LEC neurons do not show substantial spatial selectivity in their firing patterns (Deshmukh and Kneirim, 2011). Rather, LEC is thought to be involved in non-spatial memory, such as encoding object and odor information. The role of LEC in odor discrimination and how the corresponding neural activity may change with age remain unknown. In this study we aim to discover if LEC neuronal populations are active in response to distinct odors during track running, and whether age-related changes in activation patterns may provide faulty input to the hippocampus that may explain remapping in older animals.

Methods: To test this, 24 young (9 months) and 24 aged (24 months) male rats were trained to run in alternating clockwise and counterclockwise laps on a circular track in a constant spatial environment. One behavioral group (A/A) experienced the same set of 6 odors mixed with sand in ramekins in the same order around the track during two run sessions separated by 20 minutes. A second group (A/B) also experienced two run sessions, but the odor stimuli were all distinct between the two time points. A positive control group underwent Maximal Electroconvulsive Shock (MECS), and a negative control group was sacrificed from their home cages (CC). The mRNA of immediate-early gene Arc is localized to distinct cellular compartments based on the time since neuron activation. We use cellular compartment analysis of temporal activity by fluorescence in situ hybridization (catFISH; Guzowski et al., 1999) and confocal microscopy to visualize this time-dependent subcellular distribution of Arc mRNA. This method enables us to identify neurons activated during the first, second, or both running sessions in LEC.

Results: Preliminary data from LEC averaged across both treatments and age groups confirm that the track-running behavior with odors elicits 26% neural activation in comparison to low resting Arc expression (2%) for CC animals.

Conclusions: With additional animals added to treatment conditions and age groups we will be able to determine if LEC encodes odor information that can discriminate between the A/A and A/B conditions, and if population representation of odor stimuli in LEC changes across the lifespan.

Poster 66

ELUCIDATING THE INVOLVEMENT OF RBBP7 IN ALZHEIMER'S DISEASE. Dave N, Ferreira E, Piras IS, Huentelman MJ, Oddo S. Arizona State University; Translational Genomics Research Institute; Arizona Alzheimer's Consortium.

Background: Alzheimer's disease (AD) is one of the most common neurodegenerative diseases worldwide, affecting over 5 million people in the United States alone. As the prevalence of the disease continues to grow at an alarming rate, the demand for novel therapeutic targets and further understanding of the pathogenesis of the disease increases. In an attempt to address this issue and identify new potential therapeutic targets, we performed an unbiased proteomic analysis in the hippocampi of 3xTg-AD mice, a widely used animal model of AD.

Methods: Using isobaric tags for relative and absolute quantitation (iTRAQ), we analyzed the hippocampal proteome of 12-15 month-old 3xTg-AD mice (n=4) and compared it to the proteome of age-matched non-transgenic (NonTg) mice (n=4). Proteins which were significantly different between 3xTg-AD and NonTg mice were then analyzed using a protein-protein interaction (PPI) network, created by STRING database. To validate our proteins of interest in human AD, analyses were performed on an mRNA-expression data set obtained through an unbiased microarray screening of middle temporal gyrus tissue from AD cases (n=97) and non-demented (ND) cases (n=98). Furthermore, the publicly available dataset GSE5281, generated from laser capture microdissected neurons in the middle temporal gyrus, was reanalyzed to validate these RNA-expression analyses.

Results: Statistical analysis of the iTRAQ proteomic data revealed 148 proteins that are differently expressed between the 3xTg-AD and NonTg hippocampi. The STRING database was then used to identify associations between these differently expressed proteins, creating a PPI network. Of the proteins highlighted in this network, we selected Retinoblastoma Binding Protein 7 (Rbbp7). We found that Rbbp7 was significantly decreased in the aged hippocampi of 3xTg-AD mice (Log₂ FC = -0.297; FDR adj p = .0181). More importantly, analysis from human middle temporal gyrus tissue showed that Rbbp7 mRNA expression in AD patients is significantly less than that of ND patients (Log₂ FC = -0.236; FDR adj p = 0.00002). The reduced Rbbp7 levels were confirmed using an independent gene-expression data set obtained from laser captured neurons. Notably, we found a significant negative correlation between Rbbp7 mRNA expression and Braak Stage in AD patients.

Conclusions: Rbbp7 functions as a histone-binding subunit by chaperoning several chromatin remodeling factors to their histone substrates. Furthermore, Rbbp7 specifically targets lysine tails involved in learning and memory (H4K5, H4K12), interacts with CREBBP, and is an integral part of the PRC/EZH2-EED complex which is known to transcriptionally repress several genes associated with neurodegeneration. Taken together, these data suggest that reduced expression of Rbbp7 may contribute to the epigenetic changes observed in AD.

Poster 67

BIAS CORRECTION METHOD IMPROVES AUTOMATIC BRAIN EXTRACTION IN RODENT MR IMAGING. Do L, Bharadwaj P, Bernstein A, Xiao J, Alexander GE, Barnes CA, Trouard TP. University of Arizona; Arizona Alzheimer's Consortium.

Background: Magnetic resonance imaging (MRI) can provide important information in the assessment of many brain processes. Animal models allow MRI to play an important role in this characterization. Recently, an automated method for extracting brain signal from mouse MRI was described and shown to have high correlation with manual segmentation (the gold standard) (1). The authors of this method suggest that an N4 bias correction (2) improves results but showed no quantitative support. The aims of the present study were to 1) evaluate the rodent brain extraction methods when using a surface coil for reception and 2) to determine if a simple and rapid bias correction using the N4 algorithm would improve the technique.

Methods: Fisher 344 rats underwent high-resolution 3D T2-weighted turboRARE MRI scans performed on a 7T Bruker Biospec (Bruker, Billerica, MA). A volume coil was used for excitation and a surface coil for reception. The imaging parameters were as follows: FOV=3.84X2.88X1.92cm, matrix size=256X192X128, slices=1 slab, TR=1500ms, TE=10ms, time of acquisition=4608sec). The images from these animals (n=7) underwent brain extraction by both processing approaches, manual and automatic. 3D whole brain datasets were viewed in three orthogonal directions. Bias correction due to non-uniform surface coil sensitivity was performed by applying a rough mask larger than the brain followed by N4 bias correction tool in advanced normalization tools. Manual extraction was performed in a blinded fashion (MRIcron and ITK-snap). An automated method was carried out to extract the brain on bias corrected or uncorrected images. The results were quantitatively compared with dice coefficients (3).

Results: An average manual brain extraction on a slice-by-slice basis took 4 ± 1 hours whereas the automated methods took 4 ± 0.5 minutes. The set of dice coefficients (DC) indicating the spatial overlap between manually drawn masks and masks generated by the semi-automated method with a N4 bias field correction were higher than those obtained when the N4 bias field correction was not applied (Table 1). Applying the non-parametric Wilcoxon Signed Rank Test revealed a significant difference between the two sets of dice coefficients (Z -score = -2.366, p = 0.018). Visual inspection identified regions where automated extraction without bias correction were incorrectly included. As expected from the DCs, the bias corrected images followed by automated brain extraction revealed more accurate results.

Conclusions: The addition to a rapid bias field correction step in the automated method of brain extraction showed greater accuracy without adding significant time requirements, while also maintaining similar accuracy in relation to the manual method for 3D RARE brain extraction. The effectiveness of this approach most likely arises due to a better defined border in the brain.

REFERENCES: 1. Delora et al. A Simple Rapid Process for Semi-automated Brain Extraction from Magnetic Resonance Images of the Whole Mouse Head. J of Neuro Sci Med. 2016.
2. Tustison et al. N4ITK: Improved N3 Bias Correction. IEEE Transactions on Med Imag. 2010.
3. Dice LR. Measures of the Amount of Ecologic Association Between Species. Ecology. 1945.
This study was funded by support from the NIH Grants AG049464, AG049465, AG019610

Poster 68

READING COMPREHENSION NEURAL NETWORKS IN AGING. Fitzhugh MC, Braden BB, Handley P, Connor D, Sabbagh MN, Caselli RJ, Baxter LC. Arizona State University; Barrow Neurological Institute; Banner Sun Health Research Institute; Mayo Clinic Arizona; Arizona Alzheimer's Consortium.

Background: Numerous studies have explored the brain regions involved in language comprehension at its phonological, semantic, and syntactic levels using both spoken and written samples (for a review, see Price, 2012). Much of the work toward understanding this process has relied on functional MRI (fMRI) in healthy, younger adults. However, it is well known that older adults experience marked declines in brain function. Thus, this study sought to explore age-related changes in brain function associated with simple language comprehension.

Methods: Sixty-five adults aged 17 to 85 years (mean (sd) age = 52.57 (20.49) years; 43 women) were recruited from the community and, in the case of many of the older adults, from Banner Sun Health Research Institute. Participants were all native English-speaking, right-handed, and had MMSE scores greater than 27. The fMRI reading paradigm consisted of two alternating blocks: five blocks of short stories selected at a 5th grade reading level and 5 blocks of baseline letter strings. The stories were specifically designed to reflect a 5th grade reading level in order to minimize working memory load or other resources and instead focus on simple reading comprehension. The fMRI data was analyzed using an independent component analysis (ICA) technique, thus identifying independent brain networks. Regression analysis evaluated the degree to which each component was task-related across the age span.

Results: Two typical fronto-temporal language networks were identified as task-related but their relative recruitment to the task did not significantly change with age. Four networks were identified as task-related and changed with age. The beta values for three of these networks were positively correlated with age (e.g., increasing use of the network with increasing age) and included: a bilateral frontal lobe and the anterior cingulate network ($r=0.325$, $p=0.008$); a bilateral inferior parietal lobe and right inferior frontal gyrus network ($r=0.286$, $p=0.021$); and a left superior parietal lobe, left middle frontal gyrus, and left medial superior frontal gyrus network ($r = 0.271$, $p=0.029$). A bilateral cerebellum network was identified that showed smaller beta values with increased age ($r=-0.246$, $p=0.021$).

Conclusions: In response to a simple reading paradigm, adults across the age range equally recruited a well-studied, fronto-temporal network of language comprehension. However, older adults demonstrate increased recruitment of frontoparietal networks, often associated with working memory processes, in addition to a prefrontal and anterior cingulate network. These results suggest that older adults exert increased effort and attentional resources even when faced with comprehending simple sentences.

Poster 69

RELATION OF PHYSICAL SPORT ACTIVITY TO COGNITIVE PERFORMANCE IN OLDER ADULTS. Franchetti MK, Bharadwaj PK, Nguyen LA, Haws KA, Fitzhugh MC, Hishaw GA, Raichlen DA, Alexander GE. University of Arizona; Arizona Alzheimer's Consortium.

Background: It is well-known that aging is associated with differences in cognitive performance among community-dwelling older adults, with executive function, memory, and processing speed often preferentially affected. Physical activity is one factor that may have an important role in influencing observed individual differences in cognitive aging. We sought to determine whether higher levels of self-reported physical sport activity are associated with better performance on those measures of cognitive function often affected by healthy aging.

Methods: In a sample of community-dwelling, neurologically healthy older adults, self-report ratings of physical sport activity were obtained from 210 participants ages 50-89 years (mean \pm SD = 70.0 \pm 10.4 years). Subjects who reported a high level of sport activity (n=38) were compared to those who reported a low level of sport activity (n=172). The groups did not differ significantly in age, years of education, MMSE score, and hypertension status. Analysis of covariance (ANCOVA) was used to test the effects of age group, physical activity group, and their interaction. To account for multiple comparisons, a false discovery rate (FDR) correction at $p < 0.05$ was applied. Measures with a significant FDR-corrected interaction were followed by simple effects analyses.

Results: Results revealed significant FDR-corrected main effects for age across multiple cognitive measures ($0.000004 < p's < 0.05$). No main effects were significant for physical activity group ($p's > 0.73$). Significant FDR-corrected age by physical activity interactions were observed for measures of executive function (Wechsler Adult Intelligence Scale – IV (WAIS-IV) Matrix Reasoning and Digit Span and Stroop Word-Color Interference; $0.01 < p < 0.02$), memory (Rey Complex Figure Test (RCFT) Delay Recall; $p = 0.03$), visuospatial function (WAIS-IV Block Design and RCFT Copy; $p's = 0.009$), and language (WAIS-IV Similarities; $p = 0.03$). These effects remained significant after controlling for hypertension status in the sample. Follow up pairwise comparisons showed multiple age group and physical activity differences that varied across cognitive domains. Importantly, within the old-old age group (72-89 years), those with high physical activity performed better on several of these measures than those with low physical activity and they did not differ from the young-old groups (50-71 years).

Conclusions: In this community-dwelling sample of healthy older adults, the old-old group performed more poorly than the young-old group on multiple measures of cognitive functions, including executive function, memory, processing speed, and visuospatial ability. We observed age by physical activity interactions in multiple cognitive domains, including executive function, memory, visuospatial function, and language. The older adult group with high physical activity performed better than those with low physical activity. In addition, the older adult group with high physical activity performed better than, or comparable to, the younger adult group. Together, this suggests that high levels of physical sport activity can diminish the cognitive effects often observed in healthy aging.

Poster 70

AGE-RELATED REDUCTION IN SIGNAL-TO-NOISE RATIO OF SHARP-WAVE RIPPLE OSCILLATIONS FOLLOWING BEHAVIOR IN AGED RATS. Gray DT, Wiegand J, Schimanski LA, Cowen SL, Barnes CA. University of Arizona; Arizona Alzheimer's Consortium.

Background: The consolidation of episodic memories relies on the transfer of information from hippocampal networks to cortical networks, and much of this information is thought to be transferred during hippocampal sharp-wave ripple events in periods of rest.

Methods: During normative aging, we have recently shown that the rate of ripple occurrence decreases, and the mean frequency of ripple events is reduced by roughly 19 Hz prior to and following behavior on a spatial eye-blink conditioning task (Wiegand et al., 2016). To extend these recent findings, here we present an age-comparison of spectral power in the local field potential before and after performance on a spatial eye-blink conditioning task. Specifically, the time periods analyzed were: sharp-wave ripple events, the 50 ms immediately preceding and following ripple events, and quiet inter-ripple periods.

Results: In young rats, the spectral power in the 80-200 Hz frequency band during ripple events was greater in the post-behavior rest period compared to the pre-behavior rest period, although in aged rats the ripple power was not different between the rest epochs. During the inter-ripple periods spectral power was significantly lower in young rats during post-behavior rest relative to pre-behavior rest, and again the power in aged rats did not differ between rest epochs. No changes were noted between rest epochs for the 50 ms immediately preceding and following the ripple events for either age group. The observations that ripple power increases and inter-ripple power decreases following behavior only in young rats may suggest a mechanism for increased signal-to-noise in these young animals. The signal-to-noise ratios were examined by computing the ratio of the summed squared magnitudes of the 80-240 Hz spectral power of ripple events relative to inter-ripple periods. This analysis showed an increase in the signal-to-noise ratio of ripple events during post-behavior rest relative to pre-behavior rest in young animals, while aged rats did not show a change in signal-to-noise ratio following behavior.

Conclusions: Increases in the signal-to-noise ratio of ripple oscillations in rest periods following behavior may increase the efficacy by which ripple oscillations transfer information during memory consolidation. The absence of this signal to noise ratio increase in older animals suggests less efficiency in consolidation processes carried out in hippocampal networks.

Poster 71

DEEP BRAIN STIMULATION AND COGNITIVE FUNCTION AMONG PATIENTS WITH PARKINSON'S DISEASE: A HISTORICAL COHORT STUDY. Hansen A, Mehta SH, Krell-Roesch J, Kirlin KA, Velgos SN, Roesler K, Limbaeck-Stokin M, Geda YE. Mayo Clinic Arizona; Arizona Alzheimer's Consortium.

Background: Globus pallidus pars interna (GPi) and subthalamic nucleus (STN) are targets for treatment of Parkinson's Disease (PD) through deep brain stimulation (DBS). Some, but not all, studies have implicated cognitive impairment following DBS targeting STN as compared to DBS targeting GPi. Therefore, we sought to investigate the cognitive impact of DBS targeting GPi versus DBS targeting STN among patients with medically refractory PD.

Methods: We conducted a historical cohort study in the setting of the Deep Brain Stimulation Center of Mayo Clinic Arizona. We included 32 patients with medically refractory PD out of which 13 underwent GPi DBS and 19 underwent STN DBS. The diagnosis of PD was made by a movement neurologist (SHM). A board certified Neuropsychologist (KAK) oversaw the cognitive evaluation of participants that were carried out at baseline and 6 months post-DBS. The tests administered included: Boston Naming Test (BNT), Wechsler Adult Intelligence Scale – Verbal Comprehension Index (WAIS-VCI, Wechsler Adult Intelligence Scale – Working Memory Index (WAIS-WMI), and Wechsler Adult Intelligence Scale – Processing Speed Index (WAIS-PSI).

Results: We observed no significant difference in GPi patients between baseline and follow-up cognitive test scores on any of the four neuropsychological tests. STN patients showed significant cognitive decline on follow-up as compared to baseline on three of the four cognitive tests: WAIS-VCI (104.95 [SD 12.9] vs. 100.89 [12.4]; $p=0.0044$), WAIS-WMI (99.32 [14.1] vs. 92.68 [12.5]; $p<0.001$), and WAIS-PSI (92.95 [12.9] vs. 81.89 [12.2]; $p<0.001$). When we compared the changes in baseline and follow-up scores between the GPi and STN patients, we observed a significant difference on three (WAIS-VCI, WAIS-WMI, WAIS-PSI) of the cognitive tests.

Conclusions: Our results indicate that STN DBS but not GPi DBS is associated with cognitive impairment in memory, executive functioning, and language domains. From a cognitive health perspective, these findings suggest that GPi may be a preferred target for DBS in patients with medically refractory PD.

Poster 72

DNA METHYLATION AND GENE EXPRESSION PATTERN ANALYSIS FOR PARKINSON'S DISEASE BLOOD BIOMARKER DISCOVERY. Henderson-Smith AR, Meechoovet B, Siniard AL, Driver-Dunckley E, Huentelman MJ, Dunckley TL. Translational Genomics Research Institute; Mayo Clinic Scottsdale; Arizona Alzheimer's Consortium.

Background: Parkinson's disease (PD) is a progressive neurodegenerative disorder, diagnosed only at an advanced disease stage, by a series of motor deficits that manifest over years or decades. Aberrant epigenetic modifications, including hypomethylation of α -synuclein in PD, exist across a range of diseases, from cancer to schizophrenia, and are non-invasively detectable in many body fluids and blood tissue as markers of disease.

We aimed to characterize DNA methylation and gene expression patterns in blood from PD patients and matched healthy controls to identify disease-specific biomarkers that may be used to aid earlier, more accurate disease diagnosis and tracking of disease progression.

Methods: Two whole-blood samples were collected from PD patients and healthy controls, one for DNA methylation detection and one for mRNA sequencing. DNA methylation sites were probed with the Illumina Infinium HumanMethylation450 BeadChips. We used the Illumina HiSeq2000 platform for mRNA sequencing and performed separate and integrated analyses of differential expression and DNA methylation.

Results: PD methylation profiles are readily distinguishable from healthy controls, even in whole blood DNA samples. Differential expression analyses of mRNA-seq data identified global changes in gene regulation, including overall gene expression levels and expression levels of specific transcript splice variants. Combined methylation quantitative trait loci analyses (meQTL) identified cis-acting meQTLs associated with differential expression of proximal loci.

Conclusions: Establishing clear patterns of altered disease-specific DNA methylation, RNA expression and processing, and meQTL analyses from whole blood, a non-invasive tissue collection option, provides increased promise for the development of a molecular biomarker for PD with sufficient sensitivity and specificity to aid in the diagnosis and tracking of this disorder.

Poster 73

PREDICTION OF AD PROGRESSION IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT USING FDG PET BIOMARKERS AND NEUROPSYCHOLOGICAL ASSESSMENT. Jing N, Kramer H, Bi T, Ausdemore J, Chen Y, Kuang X, Luo J, Cary Savage, Reiman EM, Chen K. University of California Berkeley; Brophy College Preparatory; Desert Mountain High School; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: A pressing goal of Alzheimer's disease (AD) research is to develop tools to identify the disease during preclinical and prodromal stages. Various statistical tools have been used to predict progression to AD among individuals with mild cognitive impairment (MCI) using imaging biomarkers and neuropsychological tests, alone or in combination. However, systematic comparisons among these tools have not been conducted. In this study, we investigate and compare several, including generalized logistic modeling (GLM), decision tree, and penalized logistic regression (Lasso and Ridge) models for their ability to predict whether patients with MCI will progress to AD based on initial baseline information. In addition, we evaluated the importance of each of the measurements used in the models for their individual contribution.

Methods: We used baseline FDG PET scans obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database for 404 patients with MCI, including 151 progressors and 253 non-progressors (average follow-up time 2.61 ± 1.54 years with average baseline visit 2.63 ± 1.91 before AD diagnosis for progressors). Several logistic (GLM, Lasso, and Ridge) and decision tree (regular, pruned, and random forest) models were used to predict whether an individual with MCI would progress to AD based on neuropsychological assessment performance on the Clinical Dementia Rating Scale (CDR), Auditory Verbal Learning Test-Long Term Memory (AVLT-LTM), AD Assessment Scale-cognitive (ADAS-cog), APOE status, and FDG-PET based biomarkers including statistical regions of interest (sROI) and hypometabolic convergence index (HCI) approaches. We utilized ten-fold cross-validation on each model to calculate the accuracy, sensitivity, specificity, and the area under the curve.

Results: Among the models evaluated, we found that the GLM was the best method based on its stable distribution of accuracy (0.75), sensitivity (0.70) and specificity (0.782). The significant factors in the GLM were AVLT-LTM ($p = 0.022$), ADAS-Cog ($p = 0.000132$), APOE status ($p = 0.0091$), and FDG-PET sROI ($p = 0.04$). Using the random forest model, we also measured how important each factor was relative to the others using mean decrease in the Gini coefficient (MDG) values, where a larger MDG indicates greater importance: ADAS13 (MDG=41.44), HCI (MDG=36.41), sROI (MDG=34.32), Age (MDG=26.99), AVLT.LTM (MDG=22.85), CDR.SOB (MDG=14.44), APOE Status (MDG=10.21). We also evaluated the importance of the neuropsychological assessments (MDG = 28.20) and the biomarkers (MDG = 32.03) against each other and found that the biomarkers used in the random forest model played a slightly more crucial role in classifying progressors.

Conclusions: Our results demonstrate that future progression to AD can be predicted with up to 75% accuracy among patients who are in the prodromal stage of AD. The fact that there was not one single factor that differentiated progressors from non-progressors speaks in favor of adopting multivariate approaches to integrate information from multiple sources.

Poster 74

AD-SPECIFIC CEREBRAL GLUCOSE METABOLIC DECLINES IN COGNITIVELY NORMAL AND MCI INDIVIDUALS PRIOR TO CLINICAL PROGRESSION. Kramer H, Jing N, Ausdemore J, Bi T, Chen Y, Kuang X, Luo J, Cary Savage, Reiman EM, Chen K. Brophy College Preparatory; University of California Berkeley; Desert Mountain High School; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Introduction: We introduced the hypometabolic convergence index (HCI) and statistical region of interest (sROI) to characterize Alzheimer's disease (AD) specific cerebral metabolic rates for glucose (CMRgl) reduction. While HCI measures the degree of similarity of reduced CMRgl in an individual compared to a typical AD patient, sROI tracks CMRgl alterations in brain regions associated with longitudinal change. In this study, we use these global AD specific CMRgl indices to characterize longitudinal changes: 1) over 2.74-years in healthy individuals prior to diagnosed mild cognitive impairment (MCI; NC-MCI progressors) in comparison to the non-progressors and 2) over 2.61-years in MCI patients prior to their diagnosed dementia due to AD (MCI-AD progressors) in comparison to the patients with stable MCI. Our aim was to examine whether CMRgl decline is accelerated in the NC-MCI progressors and in MCI-AD progressors in contrast to their counterparts.

Methods: Our study included 404 MCI participants, composed of 151 progressors and 253 non-progressors (average follow-up time 2.61 ± 1.54 years) and 290 initially cognitively normal participants, consisting of 53 progressors and 237 non-progressors (average follow-up time 2.74 ± 1.58 years) who had undergone repeated FDG PET measurements, all from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. Linear mixed-effect model approaches were used to model the longitudinal data and compare the CMRgl rates of change in HCI and sROI between progressors and non-progressors separately in NC and in MCI study participants. Since we were interested in characterizing longitudinal changes, CMRgl rates of change in HCI and sROI were tracked with respect to number of years since diagnosis. Analysis of variance and effect sizes were computed to identify the presence and magnitude of the slope differences between progressors and non-progressors. In addition to the rate of change comparisons, we also examined the year-onset at which the progressors diverged from the non-progressors. Year-onset is defined as the point after which, the 95% confidence band of the fitted linear curve for the progressors is no longer intersected with the band of the non-progressors.

Results: For the study participants who were initially cognitively unimpaired, the progressors to MCI displayed accelerated rate of change compared to the non-progressors in both sROI ($p < 0.001$) and HCI ($p = < 0.001$). Similarly, significant group differences of rate of change were also found between MCI-AD progressors and stable MCI patients (sROI: $p < 0.001$, HCI: $p < 0.001$). For both sROI and HCI, MCI-AD progressors demonstrated a larger slope difference effect size (sROI: Cohen's $d = 3.32$, HCI: Cohen's $d = 3.18$) than NC-MCI progressors (sROI: Cohen's $d = 1.97$, HCI: Cohen's $d = 1.50$). For the NC-MCI progressors, the year-onset was 3.85 years for sROI and 3.73 years for HCI prior to their MCI diagnosis. For MCI-AD progressors, year-onset was 3.25 years and 3.41 years before their AD diagnosis separately for sROI and HCI.

Conclusions: Our results demonstrate that progressors and non-progressors embark on separate trajectories for cerebral metabolism years before their clinical diagnosis. In addition, significant differences more than 3 years before the appearance of the clinical symptom demonstrate the feasibility of the use of FDG-PET as a biomarker of preclinical changes in MCI and AD.

Poster 75

LONGITUDINAL CHANGES IN CEREBRAL METABOLIC RATE FOR GLUCOSE IN PROGRESSORS TO MCI. Kutchey M, Xie J, Chen Y, Luo J, Cary Savage, Caselli R, Reiman E, and Chen K. University of Arizona; Emory University; Mayo Clinic-Scottsdale; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: Previous studies have shown that brain regions including the posterior cingulate, precuneus and hippocampus had reduced glucose metabolism in patients with Alzheimer's disease (AD), mild cognitive impairment (MCI), and even in cognitively unimpaired individuals who have a higher genetic risk for AD. However, longitudinal studies, especially those with multiple-year observations, are lacking. Using a voxelwise analysis approach, this study examined cerebral metabolic rate for glucose (CMRgl), acquired every two years, in initially cognitively unimpaired individuals up to 17 years (13.93 ± 1.98) prior to progression to MCI.

Methods: FDG-PET scans from participants in the Arizona APOE Cohort study, all with a family history of dementia and between 51-70 years of age at study entry, were included in this report. Fourteen of these participants ultimately became "progressors" diagnosed with MCI and 32 matched participants remained cognitively unimpaired. SPM was used to spatially realign baseline and follow-up scans, to deform these individual images to the MNI template coordinate space, and to smooth them with an 8 mm Gaussian kernel. The CMRgl was calculated with whole brain CMRgl as a reference. Differential CMRgl rates of change between progressors and non-progressors were examined using a general linear model.

Results: Data analyses showed that CMRgl decline was faster in progressors than non-progressors in a number of regions associated with AD. Following correction for multiple comparisons, significant differences in the rates of CMRgl decline were noted in precuneus, posterior cingulate, hippocampus, parahippocampal gyrus, and middle and posterior cingulate cortex.

Conclusions: This study suggests that, more than 10 years prior to diagnosis with MCI, progressors had significantly higher rates of decline for CMRgl than non-progressors in regions affected by AD.

Poster 76

HISTOLOGY INFORMED PROBABILISTIC HIPPOCAMPAL ATLASES OF YOUNG AND OLD RHESUS MACAQUES. Kyle CT, Bennett JL, Stokes JD, Permenter MR, Vogt JA, Ekstrom AD, Barnes CA. University of Arizona; M.I.N.D. Institute; University of California, Davis; Arizona Alzheimer's Consortium.

Background: Identifying primate hippocampal subfields in vivo using structural MRI imaging relies on variable anatomical guidelines, signal intensity differences, and heuristics to differentiate between regions, and lack a clear anatomically-driven basis for subfield demarcation (Yushkevich et al., 2015).

Methods: Recent work, however, has begun to develop methods to use ex vivo histology or MRI to better inform subfield demarcations of in vivo images (Iglesias et al., 2015, Adler et al., 2014). For optimal results, though, ex vivo and in vivo images should be matched to the same subjects, with the goal to develop a neuroanatomically-driven basis for in vivo structural MRI images.

Results: Here, we address this issue in young and aging rhesus macaques (young n=2 and old n=2) using ex vivo Nissl-stained sections in which we identified the dentate gyrus, CA3, CA2, CA1, subiculum, presubiculum, and parasubiculum using morphological cell properties (30 μ m thick sections spaced at 240 μ m intervals and imaged at 161 nm/pixel). These were merged with in vivo structural MRIs (0.625 x 0.625 x 1 mm) from the same subjects via iterative rigid and diffeomorphic registration resulting in probabilistic atlases of young and old rhesus macaques.

Conclusions: These methods will inform subfield differentiation by identifying features of the MRI images that correspond to histological properties in the same animals, useful for work in both young and aging primates. Furthermore, we believe that this approach may be helpful in developing a phylogenetically-driven "ground truth" for more accurate identification of hippocampal subregions in human brains.

Poster 77

AGED RATS FAIL TO INTEGRATE CONFLICTING SPATIAL REFERENCE FRAMES. Lester AW, Kapellusch AJ, Screen RT, Barnes CA. University of Arizona; Arizona Alzheimer's Consortium.

Background: As with older adults, aged rats show robust impairments on a number of different spatial navigation tasks. There is some evidence that these navigation impairments are accompanied by a bias away from using an allothetic-based (i.e., external cue) navigation strategy towards relying on an idiothetic-based (self-motion) strategy (Rosenzweig et al., 2003).

Methods: To test the degree and timing with which aged animals utilize these two forms of spatial information, a novel behavioral arena has been developed that allows for complete and immediate control of all visual cues in the environment in order to put idiothetic and allothetic reference frames in direct conflict. The arena is composed of a circular track with a 360 degree panorama of visual cues projected on the walls. Identical feeders are spaced every 10 degrees along the perimeter of the track and animals learn to run to only one of them for food reward. By instantaneously rotating the cues we were able to characterize how quickly and accurately aged animals utilize allothetic feedback to navigate to a new rotated feeder location.

Results: Behavioral data collected from six young (9 – 15 mo) and six aged (24 - 30 mo) animals revealed that immediately following cue rotation aged rats were significantly more likely to navigate to either the exact original (idiothetically aligned) or rotated (allothetically aligned) feeder locations. Young rats, by comparison, were more significantly more likely to stop at multiple feeders, particularly those half-way between the original and rotated reward location.

Conclusions: These findings suggest that when spatial reference frames are put into conflict young rats settle on a strategy which combines the two sources of spatial information, while aged animals adhere more rigidly to only one spatial reference frame. Previous studies have shown that when spatial reference frames are put into conflict the place cells of the same CA1 network can anchor to entirely different reference frames, while CA3 place cells will align coherently with only one reference frame (Lee et al., 2004). The behavior we observe may be a consequence of the reported hyperexcitability and excessive pattern completion of aged CA3 principle cells driving an all-or-none shift to one or the other reference frame. If this is the case we expect that electrophysiological data from downstream CA1 place cells will match our behavioral findings and that CA1 place fields of aged animals will tend to snap to one or the other reference frames coherently as a population while those of young animals will show more variability in terms of which reference frame they anchor to.

Poster 78

USING BIOENGINEERING APPROACHES TO GENERATE A THREE-DIMENSIONAL (3-D) HUMAN INDUCED PLURIPOTENT STEM CELL (hiPSC)-BASED MODEL OF ALZHEIMER'S DISEASE (AD). Lundeen R, Bounds L. Arizona State University; Arizona Alzheimer's Consortium.

Background: Collectively, current hiPSC-based models of AD are limited by the use of immature, heterogeneous neuronal populations in a 2-D microenvironment that does not mimic that native brain tissue. It is well-established that in vivo cells reside within a complex 3-D microenvironment that plays a significant role in regulating cell behavior. Nonetheless, previous studies using AD hiPSCs have relied on 2-D neuronal culture models that do not reflect the 3-D complexity of native brain tissue, and therefore, are unable to replicate all aspects of AD pathogenesis. The aim of this project is to develop bioengineering methods for the generation of 3-D organoid-based cultures that mimic the architecture and complexity of in vivo cortical tissue.

Methods: The 3-D model utilizes long term culture of stem cell derived embryoid bodies. hiPSCs are cultured in suspension, using low attachment plates, in dual SMAD inhibition conditions and forced into aggregates called embryoid bodies. Culture conditions are changed to expand putative NPC populations, and then to differentiate into neuronal spheroids. Culture is continued without factors to allow for neuronal maturation. hNPCs are similarly cultured; however, neural specification and patterning is not necessary, and the expansion period begins immediately. Samples taken for analysis at long-term time points of 50 and 100 days in culture to allow for maturation.

Results: The maturity of 3-D neuronal cultures will be assessed by means of quantitative polymerase chain reaction (qPCR), immunohistochemistry (IHC), and electrophysiology as follows:

QPCR: Gene expression analysis of cells differentiated >50 days will be performed to determine the extent to which the 3-D neuronal cultures express mature neuronal and glial markers. In addition, differentiation to a cortical/forebrain neuron fate will be assessed.

IHC: Briefly, 3-D neuronal cells will be fixed, then transferred to OCT embedding medium and snap-frozen with liquid nitrogen. For immunohistochemistry, 10 μ m thick sections will be obtained using a cryostat. Sections will be double- and triple-stained for neuronal and glial markers. In particular, this analysis will be used to determine the extent to which these 3-D cultures recapitulate the spatial organization and architecture of in vivo neural tissue.

Electrophysiology: Live recordings of 3-D cultures transferred onto microelectrode arrays will be taken to determine the extent to which these 3-D neuronal cultures spontaneously fire action potentials.

Conclusions: By developing a protocol for long-term 3-D culture that recapitulates many aspects of the in vivo environment, we aim to generate a system that closely mimics the pathophysiology of AD. This will allow us to further uncover the mechanisms behind the pathology of familial and sporadic AD, and in turn, provide an accurate model for the development of treatments and therapies.

Poster 79

EFFECTS OF APOE E4 ON CHANGES IN WHITE MATTER HYPERINTENSITY VOLUME AND COGNITION IN OLDER ADULTS. Matijevic S, Walther K, Ryan L. University of Arizona; University of Erlangen; Arizona Alzheimer's Consortium.

Background: The apolipoprotein (APOE) $\epsilon 4$ allele is a strong risk factor for Alzheimer's Disease, and has been implicated in cognitive deficits in even healthy older adults. Evidence in the current literature is inconclusive as to whether the $\epsilon 4$ allele is a risk factor for developing white matter hyperintensities (WMH), which have likewise been linked to cognitive changes in aging. In the following study, we examined whether the presence of APOE $\epsilon 4$ influences changes in total WMH volume, memory and executive functioning in older adults.

Methods: We obtained neuropsychology measures and 3T MRI scans for 30 older adults (mean 70; 57-91 at time-point 1) at two time points across a 2-3 year period. Composite measures for memory and executive functioning were created to reduce data dimensionality. Total WMH volume was extracted from each subject's T2 FLAIR image. Statistical analyses were performed using the Repeated Measures General Linear Model in SPSS, with Age at time-point 1, Sex, and Delay between time-points entered as covariates.

Results: We found no significant difference between APOE $\epsilon 4$ carriers and non-carriers in the change in total WMH volume; however, there were significant Time x Age ($p < 0.05$) and Time x Sex ($p < 0.01$) interactions, and a between-subjects effect of Age ($p < 0.05$). Additionally, there were significant Time x APOE ($p < 0.01$) and Time x Sex ($p < 0.05$) interactions for the memory composite score. $\epsilon 4$ non-carriers increased their memory scores at time-point 2, presumably due to practice effects, whereas carriers remained consistent in their performance. There were no significant effects observed for the executive functioning score.

Conclusions: These preliminary results indicate that $\epsilon 4$ carriers may be selectively impaired in memory and not executive functioning. However, sex differences may serve as a potential confound, and warrant further investigation. Future analyses will also assess whether change in WMH volume might predict change in both memory and executive functioning.

Poster 80

3D WASSERSTEIN DISTANCE AS A UNIVARIATE NEUROIMAGING BIOMARKER FOR FDG-PET ANALYSIS. Mi L, Zhang W, Zhang J, Goradia D, Fan Y, Chen K, Reiman E, Gu X, Wang Y. Arizona State University; Stony Brook University; Banner Sun Health Research Institute; Arizona Alzheimer's Consortium.

Background: Fludeoxyglucose positron emission tomography (FDG-PET) is a functional imaging technique that is used to observe metabolic processes in human body, especially for early detection of Alzheimer's disease (AD). Neuroimaging biomarkers have shown their high sensitivity in tracking changes over time and thus were proposed as possible outcome measures for randomized clinical trials (RCT). There has been a desire for univariate biomarkers of FDG-PET images for neurodegenerative disease study. In this work, we develop a univariate biomarker based on 3D Wasserstein distance for FDG-PET analysis.

Methods: The Wasserstein distance has been used for measuring the similarity between two probability distributions. Our approach regards FDG-PET images as 3D distributions and computes their pairwise Wasserstein distances. First, we normalize and segment all the images by using Statistical Parametric Mapping (SPM). Then, we re-discretize the segmented images into their tetrahedral representation and map each of them into a unit sphere. After that, we compute the optimal transportation (OT) of each two unit spheres, which produces a Wasserstein distance. Finally, for each subject, we average its Wasserstein distances to all the CN subjects and treat the average as a univariate index for further analysis. We verify the potential of the obtained Wasserstein distance index in characterizing Alzheimer's disease by using both cross-sectional and longitudinal analysis on the Alzheimer's disease Neuroimaging Intuitive (ADNI) datasets.

Results: In the cross-sectional experiment involving 31 AD and 31 cognitively normal (CN) subjects, our proposed Wasserstein distance index achieves the classification accuracy of 77.42%, outperforming the hypo metabolic convergence index (HCI) which yields 75.81%. In the longitudinal experiment involving 30 AD and 30 CN subjects each having two FDG-PET scans spanning 2 years, our proposed Wasserstein distance index achieves a 5% significance with $p\text{-value} = 0.000013$ in one-sided t-test. Further more, by drawing the distance matrix, we find that the subjects with AD tend to form a similar pattern in terms of the Wasserstein distance index of their FDG-PET scans and yet the CN subjects' patterns do not converge into a single one. One explanation could be that the metabolic activities of AD subjects become similar as the disease progresses, but CN subjects have slight difference in their metabolic activities due to individual differences. Our method for computing the Wasserstein distance is 7 times faster than the traditional method.

Conclusions: Our proposed Wasserstein distance index is consistent with other studies. The statistical results show that the proposed univariate imaging biomarker is promising, as AD biomarkers for tracking AD progression and measuring responses to interventions. The application of the Wasserstein distance as an imaging biomarker to other modalities, such as diffusion MRI, may further improve pre-symptomatic diagnosis and treatment of Alzheimer's disease.

Poster 81

SEX DIFFERENCES IN METABOLIC AND NEUROLOGICAL OUTCOMES IN HUMANIZED APOE- ϵ 4 KNOCK-IN RAT MODEL. Mishra A, Yin F, Mao Z, Brinton RD. University of Southern California; University of Arizona; Arizona Alzheimer's Consortium.

Background: Women APOE- ϵ 4 carriers are at increased risk of developing Alzheimer's disease (AD) than men. Women APOE- ϵ 4 carriers are susceptible to accelerated aging and undergo faster rates of cognitive decline during Mild Cognitive Impairment (MCI). Using a novel rat model with humanized APOE- ϵ 4 gene knock-in, we conducted a longitudinal study spanning 9 months (between age 6-15 months) to characterize effect of sex and APOE- ϵ 4 genotype during the aging process by evaluating metabolic and neurological outcomes.

Methods: We used Sprague Dawley rats with humanized APOE- ϵ 4 knock-in from Horizon Discovery. For longitudinal analyses, we conducted micro PET-CT to measure glucose uptake and tail vein blood collection to estimate blood based metabolic markers- ketone bodies, triglycerides, insulin, lipidomics and inflammatory markers. Longitudinal analyses included comparisons between APOE- ϵ 4 male, female rats and Wild type (WT) male, female rats, across the following aging windows: 7-8 months (m), 9-10 m, 12-13 m and 15-16 m. Reproductive cyclicity in females was established by collection of vaginal lavages in APOE- ϵ 4 and WT rats at 6-7 m, 9-10 m and 12-13 m

Results: Longitudinal follow-up revealed in comparison to WT rats, APOE- ϵ 4 rats had significantly elevated plasma triglyceride levels across all aging windows. Male APOE- ϵ 4 rats had significantly higher triglyceride, ketone body and insulin levels across all aging comparisons. In APOE- ϵ 4 females, age related decline in insulin levels while increase in ketone body levels was evident. Glucose uptake, as determined by micro PET-CT, was significantly higher in APOE- ϵ 4 males than females at 9-10 m and 12-13 m. On undergoing the perimenopausal transition, APOE- ϵ 4 females experienced a significant decline in glucose uptake.

Conclusions: This longitudinal study helps in identifying aging windows in APOE- ϵ 4 carriers, which are predictive of metabolic and neurological changes. Female APOE- ϵ 4 rats demonstrate a state of bioenergetic deficit after the perimenopausal transition. Coincident increase in ketone body levels may reflect a metabolic state, which is shifting to a ketogenic system from a glucogenic system, and may be related to an increase in white matter catabolism. Grant support: National Institute on Aging (NIA) grants R01AG032236 and P01AG026572 to RDB, Paul Slavic Trust and Alzheimer's association.

Poster 82

DIFFERENTIAL EFFECTS OF HYPERTENSION STATUS AND WHITE MATTER HYPERINTENSITY VOLUME ON WHITE MATTER INTEGRITY IN OLDER ADULTS. Nguyen LA, Bharadwaj PK, Haws KA, Hishaw GA, Trouard TP, Alexander GE. University of Arizona; Arizona Alzheimer's Consortium.

Background: Healthy aging has been associated with a greater prevalence of vascular risk factors, such as hypertension and increased cerebral white matter hyperintensity (WMH) burden. It has been suggested that WMH may have effects that are distinct from the presence of hypertension, and previous research has not evaluated the effects of WMH on white matter integrity in the context of healthy cognitive aging. The effects of differences in hypertension status, WMH volume, and their interaction on white matter integrity measures in neurologically healthy older adults has yet to be fully investigated.

Methods: We sought to investigate whether hypertension status and WMH volume would differentially affect regional white matter integrity in a sample of 97 neurologically healthy community dwelling older adults, 70-89 years of age, with treated hypertension (N = 40) and without hypertension diagnosis or treatment (N = 57). MRI scans were acquired at 3T, including a volumetric T1, T2 FLAIR, and diffusion-weighted imaging. The volumes of WMHs were computed with a multispectral, automated lesion segmentation method to produce probability maps using Statistical Parametric Mapping (SPM12) and the lesion segmentation toolbox (LST; Schmidt et al., 2012). Diffusion-weighted MR images were processed using TRACULA with Freesurfer, which performs automated probabilistic tractography and generates estimates of fractional anisotropy (FA) and mean diffusivity (MD) for major white matter pathways (Yendiki et al., 2011). Corrections for multiple comparisons were performed using the false discovery rate with $p < 0.05$.

Results: The results indicated that greater WMH volume, but not hypertension status, was significantly associated with lower FA and greater MD in the bilateral inferior longitudinal fasciculus, bilateral superior longitudinal fasciculus temporal bundle, and right superior longitudinal fasciculus parietal bundle ($<0.00001 \leq p \leq 0.048$). Greater WMH volume was significantly associated with lower FA in forceps major ($p \leq 0.048$) and with greater MD in forceps minor, bilateral anterior thalamic radiation, left corticospinal tract, left superior longitudinal fasciculus parietal bundle, and left uncinate fasciculus ($<0.00001 \leq p \leq 0.0495$). Significant effects for MD, but not for FA, in relation to WMH remained after controlling for age. There were no interaction effects of hypertension status and WMH volume on white matter integrity.

Conclusions: Together, these findings suggest that vascular risk factors common in healthy aging may have a differential regional impact on white matter integrity in older adults. Further research is needed to evaluate the longitudinal effect of these vascular factors on metrics of white matter integrity and on cognition in the context of healthy cognitive aging.

Poster 83

CONTRASTING EFFECTS OF INDIVIDUAL VERSUS COMBINED ESTROGEN AND PROGESTOGEN REGIMENS ON COGNITIVE FUNCTION: ONE PLUS ONE DOES NOT EQUAL TWO. Prakapenka AV, Quihuis AM, Hiroi R, Carson C, Patel S, Croft C, Berns-Leone C, Fox C, Sirianni RW, Bimonte-Nelson HA. Arizona State University; Arizona Alzheimer's Consortium; Barrow Neurological Institute.

Background: There are various hormone therapy (HT) options available for women to diminish undesired physiological symptoms associated with menopause (e.g. hot flashes, vaginal atrophy). A commonly used estrogen in HT is 17 β -estradiol (E2), the most potent and naturally circulating estrogen in mammals. Studies show that E2 can benefit cognition, and that activation of extracellular signal-regulated kinase 2 (Erk2) is required for E2 to produce this effect. Estrogen-based HT for women with an intact uterus must also include a progestogen component to oppose the associated increase in risk for uterine cancer. Studies evaluating E2 plus natural progesterone treatment in ovariectomized (Ovx) rodents suggest that the addition of progesterone attenuates the beneficial cognitive effects of E2 and the increase in E2-induced Erk2 activation. Levonorgestrel (Levo) is a synthetic progestogen used in HT and contraceptives. We have previously shown that Levo treatment in middle-aged, Ovx rats enhanced cognitive performance. However, the effect of an E2 plus Levo hormone combination treatment on cognitive function has not yet been reported. This is translationally important given that E2 and Levo are administered in combination clinically, such as with the transdermal patch for HT (ClimaraPro). Thus, the aim of our study was to examine the effect of an E2 plus Levo treatment on cognitive function.

Methods: Middle-aged, Ovx rats were administered vehicle, E2 only, Levo only, or E2 plus Levo hormone treatment. Rats were then tested on a behavioral battery to assess spatial working and reference memory (water radial arm maze) and spatial reference memory (Morris water maze). Following behavior, brain regions involved in learning and memory were processed for western blot analysis of activated Erk2 expression.

Results: Results showed that both individual hormone treatments, E2 only and Levo only, enhanced learning on a working memory measure relative to vehicle control. Additionally, contrasting effects of hormone treatment were seen as the working memory demand increased, whereby at the moderate memory load, all hormone treatments enhanced performance, but at the highest working memory load, E2 plus Levo hormone combination treatment impaired performance relative to E2 only and Levo only. Preliminary analyses of activated Erk expression suggest that Erk2 activation in the frontal cortex is correlated with working memory performance, and that this relationship is impacted by whether E2 is given alone or in combination with Levo.

Conclusions: Taken together, results from this study suggest that E2 and Levo are acting through different mechanisms, resulting in contrasting effects on cognitive performance.

Poster 84

ASSOCIATIONS BETWEEN CARDIOVASCULAR RISK FACTORS AND COGNITION IN LATE MIDDLE AGE AND OLDER HISPANICS COMPARED TO NON-HISPANIC WHITES. Ryan L, Stickel A. University of Arizona; Arizona Alzheimer's Consortium.

Background: Cardiovascular health has strong associations with cognitive aging. The presence of one or more cardiovascular risk factors is associated with poorer cognitive (e.g., processing speed, executive functions, episodic memory) abilities compared to having fewer or no cardiovascular risk factors. In cohorts of Hispanics and non-Hispanic Whites with cardiovascular risk factors, Hispanics tended to live longer than Whites. This finding, known as the Hispanic paradox, is robust. However, the mechanisms underlying the Hispanic paradox are unknown, and it is unclear whether the Hispanic paradox confers protection on cognitive processes. The present study aimed to characterize differences and similarities in cognitive abilities in late-middle age and older Hispanics (n = 67) and non-Hispanic Whites (n = 67).

Methods: Participants were those identified as having no signs of dementia (n = 90) or mild cognitive impairment (n = 44) and were selected from the National Alzheimer's Coordinating Center (NAAC)* database. Hispanics and non-Hispanic Whites were matched on age (50-94 years, mean age = 72 years), gender, cognitive status (i.e., cognitively healthy versus MCI), hypertension, and apolipoprotein e4 status. Hispanics had higher body mass index (BMI) and fewer years of education, on average, than Non-Hispanic Whites. A neuropsychological battery of tests was administered to all participants. Tests of interest were Forward Digit Span, Backward Digit Span, Logical Memory Long Delay Recall, F-A-S (phonemic fluency), and Animals (semantic fluency), and the Boston Naming Test. In SPSS, univariate general linear models were performed to determine if the interaction between ethnicity and one of two cardiovascular risk factors (i.e., hypertension and BMI) predicted cognitive performance over and above the effects of age and education. Note, hypertension and BMI were not examined simultaneously.

Results: The associations between hypertension and BMI selectively differed between Hispanics and Non-Hispanic Whites, controlling for age and education. Hispanics with hypertension performed significantly worse on Animals compared to normotensive Hispanics and compared to both hypertensive groups of non-Hispanic Whites; the latter three groups did not significantly differ. BMI was related to working memory in Hispanics. In Hispanics, higher BMI was associated with lower Backward Digit Span scores (i.e., poorer working memory) but was unrelated to performance in non-Hispanic Whites. No other tests were predicted by the cardiovascular risk - ethnicity interactions. No main effects of hypertension nor BMI were detected.

Conclusions: Taken together, differences in cognition within Hispanics were more sensitive to cardiovascular health than in non-Hispanic Whites, suggesting no Hispanic Paradox-like protection on cognitive processes. Interestingly, the cardiovascular risk factors examined in the present study were predictive of specific tests and only predictive when interacting with ethnicity. Understanding how cardiovascular risk factors impact cognitive functions in various demographics is necessary for minimizing risk for cognitive impairment with age.

*The NACC database is funded by NIA/NIH Grant U01 AG016976.

Poster 85

THE IMPACT OF FAMILY HISTORY OF AD ON WHITE MATTER TRACT HEALTH. Ryan L, Stickel A, Gallegos N. University of Arizona; Arizona Alzheimer's Consortium.

Background: Alzheimer's disease (AD) is a multifactorial disease with many risk factors that are not well understood. Having a family history (FH) of AD is a risk factor that may impact the brain even before the diagnosis of AD. The uncinate fasciculus is a white matter tract that may be sensitive to Alzheimer's-related damage: Those with AD had poorer white matter integrity than those with mild cognitive impairment. While those with mild cognitive impairment had poorer white matter integrity compared to healthy controls (Kiuchi et al, 2009). However, it is unclear how healthy controls differ in white matter integrity based on FH of AD status. Our study sought to extend these findings and determine whether white matter integrity within the uncinate in late middle age and older adults (ages 53-86 years) with no signs of dementia differed between those who have a FH of AD (n=31) and those without a FH (n=30).

Methods: FH groups were matched on age, education, apolipoprotein e4status, and gender. Participants underwent magnetic resonance diffusion imaging. FSL was used to correct for eddy currents, removal of non-brain regions, and for calculation of eigen vectors and values. DTI-tk was used to convert the images into tensors and then used to extract fractional anisotropy (FA; a measure of white matter integrity) from diffusion images in the left and right uncinate fasciculus. The left and right uncinate fasciculus were extracted using Wakana's methods (Wakana et al, 2007). General linear models controlling for age and education were performed to determine if FH status predicted white matter integrity. Further analysis examined the interaction between education and FH status on uncinate white matter integrity, controlling for age. Participants were divided based on education level-- low-moderate education (an Associate's or lower degree; FH: n=5, controls: n = 11) and high education (more than an Associate's degree; FH: n=26; controls: n = 19).

Results: Our results showed no significant differences between FH groups when comparing FA measures in the left and right uncinate fasciculus, controlling for age and education. Those with FH of AD and low-moderate education had lower FA in the uncinate fasciculus than both education groups without a FH and even compared to those with a FH who had higher education. The latter three groups did not differ in uncinate white matter integrity.

Conclusions: Taken together, these results suggest that education has a protective effect on white matter integrity in those at greater risk for developing AD (i.e., those with a FH of AD). Replication with a greater sample size, particularly in the low education groups, is needed to confirm our findings. Analysis of other tracts is needed to determine if the interaction between FH and education has broad or specific impacts on white matter tracts. This study contributes to our understanding of how a family history interacts with education to impact white matter integrity. Investigations of preclinical symptoms of AD are important for understanding the development of AD and may aid in discovering ways to minimize risk of developing AD.

This project was funded by the Arizona Alzheimer's Association and McKnight Brain Institute. Nathaniel Gallegos is funded by the Western Alliance to Expand Student Opportunities, Neuroscience and Cognitive Summer Research Program, and Undergraduate Biology Research Program. Ariana Stickel is funded by the Ford Foundation and National Science Foundation.

Poster 86

CHARACTERIZATION OF ASTROCYTIC CIRCULAR RNAS IN LATE-ONSET ALZHEIMER'S DISEASE BRAINS. Sekar S, McDonald J, Cuyugan L, Mastroeni D, Liang WS. Translational Genomics Research Institute; Arizona State University; Arizona Alzheimer's Consortium.

Background: Circular RNAs (circRNAs) are a class of endogenous, non-coding RNAs that form covalently closed continuous loops and are pervasively expressed in the eukaryotic transcriptome. Although circRNAs have been found to possess potential microRNA regulatory roles and are enriched in the mammalian brain, they have not been widely characterized in the context of diseases. Given the previous evidence of astrocyte-specific contributions to Alzheimer's disease (AD), here we aim to characterize astrocytic circRNAs in the context of AD.

Methods: We laser capture microdissected astrocytes from the posterior cingulate (PC; N=10 AD, 10 controls), hippocampus (HIPP; N=6 AD, 6 controls) and substantia nigra (SN; N=6 AD, 6 controls) of clinically classified late-onset AD (LOAD) subjects and no disease (ND) healthy elderly controls. We then performed RNAseq on these samples on an Illumina HiSeq 2000. The raw fastqs generated from sequencing were run through 6 different circRNA prediction algorithms: find_circ, CIRI, DCC, Mapsplice, KNIFE and CIRCexplorer. CircRNAs unique and common to the AD and ND groups in each region were then parsed using custom bash, python and R scripts and annotated using bedtools and UCSC gene annotations.

Results: We sequenced an average of 192,081,648 reads for the PC samples, 54,121,880 reads for the HIPP samples and 62,854,856 reads for the SN samples. In total, 2,375 and 2,380 circRNAs were predicted across all tools in the AD and ND samples respectively. All 6 tools predicted 62 and 73 circRNAs in AD and ND PC samples, 35 and 15 circRNAs in AD and ND HIPP samples, and 15 and 20 circRNAs in AD and ND SN samples respectively. A widely reported circular RNA derived from the CDR1 (cerebellar degeneration-related protein 1) gene was detected across all 3 brain regions. CDR1 is overexpressed in the peripheral blood leukocytes of AD subjects, and the protein was identified in patients with paraneoplastic cerebellar degeneration. We also detected circRNAs generated from genes implicated in AD and other neurological diseases, such as insulin like growth factor 2 receptor (IGF2R), Fas apoptotic inhibitory molecule 2 (FAIM2) and bromodomain PHD finger transcription factor (BPTF). Overexpression of the receptor encoded by IGF2R can lead to an increase in the levels of amyloid precursor protein. FAIM2 confers neuronal protection in mouse models of ischemia and is regulated during the course of bacterial meningitis. In the brains of AD patients, the BPTF protein is localized in a subset of amyloid-containing plaques and its expression was found to be higher in neurodegenerative diseases.

Conclusions: In this study, we evaluated the abundance of astrocytic circRNAs in LOAD samples and healthy controls in 3 different brain regions, and demonstrate the feasibility of performing circRNA detection in whole transcriptome data using bioinformatics algorithms. Though the relevance of circRNAs in the context of AD is not well understood, we observe that certain key genes give rise to circRNAs to suggest that they could have a potential role in various cellular processes in AD. However, further functional studies are required to elucidate the role of circRNAs in AD pathogenicity and their relevance in other neurodevelopmental diseases.

Poster 87

TRACKING ALZHEIMER'S DISEASE PROGRESSION BY NON-LINEAR DIMENSION REDUCTION OF BRAIN MRI FEATURES. Seo K, Pan R, Chen K. Arizona State University; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: In addition to its potential high accuracy in diagnosis and sensitivity in tracking changes, an overall global index summarizing multiple complicated features from neuroimages should also be practically intuitive and logically explainable in the study of Alzheimer's disease. In this research, we propose a new global index, derived from non-linear dimension reduction of brain MRI features, to track AD progression over time.

Methods: Previous studies have shown that high dimensional brain MRI features could be represented well in low dimensional space by using manifold based dimension reduction techniques. In this research, we apply locally linear embedding (LLE) to a dataset from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, which includes total of 346 volumes of brain regions and the cortical thickness of 562 subjects. Each subject has 2 to 8 MRI scans over time and about 20% of them have progressed to the next level of dementia. Among the original features, 59 most important features to the diagnosis are selected by the intraclass correlation coefficient. Based on the baseline data of 177 Cognitively Normal (CN) and 110 AD subjects, LLE can reduce the feature dimension to two and the probability of belonging to AD category can be assigned to each subject by a classifier. Using this baseline template, a subject's AD progression path can be depicted in the two-dimensional LLE feature space with the probability estimated from nearest neighbors of baseline LLE template.

Results: The baseline template was constructed by two LLE coordinates and each patient's probability of AD was obtained by Support Vector Machines (SVM) with Gaussian kernel using all 59 features, which showed a sensitivity of 0.836 and specificity of 0.955 for CN and AD classification by using 10 fold cross validation. We found out that the baseline information based template can then be applied to examine the longitudinal changes over time for individual subjects. For example, when new data points of a Mild Cognitive Impairment (MCI) subject were projected into the baseline template by averaging the LLE coordinates and probabilities of nearest neighbors with suitable weights. The result shows how the MCI subject's disease progresses in terms of not only the location in two-dimensional space but also the probability of AD.

Conclusions: The proposed LLE nonlinear dimension reduction method can reduce the high dimensional brain MRI features to low space so that a patient's AD progression can be easily visualized. Meanwhile, the probability measure is calculated by SVM with fuller features so that the data points also contains information of higher dimensional features, and it leads to minimum loss of prediction power. Along with other clinical tests, this new approach has potential to integrate multiple features to assist in tracking AD progression.

3D-PATCH ANALYSIS BASED SPARSE CODING SYSTEM FOR ALZHEIMER'S CLINICAL GROUP CLASSIFICATION. Srivastava A, Singh S, Zhang J, Mi L, Goradia D, Chen K, Reiman EM, Wang Y. Arizona State University; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: Alzheimer's Disease(AD), is a chronic neurodegenerative disease in which amyloid plaques and neurofibrillary tangle accumulate in the brain. There has been a shift with urgency to find effective intervention in the pre-symptomatic stages of AD to delay the progression or even prevent its onset. To address this challenge, computer-based diagnostic classification is increasingly needed using biomarkers based on neuroimaging or other measures. For a classification algorithm based on 3D FDG-PET images the feature dimension is usually much larger than the number of subjects. With the presence of a large number of features, a learning model tends to over fit, affecting its performance. Sparse coding has proven in our earlier studies as an effective method for learning sparse representation of features. We study Sparse coding as feature learning and Principle Component Analysis (PCA) for feature extraction and compare our findings.

Methods: We studied FDG-PET from the Alzheimer's Disease Neuroimaging Initiative 2 (ADNI2), which extends the work of two previous studies (ADNI1 [1] and ADNI-GO [1]). Our experimental dataset consists of baseline data of patients including 186 healthy control (CU), 158 Early Mild Cognitive Impairment (EMCI), 178 Late Mild Cognitive Impairment (LMCI), and 146 (AD).

The FDG-PET images were processed using SPM [3], for alignment, segmentation and normalization. Then we constructed overlapping 3D patches over each data sample, and performed two types of analysis, first we used Stochastic Coordinate Coding(SCC)[1] as a feature learning approach for obtaining a Dictionary and Sparse Codes after learning the 1500D Dictionary we agglomerate the features obtained by max-pooling. We then use Ada-boost [1] as a classifier to perform binary classification over six experiments. Next we take overlapping patches and down sample the image volume again using max-pooling. Next we take overlapping patches and down-sample the image volume again using max-pooling. Theoretically we can perform classification on the selected features but the number of features is very high compared to the sample size, which might cause the data to over-fit (curse of dimensionality). To overcome this issue, we use a number of feature extraction methods like LDA, PCA and SVD and perform classification using Ada-Boost. For both our analysis we used 10-fold cross validation to estimate the Accuracy, Precision, Recall and AUC. We performed 6 different classification experiments for both analysis technique using the technique described above.

Results: We performed six classification experiments for the following comparisons: (1) AD vs. CU (2) AD vs EMCI (3) AD vs LMCI (4) CU vs LMCI (5) CU vs EMCI (6) EMCI vs LMCI. The F1-Score, Precision, Recall, AUC and ROC curves were calculated to compare results. For AD vs CU with SCC we obtained a F1-Score of 0.77 and with LDA + AdaBoost we obtained F1-Score of 0.91.

Conclusions: We performed binary classification experiments on FDG-PET images using pooling, feature extraction, and sparse coding combined with pooling and achieved competitive results. Our work may help expand our understanding of the sensitivity of FDG-FDA image in AD research and may potentially guide and improve our future FDG-PET based imaging biomarker research.

Poster 89

CENTRAL INSULIN RESISTANCE PRECEDES PERIPHERAL INSULIN RESISTANCE IN TWO MOUSE MODELS OF ALZHEIMER'S DISEASE. Tran A, Velazquez Ramon, Dave N, Ishimwe E, Denner L, Dineley KT, Oddo S. Arizona State University; University of Texas Medical Branch at Galveston (UTMB); Arizona State University; Arizona Alzheimer's Consortium.

Background: There are several risk factors that contribute to Alzheimer's disease (AD), with type 2 diabetes (T2D) being one of the most prevalent. Insulin resistance is physiologically reflected by hyperglycemia and impaired glucose clearance. Moreover, central insulin signaling dysregulation has been demonstrated in post-mortem tissue of subjects with both mild cognitive impairment (MCI) and early AD. However, it is not certain whether central insulin signaling dysregulation changes occur before or after peripheral insulin resistance. Here, we examined peripheral glucose metabolism and CNS insulin signaling in the Tg2576 and 3xTg-AD mouse model of AD at ages both prior to and after the onset of AD-neuropathology.

Methods: Published data has shown that peripheral insulin resistance is present in 9-month-old Tg2576 but not at 5-months. Here, we assessed peripheral insulin resistance via a glucose tolerance test in 10- and 16-month-old 3xTg-AD and age-matched wildtype (WT) mice. Mice were overnight fasted for 16 hours then weighed the following morning and tested for baseline fasting blood glucose levels. Next, 5- and 9-month old Tg2576 and 10- and 16- month old 3xTg-AD mice received a 2 mg/kg glucose injection into the intraperitoneal cavity, and blood glucose was sampled at 15min intervals until 210min post-injection. All brain tissue was collected from and assayed, via western blot, for various epitopes of IRS-1, PDK1, AKT, and GSK-3 β , as these components of the CNS insulin signaling pathway have been found to undergo altered function in AD.

Results: Fasting glucose levels at 10 months of age were not significantly different between WT and 3xTg-AD mice (101.12 mg/dL vs.105.45 mg/dL, respectively), nor at any of the 15min intervals after the bolus glucose injection. In contrast, 16-month-old 3xTg-AD mice had significantly elevated fasting glucose levels (132.154 mg/dL) compared to WT mice (82.727 mg/dL) and displayed impairments in their ability to restore their elevated glucose levels. Notably, the older age groups of 3xTg-AD and Tg2576 mice showed reductions in IRS-1 levels, collectively illustrating dysregulated IRS-1 activity. Additionally, downstream target PDK1, was dysregulated in both 5-month-old Tg2576 and 10-month-old 3xTg-AD mice, both prior to peripheral insulin resistance. AKT was dysregulated in both mouse models after the onset of peripheral insulin resistance. Lastly, GSK-3 β , a convergent target of the PDK1/AKT pathways and a key negative regulator of IRS-1, was upregulated consecutively with peripheral insulin resistance.

Conclusions: Our data suggest an age-dependent progression of CNS insulin dysregulation that precedes peripheral insulin resistance. Interestingly, the onset of dysfunctional central insulin signaling coincides with the onset of cognitive impairment described for these two preclinical models of AD. These results suggest that early AD may reflect engagement of different signaling networks that influence CNS metabolism, which in-turn alter peripheral insulin signaling. Peripheral insulin resistance may further contribute to brain pathology and functional abnormalities in AD. Collectively, this work suggests that reducing CNS insulin resistance in patients with MCI or early stage AD may help reduce peripheral insulin resistance.

Poster 90

ESTROGEN REGULATION OF MITOCHONDRIAL RESPIRATION IS CELL TYPE AND ER SUBTYPE SPECIFIC. Wang Y, Brinton RD. University of Southern California; University of Arizona; Arizona Alzheimer's Consortium.

Background: Human mitochondrial genome contains 37 genes, including 13 protein-encoding genes, which are all core subunits belonging to complexes I, III, IV, or V of the electron transport chain. Alterations in gene expression can lead to changes in cellular respiration and bioenergetics, which are implicated in multiple neurodegenerative diseases including Alzheimer's disease. Compared to males, females over 60 years old are at greater risk for Alzheimer's disease. Here we present the effects of endocrine transition during perimenopause and chronological aging on mitochondrial gene expression in female brain.

Methods: We used a rat model recapitulating fundamental characteristics of the human perimenopause. Specifically, female Sprague-Dawley rats between 9-10 months old were classified as either regular cycling, irregular cycling, or acyclic based on their estrus status. 6-month-old regular cycling and 16-month-old acyclic rats were included to distinguish the effects of chronological aging from endocrine aging. Changes in gene and protein expression were assessed using rtPCR and western blot respectively, and potential upstream regulators and signaling pathways were identified by RNAseq.

Results: We observed that in the hippocampus, MT-ND3 (complex I), MT-CYB (complex III), and MT-ATP6 (complex V) had significantly lower expression in both irregular and acyclic 9-month-old animals comparing to regular cyclic 9 month ones, and MT-CO1, MT-CO2, and MT-CO3 had significantly lower expression in acyclic 9-month old animals compared to regular cyclers. Although other protein coding genes did not show statistically significant differences, they did share a similar trend in decreased gene expression. In terms of chronological aging, relative to 6 month old female rats 9 month old animals exhibited mitochondrial genes were generally up-regulated in hippocampus, whereas a decline in mitochondrial gene expression occurred by 16 months of age. In contrast, the cerebral cortex exhibited a different pattern of gene expression. During perimenopause, mitochondrial gene expression patterns in irregular cycling and acyclic rats were not significantly different from regular cyclers. Rather than have a surge in expression at 9 month as seen in hippocampus, mitochondrial gene expression in cortex continuously decreased as animals aged, and at 16-month-old, 8 out of 13 protein coding genes spanning electron transport chain complexes I, III, IV, and V were significantly lower level compared to 6-month-old animals.

Conclusions: Our data suggest that in the hippocampus, mitochondrial gene expression is sensitive to both endocrine and chronological aging. We have also shown regional differences in mitochondrial gene expression between the chronological and endocrine programs with the hippocampus exhibiting both chronological and endocrine aging whereas the cerebral cortex exhibited only chronological aging.

Poster 91

MULTI-TASK DICTIONARY LEARNING FOR PREDICTING FUTURE COGNITIVE DECLINE. Zhang J, Li Q, Caselli RJ, Thompson PM, Ye J, Wang Y. Arizona State University; Mayo Clinic Arizona; University of Southern California; University of Michigan; Arizona Alzheimer's Consortium.

Background: Alzheimer's Disease (AD) is the most common type of dementia. Identifying correct biomarkers may determine pre-symptomatic AD subjects and enable early intervention. Recently, Multi-task sparse feature learning has been successfully applied to many computer vision and biomedical informatics researches. It aims to improve the generalization performance by exploiting the shared features among different tasks.

Methods: We studied longitudinal brain MRI from the Alzheimer's Disease Neuroimaging Initiative (ADNI), a study of brain aging and AD carried out at sites across North America. In our experiments, we analyzed a Longitudinal dataset consisting of brain MRI scans from adults, aged 55 to 90, including baseline (837) and 6-month (733) datasets. For the experiments, we used hippocampal surface multivariate statistics as learning features, which is a 4*1 vector on each vertex of 15000 vertices on every hippocampal surface. We propose a novel integrated unsupervised framework, termed Multi-task Dictionary Learning (MTDL) algorithm, we utilize shared and individual dictionaries to encode both consistent and changing imaging features along longitudinal time points. Our experimental results outperform some other state-of-the-art methods and demonstrate the effectiveness of the proposed algorithm.

Results: We compared MTDL with multiple state-of-the-art methods, ODL-L: the single-task online dictionary learning followed by Lasso, L21: the multi-task method called L21 norm regularization with least square loss. TGL: the disease multi-task progression model called Temporal group Lasso, as well as Ridge and Lasso.

The proposed approach MTDL outperformed ODL-L, Lasso and Ridge, in terms of both rMSE and correlation coefficient wR on four different time points. The results of Lasso and Ridge are very close while sparse coding methods are superior to them. For sparse coding models, we observe that MTDL obtained a lower rMSE and higher correlation result than traditional sparse coding method ODL-L since we consider the correlation between different time slots for different tasks and the relationship with different time points on the same patient among all tasks. We also notice that the proposed MTDL's significant accuracy improvement for later time points. This may be due to the data sparseness in later time points, as the proposed sparsity-inducing models are expected to achieve better prediction performance in this case.

We follow the same experimental procedure in the MMSE study and explore the prediction model by ADAS-cog scores. We observe that the best performance of predicting scores of ADAS-Cog is achieved by MTDL for four time points. Comparing with L21, after MTDL dealing with missing label, the results more linear, reasonable and accurate. Although the result of MTDL had bias, MTDL still achieved the best result compared with the other five methods on predicting both MMSE and ADAS-cog, which shows our method is more efficient about dealing with missing data.

Conclusions: We propose a novel Multi-task Dictionary Learning for modeling cognitive decline, which allows simultaneous selections of a common set of biomarkers for multiple time points and specific sets of biomarkers for different time points using dictionary learning.

Poster 92

GENETIC INFLUENCE OF APOE4 GEONTYPE ON MORPHOMETRIC ANALYSIS OF HIPPOCAMPUS AND LATERAL VENTRICLE IN COGNITIVELY INTACT PERSONS. Zhang W, Li B, Wu J, Schron EH, McMahon T, Shi J, Gutman BA, Chen K, Baxter LC, Thompson PM, Reiman EM, Caselli RJ, Wang Y. Arizona State University; Wellesley College; University of Southern California; Banner Alzheimer's Institute and Banner Good Samaritan PET Center; Mayo Clinic Arizona; Arizona Alzheimer's Consortium.

Background: Previous research of the brain structural changes in Alzheimer's disease (AD) attempted to find the biologically meaningful surrogates of AD from neuroimaging data to improve the AD diagnosis sensitivity and facilitate effective presymptomatic diagnosis and treatment of AD. As the major genetic risk factor for late onset AD, the apolipoprotein E (ApoE) e4 allele is associated with an increased risk of AD where the higher dose (the number of e4 alleles in a person's APOE genotype) the more chance to suffer from AD. In this study, we aim to characterize the morphometric changes of AD-related brain regions, e.g. hippocampus and lateral ventricle, with respect to the different ApoE e4 gene dose in cognitively normal elder.

Methods: The hippocampus was segmented from T1 images by using FSL software package. For ventricular segmentation, in this research, we used a novel pipeline that computed a group-wised ventricular template and used it to accurately segment continuous ventricular structures. Based on segmented binary volume masks, we extracted hippocampal and ventricular boundaries with a topology preserving level-set method and constructed triangular surface meshes with marching cubes algorithm. Later, the surfaces were further smoothed using a two-step mesh smoothing method which has been proved to be feature-preserving meanwhile effectively reduce the noise and partial volume effect. Next, a surface fluid registration algorithm was applied to non-linearly register the grid surfaces to a common template. Finally, we extracted the morphometric features with the reference of both the mTBM and the radial distance, named MADMTBM statistics. The Hotelling's T2 test was performed to evaluate the morphometric variations of hippocampal and ventricular surfaces between declining controls and stable control group on each vertex. We conducted the statistical test for both cross-sectional and longitudinal analysis.

Results: 115 normal subjects from Arizona APOE cohort were included in this study including 39 e4 non-carriers (e3/e3), 38 APOE e4 heterozygotes (e3/e4) and 38 APOE e4 homozygotes (e4/e4). We studied the cross sectional data on both baseline and 24-month follow up data. We also studied APOE e4 effects by studying the longitudinal atrophy rates.

In both longitudinal and cross sectional analysis, we observed the group differences in left hippocampus and lateral ventricle after correcting for multiple comparisons. APOE e4 homozygotes plays an important role in altering structural shape in those two regions. What's more, revealed by the longitudinal studies, patterns of APOE e4 genes effect are quite different. We found that morphological variation of hippocampus appears in heterozygotes and homozygotes but failed to found the similar trend in lateral ventricle.

Conclusions: In this study, we found that the APOE e4 genotype affects human brain structure in the normal developed individuals and this type of influence has different speed of controlling brain development. Our finding supports the idea that ApoE e4 allele as a risk gene factor of dementia also affect the normal human brain aging in two AD-related brain regions. This observation suggest the role of ApoE e4 with the reference of biological markers, e.g. hippocampal or lateral ventricular shape, in the AD early diagnosis and prevention therapies in the future.

Poster 93

DEEP LEARNING BASED CLASSIFICATION OF PET IMAGING DATA FOR ALZHEIMER'S DIAGNOSTIC CATEGORIES. Singh S, Srivastava A, Mi L, Thompson P, Reiman EM, Chen K, Goradia D, Wang Y. Arizona State University; Banner Alzheimer's Institute; Arizona Alzheimer's Consortium.

Background: Alzheimer's Disease(AD) [1], a neurodegenerative disease is a progressive disease that affects the brain gradually with time and worsens. Reliable and early diagnosis of AD and its prodromal stages (i.e. Mild Cognitive Impairment(MCI)) is essential. Deep learning has recently been applied to the analysis of structural and functional brain imaging data [2]. Here we introduce a deep learning based classification using neural networks with dimensionality reduction techniques to classify the different stages of AD based on FDG-PET image analysis.

Methods: We studied FDG-PET [1] from the Alzheimer's Disease Neuroimaging Initiative 2 (ADNI2), which extends the work of two previous studies (ADNI1 [1] and ADNI-GO [1]). Our experimental dataset consists of baseline data of patients including 186 healthy control (CU), 336 Mild Cognitive Impairment (MCI) with 158 Late MCI and 178 Early MCI, and 146 AD.

The FDG-PET images were processed using SPM [3], for alignment, segmentation and normalization. Then we constructed overlapping 3D patches over each data sample, and performed two types of pooling (Max pooling and Mean pooling) [4] to represent each data sample as a vector. The vectors were all concatenated to form a data matrix, labels were assigned to each vector (AD, EMCI, LMCI and CU). This way we had two different types of datasets (one with max pooling and one with mean pooling over patches). Dimensionality reduction using Probabilistic Principal Component Analysis (PCA) [5] was applied to the data matrix. The reduced data matrix and corresponding labels were then passed to a Multilayer Feed Forward Neural Network (with varying number of hidden layers, number of neurons and feature dimensions for some experiments). The optimizer used was the Adam optimizer [6] and Rectified Linear Units were used for activation in the neurons. The Multilayer Feed Forward Neural network was applied to 8 different binary classification problems. 10-fold cross validation was used to estimate the accuracy, precision, recall, positive and negative predictive values. We performed 8 different classification experiments using the technique described above.

Results: We performed eight classification experiments for the following comparisons: (1) AD vs. CU (2) AD vs. MCI (3) CU vs MCI (4) AD vs EMCI (5) AD vs LMCI (6) CU vs LMCI (7) CU vs EMCI (8) EMCI vs LMCI. The F1-measure, precision, recall, positive predictive value, and negative predictive value were calculated to compare results.

With our method with Mean Pooling over patches, we achieved accuracies of 0.93, 0.86, 0.73, 0.85, 0.74, 0.63, 0.57 and 0.61. With the Max Pooling over patches, we achieved accuracies of 0.92, 0.86, 0.75, 0.82, 0.72, 0.70, 0.61 and 0.64 respectively.

Conclusions: We performed binary classification experiments on FDG-PET images using pooling techniques, Probabilistic Principal Component Analysis and Artificial Neural Networks to improve classification accuracies for the 8 experiments.

Poster 94

UNCINATE FASCICULUS INTEGRITY ASSESSED IN YOUNG AND AGED BONNET MACAQUE MONKEYS. Umopathy L, Gray DT, Burke SN, Trouard TP, Barnes CA.

University of Arizona; University of Florida; Arizona Alzheimer's Consortium.

Background: Cognitive aging is known to alter reward-guided behavior, implicating dysfunction of pre-frontal cortical circuits including the orbitofrontal cortex (OFC). A decline in OFC volume with age has been reported to correlate with performance on a reward devaluation task in bonnet macaques. In that study, the volume of the basolateral amygdala (BLA) did not change with age. In the present study, the integrity of the uncinat fasciculus (UF), the primary white matter tract between the BLA and OFC is examined in a group of 11 healthy adult female bonnet macaques using high angular resolution diffusion MRI scans (HARDI) along with anatomical T1- and T2-weighted images.

Methods: High angular resolution diffusion-weighted images were acquired on 6 young and 5 aged bonnet macaque monkeys using a single-shot echo planar imaging (EPI) sequence with a diffusion-weighting of $b=1000$ s/mm² along 51 diffusion directions. Six images were also acquired with no diffusion weighting i.e., $b=0$ s/mm² with an isotropic resolution of 1.4 mm. Anatomical T1 (MPRAGE) and T2-weighted images were also acquired for reference. Diffusion-weighted images were corrected for distortions due to eddy currents using FSL's FDT toolbox and field-inhomogeneity using TORTOISE. This was followed by a local principal component analysis based noise removal. The processed images were registered to the anatomical T1-weighted images using a rigid body transformation.

FSL's BEDPOSTX was used for probabilistic tractography to identify tracts between the BLA and the OFC. Exclusion masks were identified to eliminate tracts that cross between the hemispheres or continue posterior to the BLA. An uncinat fasciculus inclusion region of interest was also identified. Tractography in FSL yields a connectivity map where the intensity of each voxel corresponds to the number of streamlines that pass through the voxel and reach the target OFC mask. A probability map is generated by dividing the connectivity map with the total number of tracts identified between OFC and BLA. The value of each voxel in the probability map is the probability of the voxel belonging to UF. A threshold is applied to eliminate voxels that have a low probability of belonging to UF ($< 40\%$ of the 95th percentile of the probability map). This thresholded probability map is used to extract parameters of interest such as fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD) along the UF.

A modified Wisconsin General Testing Apparatus was used to test the behavioral performance of these young and aged monkeys. Specifically, the monkeys were tested on a delayed response (DR) working memory task, reversal learning task (RL, affective shifting) and delayed nonmatching-to-sample (DNMS) with interference task.

Results: A significant reduction in the fractional anisotropy and axial diffusivity index was observed with increased age, along the uncinat fasciculus tract that connects the BLA and OFC. By contrast, the radial diffusivity unexpectedly reduced with age. A significant positive relationship between the FA index and the AD index with the delayed response task was also observed.

Conclusions: Using high angular resolution diffusion MRI, the white matter integrity along uncinat fasciculus was assessed in a group of young and aged bonnet macaque monkeys. The results suggest that disruptions in the interaction between the OFC and the BLA may contribute to certain age-related cognitive deficits.

Poster 95

ENABLING INTEGRATION OF ALZHEIMER DISEASE (AD) PREVENTION STUDY DATA BY APPLICATION OF CDISC STANDARDS: EXTENSION TO BIOMETRIC MONITORING DEVICE (BMD) ASSESSMENTS. Arneric SP, Kern VD, Neville J, Kaye J, Karlin D, Rhodes J, Hill D, Dorsey R, Spencer R, Nelson B, Ibara M, Mohler J, Ryan L, Barnett J, Hudson L. Critical Path Institute; University of Oregon; Pfizer; Biogen; IXICO; University of Rochester; University of Massachusetts; Clinical Data Interchange Consortium(CDISC); University of Arizona; Cambridge Cognition; Arizona Alzheimer's Consortium.

Background: In December 2016, FDA initiated the requirement of submitting data conforming to CDISC standards for all new drug applications, indicating that clinical trials initiated at the present time must adopt these standards. CDISC standards facilitate aggregation of clinical data from diverse sources, which has enabled the FDA to improve the efficiency of integrated data analyses for new drug applications by ~40%. Current clinical instruments are not sufficiently sensitive to detect changes in the pre-symptomatic stages of the disease. Biometric Monitoring Devices (e.g., wearables, smartphones, remote monitoring biosensors) offer the potential of measuring biologic events that may predict disease progression, or monitor key biologic functions in response to treatment interventions. The Coalition Against Major Diseases (CAMD), a nonprofit public-private-partnership within the Critical Path Institute focused on delivering Drug Development Tools that accelerate innovative treatments for Alzheimer disease (AD), is coordinating the development of these standards for integration into registration trials.

Methods: A partnership/collaboration between C-Path and the Arizona Alzheimer's Consortium was formed in 2016 to lay the foundation for additional data standardization and integration of data from prevention trials. On March 10, 2017, an international workshop was held to: 1) Evaluate the existing standards that apply to mobile devices that could be implemented in clinical drug trials and longitudinal disease progression studies; 2) Identify/prioritize existing gaps; 3) Develop a plan to accelerate the creation/implementation of CDISC standards required for future registration studies that assess three concepts-of-interest: mobility/frailty, sleep, and cognitive performance.

Results: High-level considerations were developed for all three concepts-of-interest (shown below is the map for cognition). Many fundamental aspects of standards development already exist. Additional work needs to be completed in detailing data provenance, annotating contextual metadata, and compliance with Good Clinical Practices (GCP).

Conclusions: Capturing critical contextual metadata to enable interpretation requires attention. Use of uniform terminology regarding sensor, device, and measurement should be a focus. High priority should be given to understanding, which functional outcomes patients and caregivers prefer. While the payer's perspective should not be ignored, this topic could be a later priority when alignment with regulators has been achieved.

Poster 96

MENOPAUSE AND THE AGING BRAIN: RELATIONSHIPS AMONG OVARIAN HORMONE LEVELS, MEMORY, AND CHOLINE ACETYLTRANSFERASE-CONTAINING NEURONS IN THE BASAL FOREBRAIN. Koebele SV, Mennenga SE, Patel S, Hiroi R, Hewitt LT, Quihuis AM, Mayer LP, Dyer CA, Demers LM, Bimonte-Nelson HA. Arizona State University; Arizona Alzheimer's Consortium; SenesTech, Inc.; The Pennsylvania State University College of Medicine.

Background: Memory changes during the menopause transition can negatively impact quality of life in women. These alterations in memory function may be related to the characteristic erratic fluctuations followed by the decline in ovarian hormone levels, including estrogens, observed during the perimenopausal period (Sherwin, 2012). Using the 4-vinylcyclohexene diepoxide (VCD) rat transitional menopause model, we investigated menopause- and age- related changes in choline acetyltransferase (ChAT) in the basal forebrain (BF), a primary synthesis site for acetylcholine. The BF is important for cognitive function, as these cholinergic neurons send long-range projections to the hippocampus, a key structure in spatial cognition. It is well established that 17-estradiol (E2) treatment increases BF ChAT levels (Luine, 1985; Gibbs, 1997, 2000) and BF lesions impair spatial memory and prevent E2-induced memory enhancements (Hagan et al., 1988; Gibbs, 1998, 2002). Thus, fluctuating ovarian hormone levels during the transition to menopause may impact acetylcholine synthesis and the BF-hippocampal cholinergic pathway.

Methods: Young (6 mo) and Middle-Aged (12 mo) Fischer-344 rats were trained on a water radial-arm maze (WRAM). Following training, rats were administered Vehicle or VCD treatment, which accelerates depletion of ovarian follicle reserve. Rats were then repeatedly tested on the WRAM for four months, across the menopausal transition to a follicle-deplete state. A subset of rats was sacrificed early in the menopausal transition to evaluate physiological changes that occur early in perimenopause. The remaining rats were sacrificed after six months, when VCD-treated rats were post-follicular depletion. The BF was stained for ChAT-immunoreactive (IR) cells, and unbiased stereology was used to estimate ChAT-IR populations in the medial septum and vertical/diagonal bands.

Results: Preliminary results suggest that the ovarian hormone fluctuations associated with follicle depletion are related to ChAT-IR BF estimates, particularly during the early menopause transition time point. Further, the relationship between ovarian hormones and ChAT-IR BF estimates changes with both aging and follicular depletion. Dynamic relationships between hormone levels and ChAT-IR estimates with memory performance will be discussed.

Conclusions: Understanding the neurobiological changes that occur early in the menopause transition period may help elucidate a critical window for hormone intervention in at-risk women so they can maintain a high quality of life, and the possibility to postpone or prevent the development of cognitive impairment or dementia later in life.

Additional Abstracts

DELIVERY OF 17 β -ESTRADIOL USING POLY(LACTIC-CO-GLYCOLIC ACID) NANOPARTICLES FOR COGNITIVE THERAPY IN A MODEL OF SURGICAL MENOPAUSE. Prakapenka AV, Quihuis AM, Hiroi R, Carson CG, Patel S, Chung E, DiPerna DM, Bimonte-Nelson HA, Sirianni RW. Arizona State University; Arizona Alzheimer's Consortium; Barrow Neurological Institute.

Background: All women undergo menopause at the average age of 51 years. There is a marked decrease in ovarian hormone secretion (estrogens and progesterone) with menopause, and these changes can be associated with several undesired symptoms (i.e. hot flashes, cognitive changes). Some women choose to take hormone therapy to decrease the presence and severity of these symptoms. A common estrogen used in hormone therapy is 17 β -estradiol (E2). E2 is the most potent endogenous estrogen, and studies have shown that E2 can play a beneficial role in cognitive performance following ovarian hormone loss. Once E2 is administered systemically, it gets rapidly metabolized and cleared from the system. Poly(lactic-co-glycolic acid) nanoparticles (PLGA NPs) can be used to encapsulate a hydrophobic agent, such as E2, to prolong its circulation time by protecting the agent from fast metabolism and systemic clearance. We hypothesized that sustained delivery of E2 from PLGA NPs would enhance cognition in ovariectomized, middle-aged rats relative to free E2.

Methods: First, biodistribution studies evaluated two routes of administration (intranasal and subcutaneous (SQ)) for payload delivery with PLGA NPs. We saw that SQ administration provided the greatest total brain exposure to encapsulated small molecules over time. Next, we examined the effect of SQ administration of free E2 treatment and the frequency (daily, weekly, biweekly) of administration on cognitive performance in ovariectomized, middle-aged rats. Results showed that the daily E2 treatment enhanced spatial working memory performance relative to vehicle control. Together, these results led us to test our hypothesis using ovariectomized, middle-aged rats with four weekly SQ treatment groups: sesame oil, free E2, blank PLGA NP, and E2 PLGA NP. Rats were tested on a battery of behavioral measures to examine spatial working and reference memory (water radial arm maze) and spatial reference memory (Morris water maze). Following behavior testing, evaluations of estrogen levels in blood serum and of markers of peripheral E2 exposure (vaginal smears, uterine horn weights) were performed.

Results: Rats that received NP-encapsulated E2 performed better on working memory relative to blank NP controls, and these rats also tended to have higher peripheral E2 exposure relative to free E2 treated rats. Interestingly, we also observed enhanced cognition with the PLGA NP as a vehicle, an effect that has not been previously reported. Analyses of neurotrophin levels and extracellular signal-regulated kinase activation in brain regions that are involved in cognitive function are currently being performed to assess whether there are changes in molecular mechanisms associated with treatment and cognitive performance.

Conclusions: In sum, these data indicate that E2 NP treatment enhances cognition relative to blank NP control, and that cognitive benefits are seen from PLGA NP as the vehicle. These results strengthen our understanding of hormone delivery in the context of cognitive decline, and raise interesting avenues for investigation of NP delivery and its effect on learning and memory.

SELECTIVELY TARGETING OLIGOMERIC ALPHA-SYNUCLEIN AS A THERAPEUTIC FOR PARKINSON'S DISEASE. Sierks M. Arizona State University; Arizona Alzheimer's Consortium.

Oligomeric forms of α -synuclein have been implicated in the progression of Parkinson's disease. We have generated antibody based reagents that selectively bind different oligomeric variants of α -syn. Here we show that selective targeting of oligomeric α -syn has therapeutic benefit in cell and mouse models of PD. The reagents are tagged with a peptide to facilitate transfer across the blood brain barrier and therefore can be administered systemically. Systemic administration of the antibody fragments showed sharp reductions in α -syn aggregates, in oligomeric α -syn levels and inflammation in brain tissue. The antibodies also restored neuronal health. We show that targeting oligomeric α -syn provides stronger therapeutic benefit than targeting monomeric α -syn, and that the preferred oligomeric targets may change with disease progression. These results suggest that selectively targeting toxic oligomeric protein variants is a promising therapeutic approach for treating Parkinson's and other related neurodegenerative diseases.

BLOOD-BASED PROTEIN VARIANT BIOMARKERS TO FACILITATE PRESYMPTOMATIC DIAGNOSIS AND STAGING OF ALZHEIMER'S DISEASE.

Williams SM, Schulz P, Rosenberry TL, Caselli RJ, Sierks MR. Arizona State University; Mayo Clinic Jacksonville; Mayo Clinic Scottsdale; Arizona Alzheimer's Consortium.

Background: Oligomeric forms of beta-amyloid, tau, and TDP-43 play important roles in Alzheimer's disease (AD), and therefore are promising biomarkers. We previously generated single chain antibody fragments (scFvs) that selectively bind disease related variants of these proteins including A4, C6T and E1 which bind different beta-amyloid oligomers, D11C which binds oligomeric tau, and AD-TDP1 and AD-TDP2 which bind TDP-43 variants.

Methods: To determine if these protein variants were useful early biomarkers for AD, we first analyzed 11 human sera samples obtained from patients ~2 years prior to an initial diagnosis of MCI followed by longitudinal human plasma from four AD (encompassing time points prior to initial MCI diagnosis to after conversion to AD) and two control cases using our phage capture ELISA.

Results: While the biomarker profile varied from case to case for the 11 pre-MCI samples, all displayed elevated reactivity relative to cognitively normal age-matched controls. To determine specific protein variant profiles indicative of different stages of AD, we next examined the longitudinal cases. Levels of A4 and C6T reactive oligomeric beta-amyloid were significantly higher with all AD stages compared to the controls; levels of D11C reactive oligomeric tau increased with AD progression, and levels of AD-TDP1 and AD-TDP2 reactive TDP-43 variants decreased with AD progression. Pre-MCI samples were characterized by high TDP-43, moderate beta-amyloid and low tau variant levels; MCI samples by moderate TDP-43 and tau, and high beta-amyloid variant levels; and AD samples by low TDP-43 and high beta-amyloid and tau variant levels. Sample time points ranged from ~7 years pre-MCI to ~9 years after AD conversion. Bivariate correlations utilizing this range showed a strong positive correlation between cumulative beta-amyloid, tau and TDP-43 levels with disease progression indicating an increase in neurodegenerative processes with time in AD.

Conclusions: Our scFv panel not only readily selects AD cases from controls, but also discriminates between AD stages, and detects the presence of blood-based AD biomarkers more than 7 years prior to an initial diagnosis of MCI.