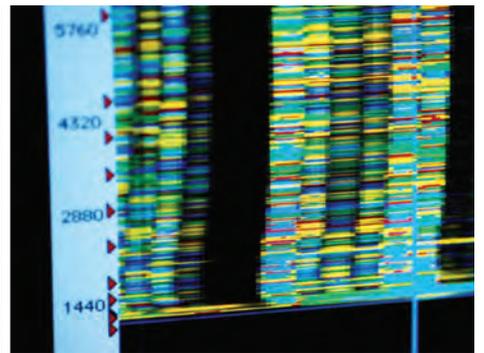
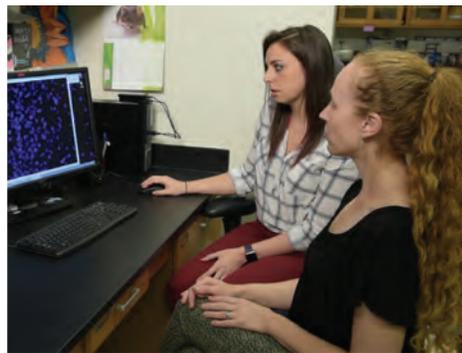


Age-related Memory Loss (ARML) Program and Cognitive Aging and Memory (CAM) Program

2016 Annual Report



UF Evelyn F. & William L.
McKnight Brain Institute
UNIVERSITY of FLORIDA

*Prepared for the McKnight Brain Research Foundation by the
University of Florida McKnight Brain Institute and Institute on Aging*

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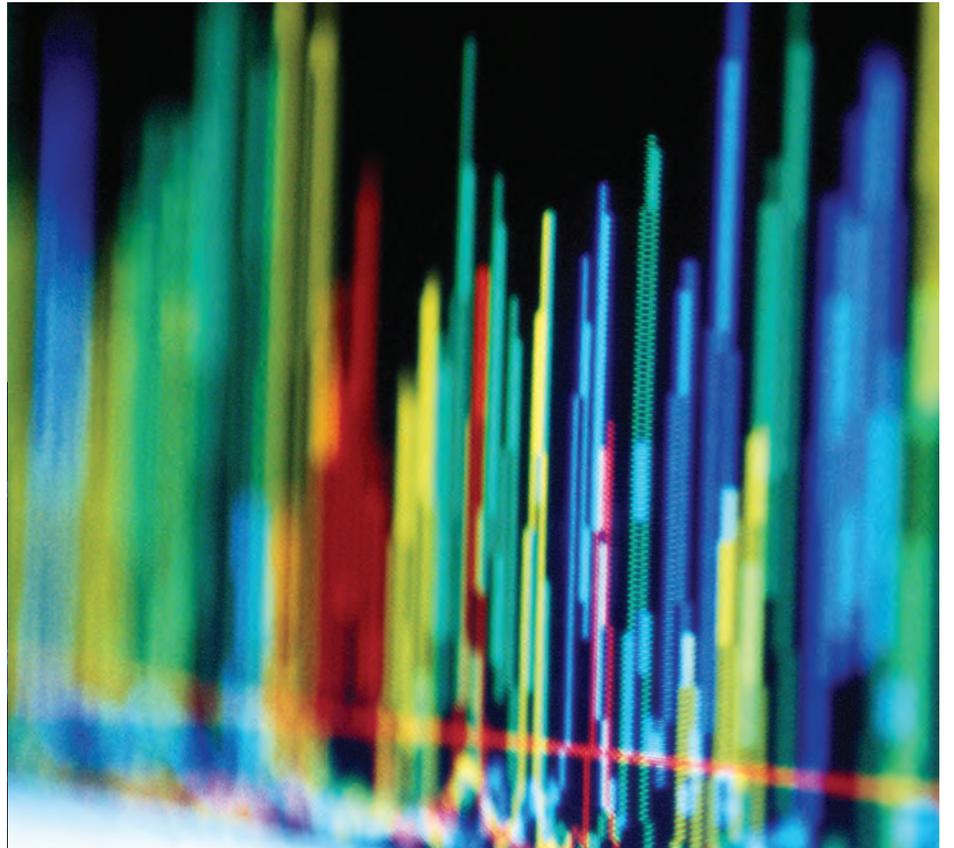
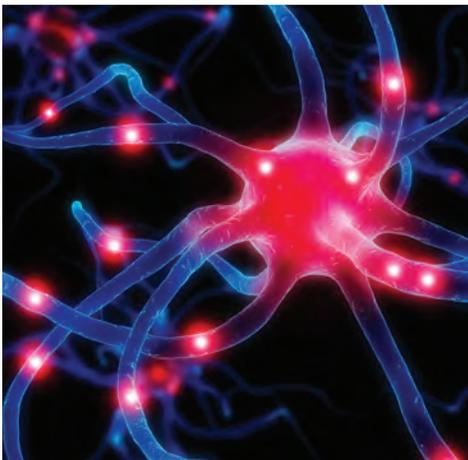
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1515 SW Archer Road, Suite 23C1
Gainesville, FL 32608
P.O. Box 100014
Gainesville, FL 32610-0014
Phone: 352.733.1700
Fax: 352.733.1201
UFHealth.org

January 12, 2016

The McKnight Brain Research Foundation
The SunTrust Bank
Mail Code FL-ORL-2160
300 South Orange Avenue, Suite 1600
Orlando, FL 32801

Dear Trustees:

We would like to share our continued gratitude to the McKnight Brain Research Foundation (MBRF) for its generous support of the University of Florida's Age-related Memory Loss (AMRL) program and Cognitive Aging and Memory – Clinical and Translational Research Program.

2016 proved to be a highly productive year for both programs. Extramural support for the MBRF-sponsored programs has grown significantly, with several investigators in each program earning and/or renewing NIH funding. The numerous publications, presentations and honors are impressive, and pilot studies are generating informative data that advances our understanding of age-related cognitive decline and serve as the basis for extramural funding of further studies.

This past year laid the groundwork for exciting opportunities for 2017, including bringing the AMRL and CAM-CTRP under one roof in the McKnight Brain Institute building, with dedicated clinical research space.

We look forward to the ongoing accomplishments from these programs and thank you again for your support.

Sincerely,



David S. Guzick, M.D., Ph.D.
Senior Vice President, Health Affairs
President, UF Health



Michael L. Good, M.D.
Dean, College of Medicine
Folke H. Peterson Dean's Distinguished Professor



Michael G. Perri, Ph.D.
Dean, College of Public Health and Health Professions
Robert G. Frank Endowed Professor

January 15, 2017

The McKnight Brain Research Foundation
SunTrust Bank
Mail Code FL-ORL-2160
300 South Orange Avenue, Suite 1600
Orlando, FL 32801

Dear MBRF Trustees,

It is my pleasure to submit this 2016 Annual Report of the Age-Related Memory Loss (ARML) program and the Cognitive Aging and Memory Clinical Translational Research Program (CAM-CTRP). In serving as the Interim Director of the Evelyn F. & William L. McKnight Brain Institute (MBI) of the University of Florida (UF) for most of this year, I sought to maintain the missions of the MBI and the associated facilities, and facilitate collaborations among researchers that would translate into therapeutic advances. I would like to express my sincere appreciation to the MBRF for your unfailing support of our programs and me during this transition period.

2016 featured significant changes in the MBI's leadership and administration. Dr. Todd Golde became the MBI's new Director on December 1, 2016, succeeding Dr. Tetsuo Ashizawa. My service as Interim Director ended commensurate with this appointment; however, I am pleased to continue serving as the MBI's Deputy Director under Dr. Golde.

Dr. Golde is professor of neuroscience and director of UF's Center for Translational Research in Neurodegenerative Disease and the 1Florida Alzheimer's Disease Research Center consortium of institutions. He has served the MBI for six years as both an investigator and a leader, pioneering programs to closely link basic-science laboratory work with patient-based studies to translate new discoveries into diagnoses and treatments. In accepting his new appointment, Dr. Golde stated, "We've made a lot of investments in neuromedicine and neuroscience research at the Health Science Center over the last five years," he said. "We have outstanding investigators, and I want to help them collaborate, find synergies, and share resources so we can continue our growth in this area. Moving forward, we'd like more connections between research at the lab bench and care at the bedside."

In September, the MBI also welcomed a new Director of Operations, Mr. Kenneth Marx. Ken served previously as chief administrator for UF's department of emergency medicine for 15 years and has over 25 years of administrative experience in academic medical centers. His knowledge of the College of Medicine and the UF Health Systems and personnel is of great value to the MBI and enables us to move forward rapidly. Ken succeeds Kelly Sharp, who is

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now executive director of administration and finance at the UF College of Pharmacy. We wish to thank Kelly for his numerous contributions to the MBI's growth and success and for all he has done to help Ken and me this past year. He did much to enable the successes the MBI has had over the past 5 years.

Other notable changes in 2016 include the reorganization of the CAM-CTRP group, directed by Dr. Ronald Cohen. Further description of these changes is provided by Dr. Cohen in his introductory letter to the CAM-CTRP section of this Report. We thank the MBRF for support in ensuring this transition was completed with minimal interruption to Dr. Cohen's group's research.

CAM's work, along with that of many other researchers at the MBI, will be augmented by the recent installation of a second 3T MRI magnet. The Siemens 3T MRI/S Prisma is a state-of-the-art \$3 million scanner that has the strongest gradients available for human imaging, advanced motion correction capabilities and increased scanning flexibility. The addition of this unit and construction of the associated facilities were key features of a broader renovation of the MBI's Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) core facility this past year.

Much excellent work has been performed this year by the members of CAM-CTRP and the ARML programs. As brief examples, the ARML members have received 5 new NIH grants—three to Dr. Burke, one to Dr. Maurer, and one to Dr. Foster. These are focused on mechanisms of aging and are the beginning of the translational pipeline to the CAM. The CAM faculty had several papers in high profile journals, addressing age-related impairment in executive function examined at the circuit and molecular levels, as well as studies addressing pharmacological treatment involving GABA_B receptors.

The ARML continued to make progress in facilitating collaborations across institutes, including establishing protocols for examining exosomal microRNA as part of the Cognition and Neuroimaging Core and development of a computational pipeline for analysis of genome-wide methylation data (DMAP2) for examining the epigenetics of age-related memory impairment <http://compbio.ufl.edu/software/dmap2/>.

ARML faculty and students also established collaborations addressing the unique and emerging idea that cognitive decline involves an interaction of the brain and peripheral systems. Peripheral factors such as systemic inflammation, exosomes in the blood, and age-related changes in peripheral organs (e.g. muscle) can result in sleep disturbances and a decline in executive function and memory, which are observed in the elderly. The exciting aspect of these investigations is that they appear approachable by therapeutic interventions.

The CAM-CTRP group also had an extraordinarily successful year. A number of large grants were funded, one of which was the ACT R01, a multisite randomized control trial funded by the NIA for combating cognitive aging using noninvasive adjunctive interventions. This will be the largest tDCS trial in history, and the first multisite clinical trial across multiple McKnight sites. CAM also was key in the establishment of an MBRF-supported Cognitive Aging and Memory Intervention Core across all McKnight Brain Institutes. This Core will facilitate multi-MBI site clinical trials and collect information on MBI-based clinical trial resources and investigators

(PIs: UA, Gene Alexander, UAB: Virginia Grissom, UM: Tatiana Rundek, UF: Ron Cohen and Adam Woods (contact PI).

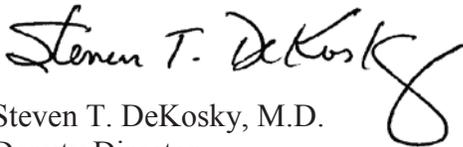
While we regularly announce publications and grant submissions, one particular CAM manuscript providing evidence that short and long-term heavy drinking behavior accelerates cognitive aging (published in *Alcoholism: Clinical and Experimental Research*) was covered by over 50 online and other media outlets, including *US News and World Report*.

Finally, Dr. Cohen received funding for the SHARC study, a U01 from NIAAA that was a renewal funding for the Southeastern HIV and Alcohol Research Center. This renewal funding was obtained with Dr. Cohen as deputy director of SHARC, with specific focus on how HIV and alcohol accelerate cognitive aging, a prime model of a co-morbid condition's impact on the aging process in the human brain.

Thus, while we continue our mechanistic basic science research and descriptive clinical studies, we are delivering on a challenge to strengthen the collaborations between CAM-CTRP and ARML programs, with the aid of new translational members of the teams. We hope to further develop our clinically meaningful translational research, which we anticipate will flow from bench to bedside, and with the help of our clinical translational colleagues, back to the bench for proof of mechanism when a relevant clinical observation is made and needs further basic studies to confirm and develop potential interventions. As discussed frequently by the Board and the leadership of CAM-CTRP and ARML, we need a number of researchers who will work closely with both basic scientists and clinical scientists to operationalize this pipeline. It is my privilege to be one of those scientists. Dr. Golde's vision includes facilitating this pipeline in aging and memory research; he and I will both be part of the clinical translational group who helps with it. Moreover, we envision having more UF faculty, industry scientists, qualified consultants and new collaborative opportunities with the Institute on Aging to aid us. New NIH grant opportunities supporting such a vision will be used for these activities, and we have already had significant moves to internal collaborations among the 4 McKnight Institutes.

Todd Golde and I share the same vision for aging and memory research, and clinically meaningful translational research on cognitive aging. It has been my great honor to be the interim executive director for the past year, and look forward to working with Todd and our superb scientific community.

Sincerely yours,



Steven T. DeKosky, M.D.
Deputy Director

January 15, 2017

The McKnight Brain Research Foundation
SunTrust Bank
Mail Code FL-ORL-2160
300 South Orange Avenue, Suite 1600
Orlando, FL 32801

Dear MBRF Trustees:

As the newly appointed director of the MBI, I would like to begin my new tenure with a quick note. As Dr. DeKosky has provided a separate detailed letter that outlines changes in the MBI leadership and other key issues relevant to the MBRF supported programs, my letter will largely be forward looking.

I think it is extremely important to acknowledge the time and effort that Dr. Steven DeKosky has spent in support of these programs as the interim MBI director. The CAM-CTRP program, in particular, has faced some challenging internal transitions. Steve played a key role coordinating the efforts of other stakeholders in the Health Science Center to ensure that the CAM-CTRP faculty made a successful transition to the Department of Clinical and Health Psychology (CHP) as full faculty members. He also developed a plan for space within the MBI that will ensure that their science can be carried out. I will work closely with Steve, who will remain on the MBI leadership team as Deputy Director, and the CAM-CTRP faculty to ensure that the final steps in this transition are moved forward. In the long run, I think that the reincorporation of the CAM-CTRP program into the MBI will have a huge positive impact on both the program and the MBI.

My own style is to be straightforward and data driven. I have a fairly simple but ambitious agenda for the MBI that can be summed up by simply saying I want to see our Neuroscience and Neuromedicine research enterprise flourish and be viewed as world-class. Both the ARML and CAM-CTRP programs are vital components of the MBI research portfolio; support from MBI's leadership will be unwavering.

I look forward to working with you to ensure that CAM-CTRP and the ARML flourish and grow. My success in this role will largely be determined by the success of the various programs under the McKnight "umbrella." Please do not hesitate to reach out to me with any concerns about the MBRF supported programs or, even better yet, if you have new ideas on how to bolster our science.

Sincerely,



Todd E. Golde, M.D., Ph.D.
Director, Evelyn F. and William L. McKnight Brain Institute

December 31, 2016

Dear Trustees of the McKnight Brain Research Foundation:

Age-Related Memory Loss Program (ARML)

The ARML program consists of researchers dedicated to understanding and alleviation of age-related cognitive decline. MBRF sponsored support of ARML researchers is overseen by the ARML Program committee consisting of Drs. Tom Foster (Evelyn F. McKnight Chair for Research on Cognitive Aging and Memory and ARML Committee Chair), Lucia Notterpek (William T. and Janice M. Neely Professor and Chair of the Department of Neuroscience) and Christiaan Leeuwenburgh (Chief, Division of Biology of Aging at the Institute on Aging and Leader of the Metabolism and Biomarkers and Research Career Development Cores). The ARML fund partially supports the faculty salaries of Drs. Thomas Foster, Jennifer Bizon, Sara Burke, and Andrew Maurer.

Major goals of the ARML program include support for collaboration and communication among researchers and nurturing scientists dedicated to the exploration and innovative research in the understanding and alleviation of age-related memory loss. During 2016, this group had 20 unique publications and received funding for five new projects.

Burke AG049722
Burke AG051004
Burke AG047266
Maurer MH11924071
Foster AG052258

The following represents a subset of collaborations related to funded projects, published papers, grants submitted to NIH, or emphasizes collaborations between ARML, CAM-CTRP, and other MBRF sponsored institutes.

Promoting Collaboration and Communication across MBRF Institutes

During the past year members of the ARML program have presented posters at the McKnight Brain Research Foundation poster session at the Society for Neuroscience. Dr. Joseph McQuail from Dr. Bizon's lab received 1st place in recognition for his poster. In addition, ARML members attended the 8th Inter-Institutional Meeting of the Evelyn F. McKnight Brain Research Foundation (April 29-May 1, 2015). At the 2016 Inter-Institute meeting, Dr. Tom Foster presented "What can the transcriptome tell us about the regional vulnerability to age and cognitive impairment?", which involved an update on results from a cross institute collaboration involving the Epigenetics Core.

Cross institute Collaborations

1) Cognitive Aging Core:

The members of the ARML program continue to interact with individuals across the four institutes and have participated in the Brain and Cognitive Health Working Group examining animals as models for age-related changes in cognition in humans. During the past year, several studies examining animal models of cognitive decline have been published by individuals from the ARML (Johnson et al., 2016; Gray et al., in press; Burke and Foster, in press). More recently, Dr. Maurer has been involved in a collaborative project with MBI faculty at the University of Arizona to do functional imaging in nonhuman primate models (Engle et al., 2016).

2) Epigenetics and Bio-Informatics Core:

This Core provides a shared Inter-institutional resource related to transcription, genomics, and epigenomics, and acts as a catalyst for discoveries across all Evelyn F. McKnight Brain Institutes. Data collection for a cross-institute study of aging, cognition, and RNA expression is complete and a manuscript is in preparation. As a group we are diminished by the loss of Dr. David Sweatt from UAB. Dr. Sweatt, was a driving force for directing the core in the examination of DNA methylation, as an epigenetic mechanism for age-related cognitive impairment. We have continued to work toward this goal and members of the ARML group published a paper describing age-related changes DNA promoter methylation in the hippocampus (Ilanov et al., 2016). In order to promote the goals of the core, the ARML group has also been involved in developing a computational pipeline for analysis of genome-wide methylation data (DMAP2) in collaboration with Dr. Alberto Riva. A full description of this pipeline can be found at <http://compbio.ufl.edu/software/dmap2/>. We are currently using this pipeline to identify alter DNA methylation associated with brain aging and cognitive decline and an initial report has been presented at the Society for Neuroscience (Foster et al., 2016).

3) Cognition and Neuroimaging Core:

The ARML group has been involved in generating standard operating procedures for collecting plasma from individuals that will be imaged and tested for cognitive function as part of the Neuroimaging Core. The current plan is to use the plasma for examination of exosome microRNA as a potential biological marker for age-related cognitive decline. In addition, we have a working group to employ imaging techniques to examine possible markers of neuroinflammation and possible mechanisms of delirium that occur in elderly. This group involves members of CAM-CTRP as well as individuals associated with ARML (Drs. Febo, Kumar, Foster).

Collaborations within UF

A number of collaborations are ongoing between ARML members and in association with other researchers at the University of Florida.

- 1) **Changes in functional connectivity during aging:** A number of studies are examining the role of functional connectivity. In particular, Drs. Burke and Maurer provide leadership for a number of studies that received funding during 2016 (Burke PI: AG049722, AG051004, AG047266 Maurer PI: MH11924071) and examine functional connectivity between memory-associated brain structures (medial temporal lobe, hippocampus, prefrontal cortex). Recent publications from the Burke lab provide an indication of the tremendous progress in this area (Hernandez et al., 2016ab). Together, Drs. Maurer and Burke provide expertise using *in vivo* recording techniques to examine network dynamics in behaving animals (Sheremet et al., 2016; Engle et al., 2016). In this way, function connectivity is examined in a manner analogous to fMRI work in humans, providing a translational perspective for linking age-related physiological changes to cognitive function.

2) **Role of systemic inflammation in age-related cognitive decline.**

A new project, funded in 2016 (Foster PI), continues the interaction between ARML members and the biomarkers core at the Institute on Aging. Blood biomarkers of inflammation are correlated with cognition and brain transcription. A similar study examining markers of systemic inflammation and cognition in humans is being conducted by CAM-CTRP and involves the same core and many of the same markers.

Projects conducted by CAM-CTRP are using magnetic resonance imaging and spectroscopy techniques (MRI and MRS, respectively) in an attempt to detect neuroinflammation. A collaboration (Febo and Foster) has been initiated to employ neuroimaging in order to examine the effects of systemic inflammation on neuroinflammation in animal models. Studies in animal models permit the use of an integrative set of neuroimaging and molecular techniques, in an attempt to bridge localized cellular/molecular phenomenon and broader *in vivo* neural network alterations (Febo and Foster, 2016).

3) **Transcriptional profiling:**

This work, funded by NIH (Foster PI), utilizes the Bio-informatics core and parallels the cross institute collaboration on transcription profiling. Transcription changes linked to regional vulnerability to aging and to a decline in executive function were detailed. In addition, age-related changes in the animal model were similar to changes reported in the dorsolateral cortex of aging humans (Ivanov et al., 2016).

A recent study involved a collaboration between ARML and CAM-CTRP (Foster and Woods) to examine human exosome RNA from individuals characterized for cognition and imaging of hippocampal volume. The initial results suggest that microRNA from exosomes may predict age, gender, and cognitive function.

4) **Treatments of age-related cognitive decline:**

A number of published studies and funded projects deal with treatment of age related cognitive decline. Prominent in this area are studies by the Bizon group examining GABA(B) signaling (Beas et al., 2016ab; Carpenter et al., 2016). This work suggests that loss of GABAergic signaling in prefrontal cortex with age is specifically associated with deficits in cognitive flexibility and that an agonist at GABA(B) receptors can improve this aspect of cognitive function in aged rats.

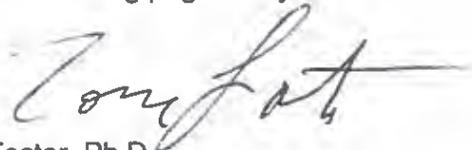
5) **Other individuals:**

Individuals previously funded through ARML (Ormerod and Frazier) continue to interact with ARML members (Johnson et al., 2016; McQuail et al. 2016; Carpenter et al., 2016). A collaboration is between Drs. Burke, Maurer, and Ormerod, received funding (R03) from NIA. Currently, animals are tested in Dr. Foster's behavioral core.

A collaboration between ARML (Foster, Rani, Kumar) with the Pepper Center (Esser and Kang) is designed to examine transcription from muscle and brain for animals with a conditional knockout of a circadian rhythm gene. The broad view is that brain aging is influenced by communication between organs. Specifically, disruption of circadian rhythms (e.g. disrupted sleep) due to muscle clocks will contribute to brain aging.

Summaries of the publications and active federal funds associated with ARML faculty efforts during 2016 are included in the following pages for your reference.

Sincerely,



Thomas C. Foster, Ph.D.

Professor, Department of Neuroscience and Genetics and Genomics Program
Evelyn F. McKnight Chair for Research on Cognitive Aging and Memory

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Publications

1. Beas, B.S., McQuail, J.A., Banuelos, C., Setlow, B., and Bizon, J.L. (2016). Prefrontal cortical GABAergic signaling and impaired behavioral flexibility in aged F344 rats. *Neuroscience*.
2. Beas, B.S., Setlow, B., and Bizon, J.L. (2016). Effects of acute administration of the GABA(B) receptor agonist baclofen on behavioral flexibility in rats. *Psychopharmacology (Berl)* 233, 2787-2797.
3. Carpenter, H.E., Kelly, K.B., Bizon, J.L., and Frazier, C.J. (2016). Age-related changes in tonic activation of presynaptic versus extrasynaptic gamma-aminobutyric acid type B receptors in rat medial prefrontal cortex. *Neurobiol Aging* 45, 88-97.
4. Engle, J.R., Machado, C.J., Permenter, M.R., Vogt, J.A., Maurer, A.P., Bulleri, A.M., and Barnes, C.A. (2016). Network Patterns Associated with Navigation Behaviors Are Altered in Aged Nonhuman Primates. *J Neurosci* 36, 12217-12227.
5. Febo, M., and Foster, T.C. (2016). Preclinical Magnetic Resonance Imaging and Spectroscopy Studies of Memory, Aging, and Cognitive Decline. *Front Aging Neurosci* 8, 158.
6. Foster, T.C., Kyritsopoulos, C., and Kumar, A. (2016). Central role for NMDA receptors in redox mediated impairment of synaptic function during aging and Alzheimer's disease. *Behav Brain Res*.
7. Gomez-Chacon, B., Gamiz, F., Foster, T.C., and Gallo, M. (2016). Increased N-Ethylmaleimide-Sensitive Factor Expression in Amygdala and Perirhinal Cortex during Habituation of Taste Neophobia. *Neural Plast* 2016, 2726745.
8. Burke, S.N. and Foster, T.C. *Animal Models of Cognitive Aging and Circuit-Specific Vulnerability*. In DeKosky and Asthana (Eds.) *Geriatric Neurology*, in press.
9. Gray, D.T., 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 macaque monkeys. *Behav Brain Res*.
10. Guadiana, S.M., Parker, A.K., Filho, G.F., Sequeira, A., Semple-Rowland, S., Shaw, G., Mandel, R.J., Foster, T.C., Kumar, A., and Sarkisian, M.R. (2016). Type 3 Adenylyl Cyclase and Somatostatin Receptor 3 Expression Persists in Aged Rat Neocortical and Hippocampal Neuronal Cilia. *Front Aging Neurosci* 8, 127.
11. Hernandez, A.R., Reasor, J.E., Truckenbrod, L.M., Lubke, K.N., Johnson, S.A., Bizon, J.L., Maurer, A.P., and Burke, S.N. (2016). Medial prefrontal-perirhinal cortical communication is necessary for flexible response selection. *Neurobiol Learn Mem* 137, 36-47.
12. Ianov, L., Rani, A., Beas, B.S., Kumar, A., and Foster, T.C. (2016). Transcription Profile of Aging and Cognition-Related Genes in the Medial Prefrontal Cortex. *Front Aging Neurosci* 8, 113.
13. Johnson, S.A., Sacks, P.K., Turner, S.M., Gaynor, L.S., Ormerod, B.K., Maurer, A.P., Bizon, J.L., and Burke, S.N. (2016). Discrimination performance in aging is vulnerable to interference and dissociable from spatial memory. *Learn Mem* 23, 339-348.
14. McQuail, J.A., Beas, B.S., Kelly, K.B., Simpson, K.L., Frazier, C.J., Setlow, B., and Bizon, J.L. (2016). NR2A-containing NMDARs in the prefrontal cortex are required for working memory and associated with age-related cognitive decline. *J Neurosci*.
15. Montgomery, K.S., Edwards, G., 3rd, Levites, Y., Kumar, A., Myers, C.E., Gluck, M.A., Setlow, B., and Bizon, J.L. (2016). Deficits in hippocampal-dependent transfer generalization learning accompany synaptic dysfunction in a mouse model of amyloidosis. *Hippocampus* 26, 455-471.
16. Orsini, C.A., Willis, M.L., Gilbert, R.J., Bizon, J.L., and Setlow, B. (2016). Sex differences in a rat model of risky decision making. *Behav Neurosci* 130, 50-61.
17. Setlow, B., and Bizon, J.L. (2016). Adolescent Cannabinoid Use and Cognition; Unexpected Results from a Rat Model of Cannabinoid Self-Administration. *Neuropsychopharmacology*.
18. Sheremet, A., Burke, S.N., and Maurer, A.P. (2016). Movement Enhances the Nonlinearity of Hippocampal Theta. *J Neurosci* 36, 4218-4230.
19. Shilnikov, A.L., and Maurer, A.P. (2016). The Art of Grid Fields: Geometry of Neuronal Time. *Front Neural Circuits* 10, 12.
20. Witharana, W.K., 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).

21. Nonuniform allocation of hippocampal neurons to place fields across all hippocampal subfields.
Hippocampus 26, 1328-1344.

Active Federal Funding:

PI	Project Title	NIH Institute	Project Number	Activity	FY	FY Total Cost
BIZON, JENNIFER LYNN	NEURAL MECHANISMS OF COGNITIVE DECLINE IN AGING	NIA	5R01AG029421-09	RD1	2016	\$ 304,143
BURKE, SARA N	NEUROGENESIS AND MEMORY NETWORK DYNAMICS DURING NORMAL AGING	NIA	5R03AG049411-02	RD3	2016	\$ 75,000
BURKE, SARA N	THE CONTRIBUTION OF DECLINES IN FUNCTIONAL CONNECTIVITY TO COGNITIVE AGING	NIA	5R01AG049722-02	RD1	2017	\$ 341,165
BURKE, SARA N	SINGLE-CELL IMAGING OF FUNCTIONAL CONNECTIVITY AS A WINDOW INTO COGNITIVE AGING	NIA	1R21AG051004-01A1	R21	2016	\$ 237,769
FOSTER, THOMAS C	SIGNALING CASCADES AND MEMORY DEFICITS DURING AGING	NIA	5R37AG036800-07	R37	2016	\$ 299,942
FOSTER, THOMAS C	ESTROGEN AND COGMITION OVER THE LIFESPAN	NIA	5R01AG037984-15	R01	2015	\$ 280,909
FOSTER, THOMAS C	SYSTEMIC INFLAMMATION IN REGULATING THE ONSET AND PROGRESSION OF BRAIN AGING	NIA	5R01AG049711-02	R01	2016	\$ 307,500
FOSTER, THOMAS C	SYSTEMIC INFLAMMATION IN REGULATING THE ONSET AND PROGRESSION OF BRAIN AGING	NIA	1R01AG052258-01	R01	2016	\$ 375,000
MAURER, ANDREW PORTER	TESTING AND FORECASTING HIPPOCAMPAL THETA WAVE PROPAGATION IN LEARNING AND MEMORY	NIMH	1R01MH109548-01A1	R01	2016	\$ 381,250

SUMMARY OF SCIENTIFIC ACHIEVEMENTS SINCE LAST REPORT:

Jennifer Bizon, PhD

Our laboratory had a productive year investigating mechanisms of age-related cognitive decline. Evidence of productivity includes 12 manuscripts in various stages of publication and over 15 presentations at national and international scientific meetings. Highlights include a paper published in the Journal of Neuroscience from a postdoctoral fellow in my laboratory, Dr. Joe McQuail. This manuscript describes specific age-associated alterations in NMDA receptors that are important for working memory decline and suggests a novel therapeutic strategy to improve working memory in older subjects. In addition, my graduate student who received her PhD earlier this year, Dr. Sofia Beas, published two manuscripts demonstrating that loss of GABAergic signaling in prefrontal cortex with age is specifically associated with deficits in cognitive flexibility and that an agonist at GABA(B) receptors can improve this aspect of cognitive function in aged rats.

In addition to continuing our grant funding from last year, we have contributed to several new awards. Dr. Caitlin Orsini, a postdoctoral fellow in the lab whom I co-mentor with Dr. Barry Setlow, received a perfect score on her K99/R00 application in July with funding expected to begin in January 2017. Dr. Orsini has been successful in establishing optogenetic tools in our laboratory, which we are now using to investigate neural circuit changes in cognitive aging. In addition, we have continued to strengthen our collaboration with Dr. Sara Burke and other ARML affiliates. I am co-I (5% effort) on Dr. Burke's new R01 as well as on Dr. Andrew Maurer's R01 (10% effort) that was awarded earlier this year. Finally, along with ARML collaborators (Burke, Maurer and Setlow), and Dr. Kevin Otto in Biomedical Engineering, we have received word that we will receive a DARPA grant (over 2.5 million dollars in direct costs over 4 years) to determine if vagal nerve stimulation can improve cognition. Dr. Burke and I also have 4 manuscripts in various stages of publication, have a pilot proposal pending at the Ed and Ethel Moore Alzheimer's Disease competition and have completed a R21 that will be submitted in the next cycle.

In addition to our accomplishments with publications and grants, I continue to serve the field of cognitive aging at the national level. I serve as a Senior Editor at Neurobiology of Aging, (Physiology, Cognition and Behavior section), where I handle approximately 100 manuscripts a year, finding appropriate reviewers and making recommendations to the Editor in Chief. I also currently serve on two study sections. First, I am chairing (until October 2018) the NIH F03 (Neurodevelopment, Synaptic Plasticity and Neurodegeneration Fellowship) Study Section. Second, I regularly serve on the neuroscience study section for the National Institute on Aging (NIA-N) and was asked to join this section as a standing member earlier this year. I also review proposals for the Alzheimer's Drug Discovery Foundation. Finally, I was asked to speak in a number of venues this upcoming year, including serving as one of the faculty members at a Neuroscience School of Advanced Studies on "Cognitive Decline and Aging" in Tuscany, Italy in September 2017.

Sara N. Burke, PhD

The focus of my research continues to be on determining the neurobiology of age-related memory loss. My lab addresses this question using multiple levels of analysis that include molecular imaging techniques, high-channel count *in vivo* neurophysiology, and behavioral assessment. Particularly, we are taking the novel approach of trying to understand how aging impacts communication across brain areas that are critical for adaptive behaviors. The past 12 months have been marked by substantial research progress. In addition to having success with funding (funded R01, R21, R03, ADRC Pilot grant, under contract for a DARPA grant, and co-I on funded R01 (PI Maurer) and an additional scored R01 pending), my lab has made progress with *in vivo* electrophysiological recordings. Furthermore, we have adopted 2 new cognitive paradigms that model behavior in humans. The first is a rat version of motor-cognitive dual task in which we monitor the rats' gait while they perform a working memory and a behavioral flexibility task. An identical behavior is being tested in humans by our collaborator David Clark, and we are seeing similar results across species. The second task is the rat variant of the mnemonic similarity task (Stark et al., 2013) in which we are using legos to parametrically vary the similarity of test objects (under review at Hippocampus). As observed in humans, the task is highly sensitive to detecting age-related deficits. We have also implemented a new technique for imaging gene expression in conjunction with anatomical tract tracing and are analyzing these data now. Finally, we are in the final stages of preparation for our first paper that has immediate-early gene data. I have also successfully recruited a new lab technician and a University Scholar Award recipient, and my postdoc was awarded the MBI fellowship. Furthermore, I have continued to strengthen my collaborations with other ARML affiliates. I am co-I (12% effort) on Dr. Maurer's new R01. Additionally, along with ARML collaborators (Bizon, Maurer and Setlow), and Dr. Kevin Otto in Biomedical Engineering, we are currently in contract negotiations for a DARPA grant (over 2.5 million dollars in direct costs over 4 years) to determine if vagal nerve stimulation can improve cognition. Once this model is established in young animals we can test if this is a potential therapeutic approach in aged animals and humans. Dr. Bizon and

I also have 4 manuscripts in various stages of publication, have a pilot proposal pending at the Ed and Ethel Moore Alzheimer's Disease competition and have completed a R21 that will be submitted in the next cycle.

In terms of disseminating research data, I have had 3 senior author publications in the last year and a co-author paper. I also gave 2 invited seminars, a data blitz at the Annual McKnight Meeting and my group presented 4 posters at SfN in San Diego and at the McKnight Poster reception and I was a collaborator on 3 additional posters.

Finally, in addition to mentoring my own trainees, Dr. Lewis and I established the UF Summer Neuroscience Internship Program (SNIP). There was a consensus among Neuroscience faculty that we should be actively trying to enhance the applicant pool to the IPD-Neuroscience concentration. It was suggested by Dr. Lewis that this could be accomplished by creating a Summer Program for undergraduates to come to UF and participate in a 10 week paid internship. Thanks to the generosity of Drs. DeKosky and Golde, the mentors, and a partnership with the Louis Stokes Florida-Georgia Alliance for Minority Participation, we recruited 11 students from around the country to participate in this program for the summer of 2016. The average GPA of our inaugural group of interns is 3.6, and 7 of the 11 students are under-represented minorities. With the guidance of Dr. Lewis, and help from Drs. McQuail and Chakrabarty we have also organized weekly seminars and career development workshops for all of our participants.

Thomas C. Foster, PhD

Several papers were published characterizing changes in the transcription profile of different brain regions (hippocampus, medial prefrontal cortex, white matter, perirhinal cortex, and amygdala), due to aging or in association with learning and memory (Ivanov et al., 2016a; Gomez-Chacon et al., 2016). Cognitive function depends on transcription; however, there is little information linking altered gene expression to impaired function during aging. One study characterized performance of our cognitive-aging rodent model on both prefrontal-dependent (attentional set shift) and hippocampal-dependent (spatial episodic memory) tasks. Transcriptional differences associated with age and cognition were examined using RNA sequencing to construct transcriptomic profiles for the medial prefrontal cortex (mPFC), white matter, and region CA1 of the hippocampus. The results indicate regional differences in vulnerability to aging. Age-related gene expression in the mPFC was similar to, though less robust than, changes in the dorsolateral PFC of aging humans suggesting that aging processes may be similar. Importantly, the pattern of transcription associated with aging did not predict cognitive decline. Rather, increased mPFC expression of genes involved in regulation of transcription, including transcription factors that regulate the strength of excitatory and inhibitory inputs, and neural activity-related immediate-early genes was observed in aged animals that exhibit delayed set shift behavior. The specificity of impairment on a mPFC-dependent task, associated with a particular mPFC transcriptional profile indicates that impaired executive function involves altered transcriptional regulation and neural activity/plasticity processes that are distinct from that described for impaired hippocampal function.

Currently, we are investigating the possibility that epigenetic mechanisms contribute to the altered transcriptional regulation. As part of this work we published research on a mechanism for age-related regulation of the expression of estrogen receptor alpha (ER α) (Ivanov et al., 2016b). For this study, we took advantage of regional differences in hippocampal ER α expression to investigate DNA ER α promoter methylation at CpG dinucleotide sites as a potential epigenetic mechanism for regulating gene expression. Young and aged female Fischer 344 rats were ovariectomized, and the level of mRNA (*Esr1*) expression and ER α promoter methylation were examined in hippocampal regions CA1 and CA3, either 3 or 14 weeks following surgery. The results indicate that reduced *Esr1* expression in region CA1 relative to CA3 was associated with an increase in DNA methylation in region CA1, particularly for the first CpG site. Additionally, differential methylation of distal CpG sites, 11-17, was associated with altered *Esr1* expression during aging or following long-term hormone deprivation. The results support the idea that methylation of site 1 may be the primary regulatory region for cross-regional patterns in ER α expression, while distal sites are modifiable across the life span and may act as a feedback mechanism for ER α activity.

As part of this focus on altered epigenetic regulation during aging, we have been involved in developing a computational pipeline for analysis of genome-wide methylation data (DMP2). A full description can be found at <http://compbio.ufl.edu/software/dmap2/>. We are currently using this pipeline to identify altered DNA methylation associated with brain aging and cognitive decline and a report has been presented at the Society for Neuroscience (Foster et al., 2016).

Andrew Maurer, PhD

Following up on last year's project report, and most pertinent to MBR foundation, we have utilized 3 sources of funding (Seed Opportunity Funding, an R03 and McKnight Seed money) for preliminary data for an R01 application titled "Age-associated changes in hippocampal circuits and cognitive function". Study section for this application meets on October 27, 2016. Interestingly, this

model takes advantage of a model proposed by Dr. Tom Foster (namely, a unilateral perirhinal pathway lesions). If successful, this application will allow a circuit level description of medial temporal lobe changes that occur over aging and how they correlate with declines in memory performance.

At the previous progress report, I only supported 20 percent of my effort. As of today, I am at ~65-70% of my total effort (nearly covering the entirety of my research effort). This has been significantly carried by an R01 in which I serve as PI from the NIMH. Nonetheless, we will continually seek out funding opportunities. Namely, I hope to submit an R21 in the next 12 months to support budding research programs in my laboratory.

The "big news" is that I have moved from Research Assistant Professor to a tenure track Assistant Professor program as of August 2016. Approximately 1,155 square feet of new lab space was allocated and prepared by the MBI to support my work on the second and fifth floors in the fall of 2016.

In the past calendar year, I have been a middle author on two manuscripts and senior author on two others. There are another two manuscripts at various levels of submission-publication of which Carol Barnes and Gyuri Buzsaki serve as senior author. Drs. Burke and I are in the final stages of preparing a manuscript in collaboration with Dr. Barnes. Finally, I hope to submit two research manuscripts which I serve as senior author in the next year.

PUBLICATIONS IN PEER REVIEWED JOURNALS:

Jennifer Bizon, PhD

1. Yoder WM, Lyman M, Munizza O, Burke SN, Setlow B, Smith DW, **Bizon JL**. "Interaction between age and perceptual similarity in olfactory discrimination learning: relationship with spatial learning impairment." *Neurobiology of Aging*. Under Revision.
2. Orsini CA, Mitchell MR, Heshmati SC, Shimp KG, Spurrell M, **Bizon JL**, Setlow B. "Effects of nucleus accumbens amphetamine administration on performance in a delay discounting task." *Behavioural Brain Research*. Under Revision.
3. Setlow B, **Bizon JL**. "Adolescent cannabinoid use and cognition; unexpected results from a rat model of cannabinoid self-administration" *Neuropsychopharmacology*. In Press.
4. McQuail JA, Beas BS, Simpson K, Kyle K, Frazier CJ, Setlow B, **Bizon JL**. (2016) "NR2A-containing NMDA receptors in the prefrontal cortex are required for working memory and predict age-related cognitive decline." *The Journal of Neuroscience*. In Press.
5. Hernandez AR, Reasor JE, Truckenbrod LM, Lubke KN, Johnson SA, **Bizon JL**, Maurer AP, Burke SN. Medial prefrontal-perirhinal cortical communication is necessary for flexible response selection. *Neurobiology of Learning and Memory*. In Press.
6. Carpenter HE, Kelly KB, **Bizon JL**, Frazier CJ. Age-related changes in tonic activation of pre- and post-synaptic GABA(B) receptors in medial prefrontal cortex. (2016) *Neurobiology of Aging*. Sep;45:88-97. doi: 10.1016/j.neurobiolaging.2016.05.015. Epub 2016 May 21.
7. Johnson SA, Sacks PK, Turner SM, Gaynor LS, Ormerod BK, Maurer AP, **Bizon JL**, Burke SN. (2016) Discrimination performance in aging is vulnerable to interference and dissociable from spatial learning. *Learning and Memory*. Jun 17;23(7):339-48. doi:10.1101/lm.042069.116.
8. Beas BS, Setlow B, **Bizon JL**. (2016) Effects of acute administration of the GABA(B) receptor agonist baclofen on behavioral flexibility in rats. *Psychopharmacology*. Jul;233(14):2787-97. doi: 10.1007/s00213-016-4321-y.
9. Beas BS, McQuail JA, Bañuelos C, Setlow B, **Bizon JL**. (2016) Prefrontal cortical GABAergic signaling and impaired behavioral flexibility. *Neuroscience*. pii: S0306-4522(16)00137-8. doi: 10.1016/j.neuroscience.2016.02.014.
10. Orsini CA, Willis ML, Gilbert RJ, **Bizon JL**, Setlow, B (2016) Sex differences in rat model of risky decision-making. *Behavioral Neuroscience*. Feb;130(1):50-61. doi: 10.1037/bne0000111.
11. Montgomery, KS, Edwards, G, Kumar, A, Levites, Y, Meyers CA, Gluck M, Setlow, B and **Bizon, JL**. (2016) Deficits in hippocampal-dependent transfer generalization learning and synaptic function in mouse models of amyloidosis. *Hippocampus*. 26(4):455-71. doi: 10.1002/hipo.22535.
12. Johnson SA, Turner SM, Santacrose LA, **Bizon JL**, Maurer AP, Burke SN. Age-related impairments on a rodent mnemonic similarity task parallel those observed in humans. *Submitted to Hippocampus*.

Sara N. Burke, PhD

1. Hernandez AR*, Maurer AP*, Reasor JE, Turner SM, Barthle SE, Johnson SA, **Burke SN** (2015). Age-related Impairments in Object-Place Associations Signify a Decline in Systems-level Neural Communication. *Behavioral Neuroscience*, 129(5):599-610.
2. Sheremet A, **Burke SN**, Maurer AP (2016) Movement Enhances the Nonlinearity of Hippocampal Theta. *The Journal of Neuroscience*, 36(15):4218-4230.
3. Johnson SA, Sacks PK, Turner SM, Gaynor LS, Ormerod BK, Maurer AP, Bizon JL, **Burke SN** (2016). Discrimination performance in aging is vulnerable to interference and dissociable from spatial memory. *Learning & Memory*, 23(7):339-48.
4. Gray DT, Smith AC, **Burke SN**, Gazzaley A, Barnes CA (2016). Attentional updating and monitoring and affective shifting are impacted independently by aging in macaque monkeys. *Behavioural Brain Research*, in press.
5. Hernandez AR, Reasor JE, Truckenbrod LM, Lubke, K, Johnson SA, Bizon JL, Maurer AP, **Burke SN** (2016). Medial Prefrontal-Perirhinal Cortical Communication is Necessary for Flexible Response Selection. *Neurobiology of Learning and Memory*, 137:36-47.
6. Yoder WM, Lyman M, Muizza O, Ormerod BK, **Burke SN**, Setlow B, Smith DW, Bizon JL. Interaction between age and perceptual difficulty in olfactory discrimination learning: relationship with hippocampal-dependent spatial learning. *Preliminary acceptance, Neurobiology of Aging*.
7. Johnson SA, Turner SM, Santacroce LA, Bizon JL, Maurer AP, **Burke SN**. Age-related impairments on a rodent mnemonic similarity task parallel those observed in humans. *Submitted to Hippocampus*.

Thomas C. Foster, PhD

1. Gomez-Chacon, B., Gamiz, F., **Foster, T.**, and Gallo, M. Increased N-ethylmaleimide-sensitive factor expression in amygdala and perirhinal cortex during habituation of taste neophobia. *Neural Plasticity*, 2016, 2726745, doi: 10.1155/2016/2726745 **PMCID: 4709763**.
2. Ivanov, L., Rani, A., Beas, B. S., Kumar, A., and **Foster, T.C.** Transcription profile of aging and cognitive-related genes in the medial prefrontal cortex. *Front Aging Neurosci*, doi: 10.3389/fnagi.2016a.00113. **PMCID: 4868850**.
3. **Foster, T.C.**, Kyritsopoulos, A., and Kumar, A., Central role for NMDA receptors in redox mediated impairment of synaptic function during aging and Alzheimer's disease. *Beh Brain Res*, in press.
4. Guadiana, S.M., Parker, A.K., Filho, G.F., Sequeira, A. Kumar, A., Semple-Rowland, S. Shaw, G., Mandel, R.J. **Foster, T.C.**, and Sarkisian, M.R. Type 3 adenylyl cyclase and somatostatin receptor 3 expression persists in aged rat neocortical and hippocampal neuronal cilia. *Front Aging Neurosci*, 2016, 8, 158. **PMCID: 4885836**.
5. Febo, M., and **Foster, T.C.** Preclinical magnetic resonance imaging and spectroscopy studies of memory, aging, and cognitive decline. *Front Aging Neurosci*, 2016, 8, 158. **PMCID: 4942756**.
6. Ivanov, L., Kumar, A., and **Foster, T.C.** Epigenetic regulation of estrogen receptor α contributes to age-related differences in transcription across the hippocampal regions CA1 and CA3. *Neurobiology of Aging*, 2016b, 49, 79-85.

Andrew Maurer, PhD

1. Engle JR, Machado CJ, Permenter MR, Vogt JA, **Maurer AP**, Bulleri AM, Barnes CA. Network Patterns Associated with Navigation Behaviors Are Altered in Aged Nonhuman Primates. *J Neurosci*. 2016 Nov 30;36(48):12217-12227.
2. Johnson SA, Sacks PK, Turner SM, Gaynor LS, Ormerod BK, **Maurer AP**, Bizon JL, Burke SN. Discrimination performance in aging is vulnerable to interference and dissociable from spatial memory. *Learn Mem*. 2016 Jun 17;23(7):339-48. doi: 10.1101/Im.042069.116. Print 2016 Jul. PubMed PMID: 27317194; PubMed Central PMCID: PMC4918781.
3. Witharana WK, Cardiff J, Chawla MK, Xie JY, Alme CB, Eckert M, Lapointe V, Demchuk A, **Maurer AP**, Trivedi V, Sutherland RJ, Guzowski JF, Barnes CA, McNaughton BL. Nonuniform allocation of hippocampal neurons to place fields across all hippocampal subfields. *Hippocampus*. 2016 Oct;26(10):1328-44. doi: 10.1002/hipo.22609. Epub 2016 Jun 24. PubMed PMID: 27273259.

- Shilnikov AL, **Maurer AP**. The Art of Grid Fields: Geometry of Neuronal Time. *Front Neural Circuits*. 2016 Mar 8;10:12. doi: 10.3389/fncir.2016.00012. eCollection 2016. PubMed PMID: 27013981; PubMed Central PMCID: PMC4782041.
- Sheremet A, Burke SN, **Maurer AP**. Movement Enhances the Nonlinearity of Hippocampal Theta. *J Neurosci*. 2016 Apr 13;36(15):4218-30. doi: 10.1523/JNEUROSCI.3564-15.2016. PubMed PMID: 27076421; PubMed Central PMCID: PMC4829647.

PUBLICATIONS (OTHER):

Sara N. Burke, PhD

Foster TC, **Burke SN** (2016). Animal Models of Cognitive Aging and Circuit-Specific Vulnerability. In *Geriatric Neurology*, eds DeKosky and Asthana.

Thomas C. Foster, PhD

Burke, S.N. and **Foster, T.C.** Animal Models of Cognitive Aging and Circuit-Specific Vulnerability. In DeKosky and Asthana (Eds.) *Geriatric Neurology*, 2016 in press.

PRESENTATIONS AT SCIENTIFIC MEETINGS:

Jennifer Bizon, PhD

Completed Talks

- "Excitatory-inhibitory imbalance and age-related decline of prefrontal-cortical dependent cognition" Tulane Aging Center, New Orleans, Louisiana. November 2015.
- "Using olfaction to understand age-related cognitive decline" Center for Smell and Taste Workshop, Gainesville, Florida. November 2015.
- "Signaling alterations in the aged prefrontal cortex: implications for cognition" Joint McKnight Age-related Memory Loss and Cognitive Aging Program, Gainesville, Florida. February 2016.
- "Shifting Excitatory-inhibitory signaling and age-related decline of prefrontal-cortical dependent cognition" Department of Physiology Seminar Series. University of Florida. Gainesville, Florida. September 2016.

Upcoming/Invited Talks

- "Glutamate signaling alterations contribute to age-associated decline in working memory." Memory Mechanisms in Health and Disease conference. Tampa, Florida, December 2016.
- "Using olfactory discrimination to understand mechanisms of age-related cognitive decline." Neurobiology of Learning and Memory Conference. Park City, Utah, January 2017.
- "Using rodent models of prefrontal cortical-mediated cognitive decline in aging to identify drug targets in GABA and glutamate systems." Panel Speaker and co-chair of session on preclinical behavioral models of age-related decline. Annual Meeting of the American Society of Pharmacology and Experimental Therapeutics at Experimental Biology. Chicago, Illinois. April 2017.
- "Hippocampal and prefrontal cortical GABA B receptors and mnemonic decline in aging." Spring Hippocampal Research Conference. Taormina, Italy. June 2017.
- "Cognitive Decline and Aging." Faculty member at Neuroscience School of Advanced Studies. Tuscany, Italy. Sept 2017.

Poster Presentations

- McQuail, JA, Bruner M, Hernandez, C, Krause E, Setlow B, Scheuer D, **Bizon JL**. Stress reactivity predicts impaired working memory in aging: Vulnerability of GABAergic synapses. *Society for Neuroscience Meeting. San Diego, CA. 2016 *First-place McKnight Brain Research Poster Award.*

2. Schwabe M, Hernandez C, McQuail J, Setlow B, **Bizon JL**. Group II and Group III metabotropic glutamate receptors are required for normal working memory and are reduced in the aged rat prefrontal cortex. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
3. Kelly K, **Bizon JL**, Frazier CJ. Functional effects of age on NR2A and NR2B containing NMDA receptors in interneurons and pyramidal cells of the rat medial prefrontal cortex. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
4. Vetere L, Orsini C, McQuail J, Burke S, Setlow B, **Bizon JL**. Age-related alterations in working memory and intertemporal choice in Fischer X Brown Norway 344 hybrid rats. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
5. Blaes S, Orsini C, Spurrell M, **Bizon JL**, Setlow B. Differential effects of D2 and D3 receptor ligands on risky decision making. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
6. A Hernandez, K Campos, L Truckenbrod, L Santacroce, C Hernandez, Y Sakarya, J McQuail, A Maurer, **Bizon, JL**, Carter, C, Burke S. The ketogenic diet as a therapeutic strategy for improving motor and cognitive functioning in a rodent model of senescence. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
7. S Johnson, Yoder W, Lubke K, Lister J, Maurer A, **Bizon JL**, Burke SN. Broad neuronal population coding in hippocampus relative to piriform cortex during difficult olfactory discriminations. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
8. Orsini C, Heshmati S, Wall S, **Bizon JL**, Setlow B. The medial prefrontal cortex is critical for flexibility necessary for adaptive risky decision making. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
9. Hernandez C, Baes S, McQuail J, Setlow B and **Bizon JL**. Biochemical evidence for altered glutamatergic signaling in the aged medial prefrontal cortex: contribution to impaired behavioral flexibility. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
10. Joseph McQuail, Ashley St. John, Caesar Hernandez, Eric Krause, Barry Setlow, Deborah Scheuer, **Jennifer Bizon**. Acute Elevation of Corticosterone Enhances Working Memory and Associates with Preserved Cognitive Ability in Aging. *American College of Neuropsychopharmacology Annual Meeting. Hollywood, FL*. 2016.
11. Caitlin Orsini, Sara Heshmati, Shannon Wall, **Jennifer Bizon**, Barry Setlow. The Medial Prefrontal Cortex is Critical for the Flexibility Necessary for Adaptive Risky Decision-Making. *American College of Neuropsychopharmacology Annual Meeting. Hollywood, FL*. 2016.
12. Barry Setlow, Shelby Blaes, Caitlin Orsini, Shandera Ferguson, Sara Heshmati, Shannon Wall, Marcelo Febo, Adriaan Buijnzeel, **Jennifer Bizon**. Effects of Acute Exposure to Cannabis Smoke on Working Memory. *American College of Neuropsychopharmacology Annual Meeting. Hollywood, FL*. 2016.

Sara N. Burke, PhD

1. Feb 29, 2016: *Behavioral Neuroscience Brown Bag Seminar Series, University of Delaware, Newark, DE*. "Working Towards a Systems-level Understanding of Cognitive and Physical Senescence."
2. Dec 10, 2015: *Keynote Speaker, Conference on Aging and Dementia, University of Rzeszow, Rzeszow, Poland*. "Cortical structure and function in aging and Alzheimer's disease"

Completed Poster Presentations (National/International Only)

1. ANDERSH K.M., D.T. GRAY, A. C. SMITH, **S. N. BURKE**, A. GAZZALEY, C. A. BARNES. Age-related attentional control and set shifting impairments arise independently in macaque monkeys. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
2. GAYNOR L.S. J. MIZELL, K. T. CAMPOS, L. SANTACROCE, C. MCEWEN, D. K. CHETRAM, A. P. MAURER, R. M. BAUER, **S. N. BURKE**. Stimulus modality affects recognition behavior during spontaneous object recognition and crossmodal object recognition tasks. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
3. Hernandez, K Campos, L Truckenbrod, L Santacroce, C Hernandez, Y Sakarya, J McQuail, A Maurer, Bizon, JL, Carter, C, **Burke SN**. The ketogenic diet as a therapeutic strategy for improving motor and cognitive functioning in a rodent model of senescence. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
4. Johnson, Yoder W, Lubke K, Lister J, Maurer A, Bizon JL, **Burke SN**. Broad neuronal population coding in hippocampus relative to piriform cortex during difficult olfactory discriminations. *Society for Neuroscience Meeting. San Diego, CA*. 2016.
5. MAURER A.P., C.-H. ELVIRA-MARTIN, **S. N. BURKE**, A. SHEREMET. Velocity modulated hippocampal local-field potential across

hippocampal lamina. *Society for Neuroscience Meeting. San Diego, CA. 2016.*

6. UMAPATHY L., D. T. GRAY, S. N. BURKE, T. P. TROUARD, C. A. BARNES. Uncinate fasciculus integrity assessed in young and aged bonnet macaques. *Society for Neuroscience Meeting. San Diego, CA. 2016.*
7. Vetere L, Orsini C, McQuail J, **Burke S**, Setlow B, Bizon JL. Age-related alterations in working memory and intertemporal choice in Fischer X Brown Norway 344 hybrid rats. *Society for Neuroscience Meeting. San Diego, CA. 2016.*
8. TURNER S.M., L. A. SANTACROCE, S. A. JOHNSON, S. N. BURKE, A. P. MAURER. A rodent model of medial temporal lobe-dependent discrimination deficits in the elderly. *Society for Neuroscience Meeting. San Diego, CA. 2016.*

Thomas C. Foster, PhD

1. The senescent synapse, Neurodegenerative and psychiatric diseases in aging - Advances in treatments and mechanisms, 2016, *International Forum on Elderly Health, Tai'an City, China.*
2. Bridging the gap between senescent synapses and genomic programs for neuronal health, *University of North Texas, Health Science Center (03/01/2016).*
3. 2016 Synaptic plasticity meets oxidative stress on the path to age-related cognitive decline, *University of Michigan, Neuroscience Graduate Program, (04/11/2016).*
4. 2016 What can the transcriptome tell us about the regional vulnerability to age and cognitive impairment? *Inter-Institute Meeting, Tucson AZ (04/28/2016).*
5. The long-term estrogen-induced facilitation of NMDA receptor synaptic function is mediated through altered redox state. (2016) Kumar, A., Rani, A., Bean, L., **Foster, T.C.** *Soc for Neurosci.* 177.03
6. Expression of G-protein estrogen receptor 1 (GPER1) in the hippocampus and prefrontal cortex over the oestrous cycle: Influence of ovariectomy and aging. (2016) Rani, A., Keric, S. Bean, L., Barter, J. **Foster, T.C.**, Kumar, A. *Soc for Neurosci.* 177.04
7. Systemic inflammation contributes to the onset of cognitive impairment associated with senescence. (2016) Barter, J.D., Kumar, A., Rani, A., **Foster, T.C.** *Soc for Neurosci.* 177.05
8. Up regulation of GluN2B type NMDA receptor in CA1 region of hippocampus and its influence on cognitive and synaptic function. (2016) Kyritsopoulos, C., Kumar, A., **Foster, T.C.** *Soc for Neurosci.* 177.06
9. Epigenetic regulation of medial prefrontal cortex transcription associated with aging and impaired executive function. (2016). **Foster, T.C.**, Ianov, L. Riva, A. *Soc for Neurosci.* 182.04.
10. Transcriptomic profile for determining regional vulnerability to age and cognitive impairment. (2016). Ianov, L., De Both, M.D., Chawla, M.K., Rani, A., Kennedy, A.J., Piras, I., Day, J.J., Siniard, A.L., Kumar, A., Sweatt, J.D., Barnes, C.A., Huentelman, M.J., **Foster, T.C.** *Soc for Neurosci.* 182.05
11. Transcriptional differences among hippocampal subregions. (2016) De Both, M.D., Ianov, L., Chawla, M.K., Rani, A., Kennedy, A.J., Piras, I., Day, J.J., Siniard, A.L., Kumar, A., Sweatt, J.D., **Foster, T.C.**, Barnes, C.A., Huentelman, M.J. *Soc for Neurosci.* 182.06.
12. NSAID treatment reverses age-related changes in hippocampal neurogenesis. (2016) McGuinness, Scheinert, R.B., Schwingel, V-C., Rani, A., Kumar, A., **Foster, T.C.**, Ormerod, B.K. *Soc for Neurosci.* 592.09.
13. A high-performance pipeline for differential methylation analysis (2016) Riva, A., Poudyal, R., Ianov, L., Gu, T., Kladdde, M., **Foster, T.C.** *International Society for Computational Biology.*

Andrew Maurer, PhD

1. *Velocity modulated hippocampal local-field potential across hippocampal lamina* *A. P. MAURER¹, C.-H. ELVIRA-MARTIN¹, S. N. BURKE¹, A. SHEREMET²
2. *Broad neuronal population coding in hippocampus relative to piriform cortex during difficult olfactory discriminations* *S. A. JOHNSON^{1,2}, W. M. YODER³, K. N. LUBKE², J. P. LISTER⁶, A. P. MAURER⁴, J. L. BIZON², S. N. BURKE^{2,5}
3. *A rodent model of medial temporal lobe-dependent discrimination deficits in the elderly* *S. M. TURNER¹, L. A. SANTACROCE¹, S. A.

JOHNSON¹, S. N. BURKE^{1,3,4}, A. P. MAURER^{1,2}

4. *Stimulus modality affects recognition behavior during spontaneous object recognition and crossmodal object recognition tasks*
*L. S. GAYNOR^{1,2}, J. MIZELL¹, K. T. CAMPOS¹, L. SANTACROCE¹, C. MCEWEN¹, D. K. CHETRAM¹, A. P. MAURER¹, R. M. BAUER², S. N. BURKE¹
5. *The ketogenic diet as a therapeutic strategy for improving motor and cognitive functioning in a rodent model of senescence* *A. R. HERNANDEZ, K. CAMPOS, L. TRUCKENBROD, L. SANTACROCE, C. M. HERNANDEZ, Y. SAKARYA, J. A. MCQUAIL, A. P. MAURER, J. BIZON, C. CARTER, S. N. BURKE
6. **Faculty for Undergraduate Neuroscience.** Mentoring Charles Martin on “*Velocity-modulated hippocampal local-field potential across hippocampal lamina.*” as a part of the SfN San Diego conference.

PRESENTATIONS AT NON-SCIENTIFIC MEETINGS OR EVENTS:

Sara N. Burke, PhD

UF College of Medicine Research Landscapes

https://www.youtube.com/watch?v=dliA7Y6xoVc&list=PLr_fqSI-B7YBAR-X88_vCO2V5FMIs35F6&index=13

AWARDS (OTHER):

Jennifer Bizon, PhD

UF College of Medicine Excellence in Teaching Award

Sara N. Burke, PhD

1. 2015-2016, Exemplary Teaching Award, University of Florida College of Medicine
2. 2016, University of Florida Excellence Award for Assistant Professors

FACULTY BIOGRAPHICAL SKETCHES: See page 82

TRAINEES:

Jennifer Bizon, PhD

a. Post-doctoral

Dr. Joseph McQuail Dr. McQuail is funded by a three-year F32 NRSA from the National Institute on Aging that was awarded last year. In the past year, he published several manuscripts, including one that recently appeared in the *Journal of Neuroscience* describing specific synaptic alterations in the aged prefrontal cortex that contribute to age-related cognitive decline. Dr. McQuail has received several awards in this past year, including one given for the best poster presented by a postdoctoral fellow at the Department of Neuroscience annual retreat, as well as 1st place at the McKnight reception at the annual Society for Neuroscience meeting in San Diego.

Dr. Caitlin Orsini (co-Mentored with Dr. Barry Setlow) During the past year, Dr. Orsini has been funded by a Maren Postdoctoral fellowship from the UF-College of Medicine. In July, she received a perfect score on her K99/R00 award from NIH to fund her research related to the neural mechanisms of decision making. She has been instrumental in establishing optogenetic tools in our laboratory, which we are now applying to questions pertaining to cognitive aging. Dr. Orsini has published several manuscripts in the past year, including one in *Behavioral Neuroscience* that describes sex differences in cost-benefit decision making.

b. Pre-doctoral

Caesar Hernandez Caesar entered my laboratory in 2014 to pursue his Ph.D. on topics related to age related cognitive decline. He has been exceptionally productive and is in the process of writing two manuscripts that will be submitted this upcoming year. This year, he received a travel fellowship to the annual Society for Neuroscience meeting from the national chapter.

Sara N. Burke, PhD

a. Post-doctoral

Sarah A. Johnson, PhD: Dr. Johnson is funded by the MBI fellowship and my R01. She is a third-year postdoc that is examining how the discrimination of perceptually similar spatial and object stimuli is related to memory performance in old age. She has recently found that one of the hallmarks of superior mnemonic discrimination performance is the ability to flexibly regulate behavior. Dr. Johnson will be pursuing this link further with the K99/R00 proposal she is currently working on. Currently, she has published one first author paper in my lab, has another paper under review, and has published two 2 co-author papers.

b. Pre-doctoral:

Abigail R. Hernandez (Rosen), M.S.: Ms. Hernandez is a third-year graduate student that is funded from my Pepper Pilot Grant and MBI-ARML institutional startup funds. She is investigating how a ketogenic diet intervention could potentially improve function across multiple systems in the elderly. Since joining my lab, she has published 2 first-author papers.

Katelyn N. Lubke: Ms. Lubke is a first-year graduate student in biomedical engineering that is funded from R01. She is examining functional connectivity that is related to an animal's ability to perform flexible bi-conditional associations in aged rats.

Leslie S. Gaynor: Leslie Gaynor is a second-year Neuropsychology graduate student in the laboratory of my collaborator Russell Bauer. I co-mentor her on an animal project that measures perirhinal-cortical dependent behaviors in young and aged rats and humans.

c. Other:

Sean Turner: Mr. Turner is a post baccalaureate student that has been working in my laboratory for 3 years. He is funded off of my R01 and will be attending graduate school next year. He has co-authored multiple papers, presented at national meetings and was invited to participate in the Council for Undergraduate Research Advocacy Event Posters on the Hill last spring. As part of this event he met with Florida Congressman Ted Yoho and other Congressional staff in Washington D.C.

Keila Campos: Ms. Campos is a third-year undergraduate and a University Scholar awardee. She is assisting Abbi Hernandez with her dissertation research and will be completing and honor's thesis with me next year.

Leah Truckenbrod: Ms. Truckenbrod is a third-year undergraduate that will be applying for a University Scholar Award next year and writing an honor's thesis with me.

Thomas C. Foster, PhD

a. Post-doctoral

Brittney Yelga, PhD

b. Pre-doctoral

(i) **Lara Ianov**, Graduate Student, Department of Genetics, University of Florida

(ii) **Constantinos Kyritsopoulos**, Graduate Student, Department of Neuroscience, University of Florida

(iii) **Jolie Barter**, Graduate Student, Department of Neuroscience, University of Florida

c. Other

Ashok Kumar, PhD

Asha Rani (Technical)

Andrew Maurer, PhD

- b. Pre-doctoral:
Co-mentor, **Douglas Miller**, Neuroscience IDP

CLINICAL/TRANSLATIONAL PROGRAMS:

Thomas C. Foster, PhD

- a. **New programs**

A new NIH grant has been funded to test a hypothesized mechanism that links systemic inflammation to brain aging through the cytokine IL-6.

A project is underway to examine RNA from serum exosomes collected from individuals that have been characterized for cognition as part of the ACTIVE study.

TECHNOLOGY TRANSFER: NA

BUDGET UPDATE: See page 62

EDUCATIONAL PROGRAMS FOCUSING ON AGE RELATED MEMORY LOSS: NA

COLLABORATIVE PROGRAMS WITH OTHER MCKNIGHT INSTITUTES, INSTITUTIONS AND RESEARCH PROGRAMS:

Sara N. Burke, PhD

I am involved in the Behavioral and Cognitive Health Working Group along with colleagues at the University of Arizona, University of Miami and University of Alabama. We had a workshop last summer, of which I was a participant, and our group recently completed a white paper that was submitted to the MBRF trustees.

Thomas C. Foster, PhD

Relationship between transcription and cognitive decline: Inter-institute collaboration. The results have been presented at the Society for Neuroscience meeting (2016) and a manuscript related to this work is currently in the process of construction.

In collaboration with the neuroimaging group, the protocols have been developed for collection and processing of serum for examine RNA from serum exosomes.

Andrew Maurer, PhD

Dr. Sara Burke (UF), Dr. Carol Barnes (U of A) and I may utilize an older database to conduct a small, collaborative analysis on perirhinal dynamics and aging.

COLLABORATIVE PROGRAM WITH NON-MCKNIGHT INSTITUTES, INSTITUTIONS AND RESEARCH PROGRAMS:

Jennifer Bizon, PhD

This past year, Dr. Carol Barnes at the University of Arizona asked me to participate in an inter-institutional “Brain and Cognitive Health Working Group” that was an extension of a cognitive working group that I was a part of several years ago. This new working group (which included faculty members across each of the four institutes) had our first meeting prior to the annual inter-institutional meeting in Arizona and resulted in 3 pilot projects, including one that I led on the topic of stress as a contributing factor to age-related cognitive decline. I organized the second meeting of this working group in Jacksonville, FL in August and, along with Carol, coordinated the writing of white papers from the each of the three subgroups. These white papers were delivered to the trustees earlier this month. The white paper I led is focused on collecting pilot data (in both rats and humans) across McKnight institutions to provide the foundation for an extramurally funded project to investigate chronic stress and HPA axis dysfunction as a modifiable risk factor for cognitive decline. Assuming some additional funding is secured to obtain key inter-institutional preliminary data, this working group plans to begin work on NIH grant submissions over the next year.

Thomas C. Foster, PhD

We currently have a collaboration with Drs. Esser and Kang to examine transcription from muscle and brain for animals with a conditional knockout of a circadian rhythm gene. The broad view is that brain aging is influenced by communication between organs. Specifically, disruption of circadian rhythms (e.g. disrupted sleep) due to muscle clocks will contribute to brain aging. This work represents a collaboration between the MBRF and the Pepper Center.

BRIEFLY DESCRIBE PLANS FOR FUTURE RESEARCH AND/OR CLINICAL INITIATIVES:

Jennifer Bizon, PhD

As described above, Dr. Sara Burke and I are currently preparing a new R21 as MPIs (target submission February 2016) that is focused on identifying the mechanisms of perceptual deficits in aging and their contribution to episodic mnemonic decline. Dr. Maurer and I will continue to advance our collaboration related to basal forebrain and aim to publish at least one paper together this year. I will further continue to serve on the mentoring committee for Dr. Eric Porges, a member of CAM, as I have co-Mentored several of his recent K-grant submissions.

Molecular and Circuit Mechanisms of Cost-Benefit Decision Making. Given that we have recently been successful in collecting both *in vitro* and *in vivo* optogenetic data, we are now positioned to write R01 proposals utilizing these techniques in combination with some of our novel behavioral models. My initial priority is to focus on a MPI R01 with Barry Setlow and Jason Frazier in which we will propose experiments to test the hypothesis that alterations in medial prefrontal cortex - basolateral amygdala circuits mediate age-associated changes in decision making. My graduate student, Caesar Hernandez, will also submit a F31 in the next year testing ideas related to this line of research.

Cognitive Impact of Marijuana Use at Advanced Ages. The NIDA program officer who manages the NIDA R01 that Barry and I share (Setlow PI, Bizon co-I) recently expressed interest in my developing a line of research investigating the cognitive impact of marijuana use in older adults. With the recent legalization of marijuana in several states, there has been a dramatic escalation in the number of individuals over age 65 using this drug recreationally or for medical purposes. To date, however, there is no information regarding the impact of such drug use on cognitive functioning in this population of individuals already at increased risk for decline. Given my expertise in cognitive aging and the fact that Barry already holds several NIDA-funded grants on the topic of cannabis exposure, the program officer specifically asked me to submit a new grant on this topic with Barry as a co-I. We are perfectly positioned to conduct these types of experiments and we plan to write an R21 on this topic in the coming months.

Sara N. Burke, PhD

I want to focus on developing collaborative research grants with my EMBL colleagues at UF, Miami, Arizona and Alabama. Dr. Bizon and I have a pending grant together (Ed and Ethyl Moore) and will be submitting an R21 and R01 within the next year. These will

be focused on defining the mechanisms of multi-modal discrimination deficits in the elderly as well as defining the boundary between normal aging and pathology with state-of-the-art behavioral analysis.

Thomas C. Foster, PhD

Continue research on the hypothesis that systemic inflammation contributes to the onset and progression of age-related cognitive decline.

Continue research on the hypothesis that redox mediated NMDA receptor hypofunction mediates impaired hippocampal and prefrontal function during aging.

Continue research on epigenetic changes associated with age-related cognitive decline.

Complete analysis of exosome RNA from human serum samples.

Continue collaborations examining organ-systems interactions as a mechanism influencing brain aging.

IF APPLICABLE, PLEASE PROVIDE ENDOWMENT INVESTMENT RESULTS FOR THE REPORT PERIOD: See page 71

WERE ANY FUNDS USED FOR A PROHIBITED PURPOSE DURING THE REPORT PERIOD? No

DO YOU RECOMMEND ANY MODIFICATION TO THE PURPOSE OR MANDATES IN THE GIFT AGREEMENT? No

DID ALL ACTIVITIES DURING THE REPORT PERIOD FURTHER THE PURPOSE? Yes

NEGATIVE EVENTS (LOSS OF PERSONNEL, SPACE, BUDGET ETC.): NA

ADDITIONAL COMMENTS: See letter on page 9

SIGNATURE, DATE AND TITLE OF PERSON SUBMITTING THE REPORT:



Thomas C. Foster, PhD

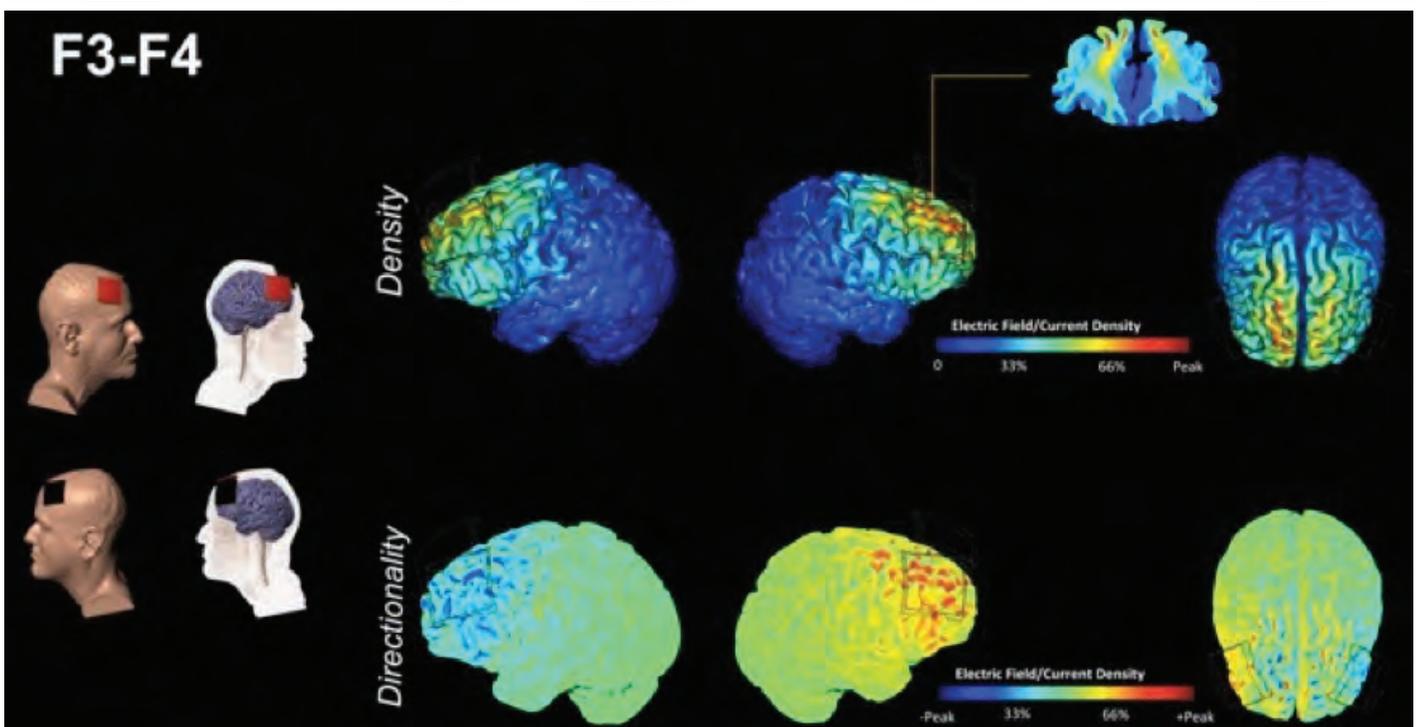
Professor, Department of Neuroscience and Genetics and Genomics Program
Evelyn F. McKnight Chair for Research on Cognitive Aging and Memory

Cognitive Aging and Memory – Clinical Translational Research Program and the Evelyn F. McKnight Chair for Clinical Translational Research in Cognitive Aging

2016 Progress Report



F3-F4



December 31, 2016

Dear Trustees of the McKnight Brain Research Foundation:

We are pleased to provide you with a progress report of the Cognitive Aging and Memory-Clinical Translational Research Program (CAM-CTRP) for the year ending December 31, 2016. The past year has been extremely busy and productive, but also had challenges which are now being overcome. The CAM-CTRP continues to make significant progress in the areas of research outlined in last year's report, as well as the initiation of several new major initiatives. The CAM-CTRP has enjoyed many successes that will keep the program on track for a future of exciting and groundbreaking clinical-translational research on cognitive aging. This has occurred against the backdrop of a major change in the administrative structure of CAM-CTRP at UF. These changes will be reviewed first, which will be followed by research and programmatic developments.

Considerable effort was directed over the past twelve months towards resolving issues related to the structure and operation of CAM-CTRP within the UF Institute on Aging, including budgetary issues. These difficulties will not be reviewed in this Annual Report, but had a significant impact on our activity during this year period. Fortunately, a positive resolution has been achieved, resulting in the CAM-CTRP moving back into the direct administrative structure of the McKnight Brain Institute (MBI). Space has been identified on the ground floor of the MBI, which will be the new physical location of the CAM-CTRP. This space (approximately 700 sq. ft.) will be renovated for use as the primary clinical research facility and central office for the CAM-CTRP. It will be subdivided into a reception/administrative area, four clinical assessment/ testing rooms, and a shielded room and console area. These latter two areas in the back of the space will contain the electrophysiology and neuromodulation laboratory and equipment, along with computers for the technicians running electrophysiology and neuromodulation studies. There is also adjacent space that we have been given and will convert into a phlebotomy lab with a centrifuge and freezer. Overall, this space should meet our operational needs well for conducting CAM-CTRP related research.

In addition to the new MBI clinical research space, the current core faculty of the CAM-CTRP (Drs. Cohen, Woods, and Porges) have joined the Department of Clinical and Health Psychology (CHP) in the College of Public Health and Health Professions as full faculty members, with Dr. Cohen transferring his tenure to this department. Office space has been provided for the three faculty members, along with three larger spaces to house a total of eleven study coordinators and graduate students. In the short-term, the CAM-CTRP administrator, Tina Lacy is working out of one of these spaces, though the plan is for her to be located in the CAM-CTRP research area in the MBI once renovations are complete.

As part of this transition, we were encouraged by the UF Health administration to seek official UF center status, and are in process of submitting the application and paperwork for creation of this center. With the move to the department of CHP, discussions have also been initiated with Drs. Smith and Marsiske to integrate them into the CAM-CTRP. Several other faculty members (Price, Bowers, Bauer) have ongoing collaborations with our group, which we hope will be enhanced as a function of the changes that have occurred.

In spite of the time associated with managing these transitions, the CAM-CTRP has been extremely productive and successful in obtaining extramural funding over the past year, as well as publishing numerous manuscripts on topics related to cognitive and brain aging. Of particular note was NIA funding of the ACT grant, a multi-center study to examine the augmenting effects of tDCS brain stimulation on cognitive training in older adults without AD or other neurodegenerative disease. Dr. Woods received a career development (K) award from NIA to examine specific brain mechanisms associated with cognitive changes in the context of brain stimulation. Dr. Porges obtained KL-2 funding to study glutamate and GABA changes in the brain in HIV and aging using magnetic resonance spectroscopy (MRS). Cohen's RO1, funded by NIDDK on, "Obesity and type II diabetes: bariatric surgery effects on brain function and aging", is underway, providing insights into the metabolic factors associated with obesity and diabetes on brain structure and function, and the brain effects resulting from reductions obesity and DM severity after bariatric surgery and significant weight loss. This clinical study provides an excellent experimental model for testing whether caloric reduction improves brain health, with major implications for healthy cognitive aging. Dr. Cohen's ARCH-2 grant from NIAAA to study HIV and alcohol consumption effects on the brains in the context of aging is funded. This fall he and Robert Cook, MD (Co-PIs) received a large U01 to study the beneficial effects of reducing alcohol consumption, via contingency management, on cognitive and brain function of older adults with HIV. This project involves investigators at the University of Miami, another MBI site. We also initiated a R56 grant through NHLBI (Cohen, Williamson) that examines the effects of increasing cerebral blood flow on the brain and cognitive function in people with cardiovascular disease. This project is likely to lead to a larger R01 project, and must be set against a background of such function in normalizing and potential improvement in cognition with improved blood flow.

In addition, CAM-CTRP faculty have over 15 pending grants in PI and Co-I roles. These include collaborations with UCLA, other investigators in the UF-College of Public Health & Health Professions, the Malcolm Randal VAMC, the University of Southern California, Stanford, Georgia Tech, the University of Miami, Florida International University, the University of Alabama at Birmingham, the University of Arizona, the University of Arkansas for Medical Sciences, City University of New York, the University of Pennsylvania, and Brown University. The topics span a broad range of important areas for study of the aging brain, such as predicting brain changes, multi-modal brain training for broad cognitive transfer in elders, white matter integrity using ultra-high field neuroimaging of the aging brain, and non-invasive interventions for cognitive aging. The full scope of our extramural activity is outlined in the full Report.

Two areas of study on which we have placed particular emphasis over the past year are: 1) Clinical-translational research development; and 2) greater collaboration with physician-scientists, including efforts to recruit a physician-scientist with specific interests in cognitive aging.

Collaborations with physician researchers has progressed with major involvements with Dr. Cook (Internal Medicine), cardiologists involved in our heart failure project, bariatric surgeons and endocrinologists in the bariatric surgery project, and neurologists on several different studies. Dr. Steven DeKosky has been involved in co-mentoring career development award applications for faculty members, and also will be the study physician on the ACT grant. He is also developing a new project with several CAM-CTRP faculty that examines an intervention that uses intermittent low level hypoxia to enhance brain function in normal aging.

We continue to focus on recruitment of a physician scientist who has a focus on cognitive aging, and have identified a possible candidate who is currently a post-doctoral fellow in neurology at UF, though she is a year

away from being ready to pursue a faculty position. Significant strides have been made on our clinical-translational objectives, most notably the ACT grant.

Others include: 1) Pilot laboratory studies with Drs. Bizon, Febo, Setlow, Burke, and Mauer in neuroscience; 2) Epigenetic analyses of blood from CAM-CTRIP cohort (Foster); 3) a Nutraceutical trial in cognitive function (Woods); 4) Oxytocin trials (Ebner); 5) the MBAR project (inter-MBI); and, 6) the NSF CANE neuro-engineering grant, which is likely to be funded in February 2017. These projects include both basic laboratory science translation to human experimental studies and human studies translation to clinical trials. These efforts will be supported by recent work to create an inter-MBI Clinical Translation Core. While we have had to overcome significant challenges, the CAM-CTRIP has enjoyed a wonderful year of successes, which we expect to continue over the coming years.

Summaries of the publications and active federal funds associated with CAM faculty efforts during 2016 are included in the following pages for your reference.

Sincerely,

A handwritten signature in black ink, appearing to read 'R. Cohen', is positioned above the typed name.

Ronald Cohen, Ph.D., ABPP, ABCN
Professor, Aging, Neurology, and Psychiatry
Director, CAM-CTRIP,
Evelyn McKnight Chair
for Clinical Translation in Cognitive Aging

Publications

1. Alosco ML, Brickman AM, Spitznagel MB, Narkhede A, Griffith EY, Cohen R, Sweet LH, Josephson R, Hughes J, Gunstad J. Reduced Gray Matter Volume Is Associated with Poorer Instrumental Activities of Daily Living Performance in Heart Failure. *J Cardiovasc Nurs*. 2016 Jan-Feb;31(1):31-41. doi: 10.1097/JCN.0000000000000218. PubMed [citation] PMID: 25419946, PMCID: PMC4440850
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5. Cohen RA, Navia B. Comment: Getting a handle on HAND in the era of cART. *Neurology*. 2016 Jan 26;86(4):339. doi: 10.1212/WNL.0000000000002279. No abstract available. PubMed [citation] PMID: 26718565.
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10. Monnig MA, Kahler CW, Lee H, Pantalone DW, Mayer KH, Cohen RA, Monti PM. Effects of smoking and alcohol use on neurocognitive functioning in heavy drinking, HIV-positive men who have sex with men. *AIDS Care*. 2016;28(3):300-5. doi: 10.1080/09540121.2015.1093595. PubMed [citation] PMID: 26444260, PMCID: PMC4821065
11. Okafor CN, Kelso NE, Bryant V, Burrell LE 2nd, Míguez MJ, Gongvatana A, Tashima KT, de la Monte S, Cook RL, Cohen RA. Body mass index, inflammatory biomarkers and neurocognitive impairment in HIV-infected persons. *Psychol Health Med*. 2016 Jun 20:1-14. [Epub ahead of print] PubMed [citation] PMID: 27319430
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15. Swami S, Cohen RA, Kairalla JA, Manini TM. Anticholinergic Drug Use and Risk to Cognitive Performance in Older Adults with Questionable Cognitive Impairment: A Cross-Sectional Analysis. *Drugs Aging*. 2016 Nov;33(11):809-818. PubMed PMID: 27638818.
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73. Nissim, N., Szymkowitz, S.M., Woods, A.J. Edge Detection. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
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75. Nissim, N., Woods, A.J. Visual Psychophysics. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
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77. Sinha, P., Bowers, D., Woods, A.J. D2 Test of Attention. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.

78. Suryadevara, U., Woods, A.J. Motion Parallax. Encyclopedia of Clinical Neuropsychology, 2nd Ed. Springer New York, in press.
79. Suryadevara, U., Woods, A.J. Eye Dominance. Encyclopedia of Clinical Neuropsychology, 2nd Ed. Springer New York, in press.
80. O'Shea, A., Woods, A.J. Useful Field of View. Encyclopedia of Clinical Neuropsychology, 2nd Ed. Springer New York, in press.
81. Altmomare, L.G., Woods, A.J. Visual Convergence. Encyclopedia of Clinical Neuropsychology, 2nd Ed. Springer New York, in press.
82. Richards, L., Woods, A.J. Posterior Cortical Atrophy. Encyclopedia of Clinical Neuropsychology, 2nd Ed. Springer New York, in press.
83. Polejaeva, E., Woods, A.J. Behavioral Inattention Test (BIT). Encyclopedia of Clinical Neuropsychology, 2nd Ed. Springer New York, in press.
84. Polejaeva, E., Woods, A.J. Auditory Selective Attention Test. Encyclopedia of Clinical Neuropsychology, 2nd Ed. Springer New York, in press.
85. O'Shea, D., Woods, A.J. Tests of Variables of Attention. Encyclopedia of Clinical Neuropsychology, 2nd Ed. Springer New York, in press.

Active Federal Funding:

PI	Project Title	020403-02	Project Number	Type	Activity	IC	Organization Name	FY	FY Total Cost
COHEN, RONALD A	OBESITY AND TYPE-2 DIABETES: BARIATRIC SURGERY EFFECTS ON BRAIN FUNCTION	NIDDK	5R01DK099334-03	5	R01	DK	UNIVERSITY OF FLORIDA	2016	526,600.00
COHEN, RONALD A/COOK, ROBERT L	EFFECTS OF EXPERIMENTALLY-INDUCED REDUCTIONS IN ALCOHOL CONSUMPTION ON BRAIN COGNITIVE; AND CLINICAL OUTCOMES AND MOTIVATION FOR CHANGING DRINKING IN OLDER PERSONS WITH HIV INFECTION	NIAAA	2U01AA020797-06	2	U01	AA	UNIVERSITY OF FLORIDA	2016	747,203.00
COHEN, RONALD A	ALCOHOL and HIV: Biobehavioral INTERACTIONS AND INTERVENTIONS	NIAAA	2P01AA019072-07		P01		UNIVERSITY OF FLORIDA	2016	329,620.00
COHEN, RONALD A	ENIGMA	NIH/NIBIB	5U54EB020403-02		R01		UNIVERSITY OF FLORIDA	2016	40,914.00
COHEN, RONALD A	PREDICTING BRAIN CHANGES IN HIV/AIDS	NINDS	5R01NS080655-06		R01		UNIVERSITY OF FLORIDA	2016	35,337.00
CRUZ-ALMEIDA, YENISEL	NEUROIMAGING AGE-RELATED CHANGES I PAIN MODULATION	NIA	5K01AG048259-02	5	K01	AG	UNIVERSITY OF FLORIDA	2016	121,779.00
WOODS, ADAM J.	NEUROMODULATION OF COGNITION IN OLDER ADULTS	NIA	1K01AG050707-01A1	1	K01	AG	UNIVERSITY OF FLORIDA	2016	119,760.00
WOODS, ADAM J./COHEN, RONALD A	AUGMENTING COGNITIVE TRAINING IN OLDER ADULTS - THE ACT GRANT	NIA	1R01AG054077-01	1	R01	AG	UNIVERSITY OF FLORIDA	2016	1,339,215.00
WOODS, ADAM J.	THE INFLUENCE OF FERMENTED PAPAYA PREPARATION (FPP) ON CEREBRAL ENERGY METABOLISM, NEURONINFLAMMATION, AND COGNITION		N/A		Industry		UNIVERSITY OF FLORIDA	2016	134,089.00
									3,394,517.00

SUMMARY OF SCIENTIFIC ACHIEVEMENTS SINCE LAST REPORT:

Ronald A. Cohen, PhD

1. The award of the ACT grant from NIA, a major multi-site R01 aimed at examining the augmenting effects of tDCS brain stimulation on cognitive training in the elderly (Cohen, Woods, Marskike, MPis);
2. The award of a U01 grant to study the effects of reducing alcohol consumption among HIV-infected people who are reaching more advanced age (Cohen, Cook, MPis);
3. Initiation of the McKnight Brain Research Foundation Inter-Institutional neuroimaging and cognitive initiatives to creates a brain aging registry (MBAR) and establish normative databases for successful cognitive aging in people over 85 years;
4. Continuation of several ongoing R01 projects (e.g., WISE study that examines bariatric surgery induced weight loss effects on the brain and cognition;
6. Publications and initiation of a pilot project to study chemotherapy effects for breast cancer in older women (CAM-Nursing-Cancer Institute initiative);
7. K01 award to Dr. Woods (Cohen, Primary mentor)
8. KL-2 award to Dr. Porges (Cohen, Primary mentor);
9. Multiple manuscripts related to current CAM lines of research.

Note: These achievements have been occurring in the context of a major change in the CAM-CTRP administrative structure. Specific scientific achievements, including manuscripts linked to these research efforts are outlined later in this report.

Yenisel Cruz-Almeida, PhD

This past year, I started my K01 funded study which included IRB preparation, submission and approval, and protocol development and refinement. My laboratory has been very successful this period with recruitment and enrollment of 84% of the study's proposed sample. We are currently working on data management and data analysis for manuscript and grant development (R01s) and submissions next year. I submitted three manuscripts that are currently in peer review.

1. **Cruz-Almeida Y**, Aguirre M, Sorensen H, Wallet SM, Riley JL. Age differences in salivary markers of inflammation: Does venipuncture matter? *Journal of Pain Research*.
2. **Cruz-Almeida Y**, Rosso AL, Marcum Z, Harris T, Satterfield S, Newman A, Yaffe K, Rosano C. Associations of musculoskeletal pain with mobility in older adults: potential cerebral mechanisms. *The Journals of Gerontology: Medical Sciences*.
3. **Cruz-Almeida Y**, Cardoso J, Sibille KT, Riley JL, Glover TL, King CD, Goodin BR, Bartley EJ, Herbert MS, Sotolongo A, Fessler BJ, Redden DT, Staud R, Bradley LA, Fillingim RB. Physical performance profiles in persons with knee osteoarthritis. *The Journals of Gerontology: Medical Sciences*.

Natalie C. Ebner, PhD

Since the last report, I have acquired additional funding for my research to complete the ongoing 4-week clinical trial on oxytocin and aging. A PRICE-CTSI-IOA (ARG DTD 03-26-2008) pilot grant on *Neurobiological Mechanisms of Oxytocin's Pain-Modulatory Role in Aging* (\$24,907) as well as a University of Florida Cognitive Aging and Memory Clinical Translational Research Program (CAM-CTRP) (F016327 & NIH/NIA University of Florida Claude D. Pepper Older Americans Independence Center P30 AG028740) supplement on the *Role of Oxytocin in Inflammation* (\$8,567). I currently still have two active NSF grants and one active MIT Lincoln Lab grant ongoing. I got 14 papers accepted in peer-reviewed journals over the course of the last year. This year, I was awarded a UF Excellence Award for Assistant Professors by the Provost, selected as one of 10 faculty university-wide to receive this award and I was nominated recipient of a CLAS International Educator of the Year Award for 2016. In addition, I have served as Member of the Organizing Committee for the 6th Indonesian-American Kavli Frontiers of Science (KFoS) symposium in East Java, Indonesia, sponsored by the Indonesian Academy of Sciences, the U.S. National Academy of Sciences, the U.S. Agency for International Development (USAID), and the Australian Academy of Science. The KFoS symposia are designed for outstanding early-career

scientists to share ideas across disciplines and to build national and international networks that will serve them as they advance in their careers. The joint program with Indonesia is part of a set of bilateral symposia that have connected young U.S. scientists with their counterparts in China, England, France, Germany, India, and Japan. Upon return from this year's conference in Indonesia, the program directors invited me to serve again on the Organizing Committee for next year's (2017) meeting in Indonesia.

Robert A. Fieo, PhD

Robert Fieo, PhD, joined the CAM team in 2015 as a Research Assistant Professor. He is housed within the UF Institute on Aging. He is primarily interested in how Cognitive Enrichment models can serve to attenuate cognitive decline in older adults. Dr. Fieo is also interested in the application of psychometrics to help enhance health outcome measures, particularly, hard to define subjective constructs of self-report, e.g., fatigue or motivation. He is also affiliated with the UF Department of Health Outcomes and Policy.

1. NIH K01 application, June, 2016—resubmission stage.
2. NIH KL2 application, December, 2016

Damon Geoffrey Lamb, PhD

Wrapped up analysis of several data sets looking at alterations of attention with healthy aging and published work investigating the impact of aging on motor control. Furthermore, received funding to examine the impact of vagal nerve stimulation on learning and memory in animal models as part of a large collaborative DARPA project. Proposals to investigate safe, non-invasive forms of this stimulation in an aging human population have been submitted to NIH and are currently pending review.

Eric Porges, PhD

Publication in Biological Psychiatry: CNNI of the first report that cognitive function is sensitive to cerebral GABA concentrations in the frontal cortex, and GABA concentration in frontal and posterior regions continue to decline in later age. These effects suggest that proton MRS may provide a clinically useful method for the assessment of normal and abnormal age-related cognitive changes and the associated physiological contributors.

John B. Williamson, PhD

Since the last report, I have been active in completing data collection on my VA funded CDA-2 (VA K award equivalent) designed to assess the impact of neurological contributions of mild traumatic brain injury to the development of emotional dysregulation in the continuum of Post Traumatic Stress Disorder. We have enrolled 82 subjects in the protocol. We have multi-modal, cognitive, neuroimaging and autonomic data in these patients. Both mild traumatic brain injury and post traumatic stress disorder are significant risk factors for accelerated aging and early cognitive decline in older adults. We have identified white matter damage in pathways that project from prefrontal cortex to the amygdala such that lower white matter integrity in these systems that is associated with the presentation of symptoms of post traumatic stress disorder. These results are currently being written up for publication.

Following up on this mechanistic study, we have completed a pilot study examining the impact of vagal nerve stimulation on symptoms of post traumatic stress disorder in combat exposed Veterans with and without history of post traumatic stress disorder and mild traumatic brain injury. Our preliminary data are promising and we have submitted a Merit Review grant to the VA to conduct a larger scale clinical trial in this population using this tool. The first score on this proposal was good, but not below the funding line (impact score ~2.5; scale mirrors NIH, 1-9). The resubmission is currently under review.

Relatedly, due to overlapping functional neuroanatomy and based on other pilot data from our lab, we have submitted two proposals to NIH (an R21 and an R01) to evaluate the efficacy of an electroceutical intervention to improve cognitive performance in patients with amnesic mild cognitive impairment. This is a center for Cognitive Aging and Memory (CAM) affiliated initiative and includes Drs. Cohen, DeKosky, Lamb, and Porges, among others. The R21 was 3 percentile from the funding line and was resubmitted. The R01 has not been scored yet (first submission).

In healthy younger individuals, we submitted a proposal to DARPA to enhance cognitive performance and map the functional neuroanatomical impact of a nerve stimulation technology. The animal neuroscience component of this proposal was funded and efforts to start this project are underway.

In a heart failure population, capitalizing on my interest in peripheral and central nervous system interactions in the support of cognitive and emotional behaviors, I started an NIH funded project (discontinuation date, August, 2017). This is an R56 supported feasibility study to demonstrate cognitive and brain health improvement mechanisms via cardiac resynchronization therapy (CRT). This project is designed to longitudinally assess brain, cognitive and cardiovascular changes associated with CRT using MRI and MRS. MRI safe devices have just recently come to market and thus, this is a timely and cutting edge study. We have IRB approval. We have piloted all methodologies. We have enrolled our first subject and are currently actively recruiting at UF and at the VA. This study involves CAM members (Cohen, Heilman, Porges, Lamb, Woods) and collaborators from Cardiology.

I was involved in the conceptualization of an NSF proposed engineering research center, the Center for Autonomic Neural Engineering (CANE). I am co-lead on a test-bed along with Dr. Cohen. I proposed to get control of specific hypothalamic nuclei using newly developed technology in order to control appetitive behaviors. Our proposal was one of 19 out of 200 selected for the full proposal. We were then one of 8 selected for a site visit. Five members of our team are currently in Washington D.C. at the reverse site visit to NSF. We should know within the next few months whether this initiative will be funded.

I have three technological innovation disclosures that the University of Florida has exercised their rights on for further development. The first one of these has been submitted for full patent consideration and is currently pending. The other two are in preparation for provisional patent application. As these are pending, I cannot discuss their content, but they should prove interesting.

I spearheaded, along with collaborators including Drs. Bauer and Perlstein the development of a traumatic brain injury registry at the Brain Rehabilitation and Research Center at the VA. This registry has IRB approval and we have reviewed our first few potential entrants. The registry will serve the investigational needs of the TBI researchers in the community.

Refereed Articles

1. Porges E.C., A.J., Edden, R., Harris, A., Chen, H., Garcia, A., Lamb, D., **Williamson, J.B.**, Cohen, R.A. Frontal GABA concentrations are associated with cognitive performance in older adults. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. 2016.
2. Kesayan T, **Williamson, J.B.**; Falchook, A.D.; Skidmore, F.M.; Heilman, K.M. Allocentric but not egocentric pseudoneglect of peripersonal space. *Cognitive and Behavioral Neurology*. 2016.
3. Harciarek, M., Jarosław B, **Williamson, J.B.**; Dębska-Ślizień, A.; Rutkowski, B.; Heilman, K.M. Disorders of the anterior attentional-intentional system in patients with end stage renal disease: evidence from reaction time studies. *Brain and Cognition*. 2016.
4. Harciarek M.; Biedunkiewicz, B.; **Williamson, J.B.**; Dębska-Ślizień, A.; Rutkowski, B.; Heilman, K.D. Slowing with end stage renal disease: Attentive but not prepared to act. *International Journal of Psychophysiology*. 2016.

Refereed Abstracts

1. Milano, N., Goldman, A., Woods, A., **Williamson, J.**, Acosta, L., Lamb, D., Zhang, H., Heilman, K. The influence of right and left frontotemporal stimulation on visuospatial creativity. *Neurology*, 2016.
2. Kesayan, T. Lamb, D., **Williamson, J.B.**, Heilman, K.M. Perceptual pseudoneglect: Laterality and the perception of tactile pressure. *Neurology*, 2016
3. Kesayan, T. Lamb, D., **Williamson, J.B.**, Heilman, K.M. Reduced tactile pressure perception with age. *Neurology*, 2016

Adam Joshua Woods, PhD

Since last report, I have acquired significant funding for my research on the use of transcranial direct current stimulation to combat cognitive aging. This includes a multisite R01 randomized clinical trial across three of the McKnight Brain Institutes (The ACT study, n=360, \$5.8 million) and a K01 performing a dose response companion study to my R01 (Stimulated Brain, n=80, \$612K). The ACT study is the largest Phase III tDCS trial in history and the Stimulated Brain study (Phase II) is one of the five largest tDCS trials in history. Both trials are focused on combating cognitive aging. This represents a significant advance in the fields of cognitive aging and non-invasive brain stimulation. In addition, I acquired an industry pilot clinical trial (Phase II) using a novel natural compound

to investigate reduction in neuroinflammation to enhance cognitive function in older adults (Efficient Brain study, n=30, \$275k). This body of work represents the CAM's efforts to pioneer novel non-invasive interventions for combating cognitive aging in older adults. In addition, some of my recent publications have received significant attention in the press and scientific community. For example, my recent paper on the effects of alcohol on cognitive function in older adults was covered by over 50 websites and news agencies around the world, highlighting an important factor underlying cognitive aging.

In addition to the faculty listed above, Dr. Huaihou Chen, Dr. Samuel Wu and Dr. Steven T. DeKosky provided MBRF funded support in 2016 to the CAM-CTRP. Drs. Wu and Chen serve as CAM-CTRP statisticians, working closely with all CAM-CTRP faculty on data analyses, statistical plans for grant submissions, and manuscript generation. Dr. Wu serves as the lead statistician for the CAM-CTRP, while Dr. Chen was both a junior scholar under Dr. Cohen and worked under the supervision of Dr. Wu for statistical support of the CAM. In the context of this work, Dr. Wu was instrumental in acquisition of the ACT grant, serving as director of the ACT Data Management and Quality Control (DMAQC) Core in the grant. His adaptive clinical trial design was highlighted as a significant novel achievement in the ACT grant. Dr. Chen, as of August, chose to take a position in industry as a biostatistician. However, in his time in the CAM-CTRP he was instrumental in statistical analyses on several important cognitive aging papers published by our team. Dr. Wu continues to serve as the lead statistician for the CAM-CTRP.

In addition to serving as the MBI interim executive director in 2016, Dr. DeKosky serves as the CAM-CTRP MD and key liaison for clinical neuroscience research between the CAM-CTRP and Department of Neurology. In this role, he has facilitated both ongoing and planned cognitive aging and memory research projects within the CAM-CTRP. He has also been instrumental in several grants recently submitted to the NIA (e.g., ENRGISE-COG). Dr. DeKosky also serves as an important bridge between our basic science and clinical translational research ongoing in the CAM-CTRP and is a central conduit for identifying and attracting promising young clinician-researchers to the CAM-CTRP. He works with both Drs. Cohen and Woods in a variety of ongoing projects that aim to translate novel intervention approaches to clinical application (e.g., neuroplastic facilitation through oxygenation alteration). He also serves as a key faculty mentor to junior faculty in the CAM-CTRP (e.g., Fieo, Porges).

PUBLICATIONS IN PEER REVIEWED JOURNALS:

Ronald A. Cohen, PhD

1. Alosco ML, Brickman AM, Spitznagel MB, Narkhede A, Griffith EY, **Cohen R**, Sweet LH, Josephson R, Hughes J, Gunstad J. Reduced Gray Matter Volume Is Associated With Poorer Instrumental Activities of Daily Living Performance in Heart Failure. *J Cardiovasc Nurs*. 2016 Jan-Feb;31(1):31-41. doi: 10.1097/JCN.0000000000000218. PubMed [citation] **PMID: 25419946, PMCID: PMC4440850**
2. Chen H, Zhao B, Porges EC, **Cohen RA**, Ebner NC. Edgewise and subgraph-level tests for brain networks. *Stat Med*. 2016 Nov 30;35(27):4994-5008. doi: 10.1002/sim.7039. PubMed [citation] **PMID: 27397632, PMCID: PMC5096985**
3. Chen H, Zhao B, Cao G, Porges EC, O'Shea A, Woods AJ, **Cohen RA**. Statistical Approaches for the Study of Cognitive and Brain Aging. *Front Aging Neurosci*. 2016 Jul 19;8:176. doi: 10.3389/fnagi.2016.00176. PubMed [citation] **PMID: 27486400, PMCID: PMC4949247**
4. **Cohen RA**, Navia B. Comment: Getting a handle on HAND in the era of cART. *Neurology*. 2016 Jan 26;86(4):339. doi: 10.1212/WNL.0000000000002279. No abstract available. PubMed [citation] **PMID: 26718565**
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6. Ebner NC, Chen H, Porges E, Lin T, Fischer H, Feifel D, **Cohen RA**. Oxytocin's effect on resting-state functional connectivity varies by age and sex. *Psychoneuroendocrinology*. 2016 Jul;69:50-9. doi: 10.1016/j.psyneuen.2016.03.013. PubMed [citation] **PMID: 27032063, PMCID: PMC4942126**
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Yenisel Cruz-Almeida, PhD

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2. Naugle KM, **Cruz-Almeida Y**, Fillingim RB, Staud R, Riley JL 3rd. Increased spatial dimensions of repetitive heat and cold stimuli in older females. *Pain*. 2016 Aug 31.
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4. Cardoso JS, **Cruz-Almeida Y***. Moving beyond the eigenvalue greater than one retention criteria in pain phenotyping research. *Pain*. 2016 Jun;157(6):1363-4. doi: 10.1097/j.pain.0000000000000520. ***Senior/Corresponding Author**
5. Naugle KM, **Cruz-Almeida Y**, Fillingim RB, Staud R, Riley JL 3rd. Novel method for assessing age-related differences in the temporal summation of pain. *J Pain Res*. 2016 Apr 8;9:195-205. doi: 10.2147/JPR.S102379.
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Natalie C. Ebner, PhD

* INDICATES UNDERGRAD/GRAD STUDENT OR POSTDOC AUTHOR

1. Chen, A., Brahma, P., Wu, D. O., **Ebner, N. C.**, Matthews, B., Crandall, J., Wei, X., Faloutsos, M., & Oliveira, D. Cross-layer personalization as a first class citizen for situation awareness and computer infrastructure security. Accepted to the ACM New Security Paradigms Workshop (NSPW 2016). September 26-29, 2016. C Lazy U Ranch Colorado, USA. Acceptance rate (46%).

2. Chen, H., Zhao, B., *Porges, E. C., Cohen, R. A., & **Ebner, N. C.** (in press). Edgewise and subgraph level tests for brain networks. *Statistics in Medicine*.
3. Rana, M., Varan, A. Q., Davoudi, A., Cohen, R. A., Sitaram, R., & **Ebner, N. C.** (2016). Real-time fMRI in neuroscience research and its use in studying the aging brain. [Research topic] *Frontiers in Aging Neuroscience*, 8(239), 1-16. DOI: 10.3389/fnagi.2016.00239
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7. *Ziaei, M., **Ebner, N. C.**, & Burianová, H. A. (in press). Functional brain networks involved in gaze and emotional processing. *European Journal of Neuroscience*.
8. **Ebner, N. C.**, Bailey, P. E., *Horta, M., *Joiner, J., & Chang, S. W. C. (2016). Multidisciplinary perspective on prosociality in aging. In J. A. Sommerville & J. Decety (Eds.), *Social Cognition: Developmental across the life span*, Frontiers in Developmental Science Series. (pp. 185–202). Psychology Press: Taylor and Francis Group.
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11. **Ebner, N. C.**, Chen, H., *Porges, E., *Lin, T., Fischer, H., Feifel, D., & Cohen, R. A. (2016). Oxytocin's effect on resting-state functional connectivity varies by age and sex. *Psychoneuroendocrinology*, 69, 50-59. DOI: 10.1016/j.psyneuen.2016.03.013

Robert A. Fieo, PhD

1. Seider TR, **Fieo RA**, O'Shea A, Porges E, Woods AJ, Cohen RA. Cognitively Engaging Activity Is Associated with Greater Cortical and Subcortical Volumes. *Frontiers in Aging Neuroscience*. 5/2/2016, p1-10. 10p.
2. O'Shea DM, Dotson VM, Fieo RA. Aging perceptions and self-efficacy mediate the association between personality traits and depressive symptoms in older adults. *Int J Geriatr Psychiatry*. 2016 Sep 21.

Damon Geoffrey Lamb, PhD

Lamb DG, Correa L, Seider TR, Mosquera DM, Rodriguez JA, Salazar Bejarano L, Schwartz ZJ, Cohen RA, Falchook AD, Heilman KM. The Aging Brain: Movement Speed and Accuracy. *Brain and Cognition*, 109 105-111, November 2016.

Eric Porges, PhD

1. O'Shea, A., Cohen, RA, **Porges, E.C.**, Nissim, N., Woods, A.J. Age-related contribution of the hippocampus to cognitive function. *Frontiers in Aging Neuroscience* 2016 in press.
2. **Porges, E.C.**, Woods A.J., Edden R.A., Harris A.D., Chen H., Garcia A.M., Lamb D.G., Williamson J.W., Cohen R.A. Frontal GABA concentrations are associated with cognitive performance in older adults. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. 2016 in press
3. Woods, A.J., **Porges, E.C.**, Bryant, V., Seider, T., Gongvatana, A., Kahler, C.W., de la Monte, S., Monti, P.M., Cohen, RA. Heavy alcohol consumption is associated with greater cognitive impairment in older adults. *Alcoholism: Clinical and Experimental Research*. 2016 in press

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6. Zamzow, R. M.; Ferguson, B. J.; Stichter, J. P.; **Porges, E.C.**; Ragsdale, A. S.; Lewis, M. L.; Beversdorf, D. Q. Effects of Propranolol on Conversational Reciprocity in Autism Spectrum Disorder: A Pilot, Double-Blind, Single-Dose Psychopharmacological Challenge Study. *Psychopharmacology* 2016, 233, 1171–8.
7. Ebner, N. C.; Chen, H.; **Porges, E.**; Lin, T.; Fischer, H.; Feifel, D.; Cohen, R. A. Oxytocin's Effect on Resting-State Functional Connectivity Varies by Age and Sex. *Psychoneuroendocrinology* 2016, 69, 50–59.
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Adam Joshua Woods, PhD

1. O'Shea, A., Cohen, RA, Porges, E.C., Nissim, N., **Woods, A.J.** Age-related contribution of the hippocampus to cognitive function. *Frontiers in Aging Neuroscience*. Accepted November 2016.
2. Szykowitz, S.M., McLaren, M.E., Suryadevara, U., **Woods, A.J.** The use of transcranial direct current stimulation (tDCS) in the treatment of neuropsychiatric disorders: A brief review. *Psychiatric Annals*. 46(11): 642-646.
3. **Woods, A.J.**, Porges, E.C., Bryant, V., Seider, T., Gongvatana, A., Kahler, C.W., de la Monte, S., Monti, P.M., Cohen, RA. Heavy alcohol consumption is associated with greater cognitive impairment in older adults. *Alcoholism: Clinical and Experimental Research*. Accepted August 2016.
4. Szykowitz, S.M., McLaren, M.E., O'Shea, A., **Woods, A.J.**, Anton, S., Dotson, V. Depressive Symptoms Modify Age Effects on Hippocampal Subfields. *Geriatrics and Gerontology International*. Accepted July 2016.
5. Chen, H., Zhao, B., Cao, G., Porges, E.C., O'Shea, A., **Woods, A.J.**, Cohen, R. (2016). Statistical approaches for the study of cognitive and brain aging. *Frontiers in Aging Neuroscience*. 8:176.
6. Porges, E.C., **Woods, A.J.**, Edden, R., Harris, A., Chen, H., Garcia, A., Lamb, D., Williamson, J.W., Cohen, RA. Frontal GABA concentrations are associated with cognitive performance in older adults. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*.
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8. Seider, T., Fieo, R., O'Shea, A., Porges, E.C., **Woods, A.J.**, Cohen, R.A. (2016). Cognitively engaging activity is associated with greater cortical and subcortical volume. *Frontiers in Aging Neuroscience*. 8:94.
9. McLaren, M.E., Szymkowitz, S.M., O'Shea, A., **Woods, A.J.**, Anton, S.D., Manini, T.M., Dotson, V.M. (2016). Symptom dimensions of subthreshold depression and cingulate volumes in older adults. *Translational Psychiatry*. 6(4):e788.
10. Nissim N, O'Shea A, Bryant V, Porges E, Cohen R, **Woods, A.J.** Frontal structural neural correlates of working memory performance in older adults. *Frontiers in Aging Neuroscience*. Accepted December 2016.

PUBLICATIONS (OTHER):

Ronald A. Cohen, PhD

20 Chapters in ECNP (in press)

Yenisei Cruz-Almeida, PhD

Books/Chapters:

1. Lysne P & **Cruz-Almeida Y***. *Macular degeneration*. In: *Encyclopedia of Clinical Neuropsychology, 2nd Edition*. Kreutzer J, DeLuca J, Caplan B (Eds.) Springer, In Press. ***Senior/Corresponding Author**
2. Lysne P & **Cruz-Almeida Y***. *Retinotopic Mapping*. In: *Encyclopedia of Clinical Neuropsychology, 2nd Edition*. Kreutzer J, DeLuca J, Caplan B (Eds.) Springer, In Press. ***Senior/Corresponding Author**

Natalie C. Ebner, PhD

1. **Ebner, N. C.**, *Frazier, I., & *Ellis, D. (in press). Visual search and attention test. In J. S. Kreutzer, J. DeLuca, & B. Caplan (Eds.), *Encyclopedia of Clinical Neuropsychology*. New York, London: Springer.
2. **Ebner, N. C.**, *Gulliford, D., & *Yumusak, S. (in press). Saccadic eye movements. In J. S. Kreutzer, J. DeLuca, & B. Caplan (Eds.), *Encyclopedia of Clinical Neuropsychology*. New York, London: Springer.
3. **Ebner, N. C.**, *Weir, D., & *Rainer, R. (in press). Eye tracking. In J. S. Kreutzer, J. DeLuca, & B. Caplan (Eds.), *Encyclopedia of Clinical Neuropsychology*. New York, London: Springer.

Eric Porges, PhD

Carter CS, Bartal IB, **Porges EC**. The Roots of Compassion: An Evolutionary and Neurobiological Perspective. *Frailty: Oxford Handbook of Compassion Science*. 2016. In Press. New York, NY: Oxford University Press.

Adam Joshua Woods, PhD

1. **Woods, A.J.**, Cohen, R.A., Pahor, M. Cognitive frailty: frontiers and challenges. Vellas, B (eds). *White Book on Frailty*. 2016. pp. 44-47. International Association on Gerontology and Geriatrics.
2. **Woods, A.J.**, Martin, D. Clinical Research and Methodological Aspects for tDCS Research. In Brunoni, A., Nitsche, M., Loo, C. (Eds.) *Transcranial Direct Current Stimulation in Neuropsychiatric Disorders: Clinical Principles and Management*, Springer, New York, 2016, pp. 393-404.
3. Nissim, N., **Woods, A.J.** Mach Bands. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
4. Ochoa, C., **Woods, A.J.** Visual Search. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
5. Szymkowicz, S.M., **Woods, A.J.** Binocular Disparity. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
6. Nissim, N., Szymkowicz, S.M., **Woods, A.J.** Edge Detection. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
7. Nissim, N., **Woods, A.J.** Digit Vigilance Test (DVT). *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
8. Nissim, N., **Woods, A.J.** Visual Psychophysics. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
9. McLaren, M., **Woods, A.J.** The Brief Test of Attention. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
10. Sinha, P., Bowers, D., **Woods, A.J.** D2 Test of Attention. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
11. Suryadevara, U., **Woods, A.J.** Motion Parallax. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
12. Suryadevara, U., **Woods, A.J.** Eye Dominance. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
13. O'Shea, A., **Woods, A.J.** Useful Field of View. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.

14. Altmomare, L.G., **Woods, A.J.** Visual Convergence. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
15. Richards, L., **Woods, A.J.** Posterior Cortical Atrophy. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
16. Polejaeva, E., **Woods, A.J.** Behavioral Inattention Test (BIT). *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
17. Polejaeva, E., **Woods, A.J.** Auditory Selective Attention Test. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.
18. O'Shea, D., **Woods, A.J.** Tests of Variables of Attention. *Encyclopedia of Clinical Neuropsychology*, 2nd Ed. Springer New York, in press.

PRESENTATIONS AT SCIENTIFIC MEETINGS:

Ronald A. Cohen, PhD

1. McKnight Brain Institute Annual Meeting- Tucson, Az.: Update on the MBAR cognitive initiative
2. Florida Neurological Association: Orlando, FL: Cardiovascular effects on cognitive and brain function
3. ARCH annual summer meeting: Brown University, Research Component 1 update
4. SHARC annual meeting: Miami Florida

Natalie C. Ebner, PhD

1. *Lin, T., *Muradoglu, M., *Weir, D., Lillard, T., Carter, S., Cohen, R. A., Connelly, J., & **Ebner, N. C.** (November, 2016). *OXTR methylation as a predictor of variations in attachment in young and older adults*. Poster at the 9th Annual McKnight Brain Research Foundation Social at Society for Neuroscience, San Diego, CA, USA.
2. Oliveira, D., *Ellis, D., *Rocha, H., *Weir, D. H., *Dommaraju, S., *Yang, H., *Muradoglu, M., & **Ebner, N. C.** (January, 2017). *Security for vulnerable populations—On the interplay of weapons of influence and life domains in predicting older adults susceptibility to spear-phishing emails*. Enigma—A USENIX conference 2017, Oakland, CA, USA.
3. *Lin, T., *Horta, M., Fischer, H., Feifel, D., Cohen, R. A., & **Ebner, N. C.** (November, 2016). *Effects of intranasal oxytocin on perceptions of trustworthiness in aging*. Poster at the Gerontological Society of America's 69th Annual Scientific Meeting, New Orleans, LA, USA.
4. *Ellis, D., *Rocha, H., *Weir, D. H., *Dommaraju, S., *Yang, H., *Muradoglu, M., Oliveira, D., & **Ebner, N. C.** (September, 2016). *Age-related vulnerabilities to social-engineering attacks*. Poster at the 7th Annual Spotlight on Research in Aging, University of Florida, Gainesville, FL, USA.
5. *Lin, T., *Muradoglu, M., *Weir, D., & **Ebner, N. C.** (September, 2016). *OXTR methylation as a predictor of variations in attachment in young and older adults*. Poster at the 7th Annual Spotlight on Research in Aging, University of Florida, Gainesville, FL, USA.
6. *Rainer, R., *Ellis, D., *Rocha, H. A., *Frazier, I. R., Cohen, R. A., & **Ebner, N. C.** (September, 2016) *Associations between hormone concentrations and inflammation biomarker levels in young and older adults*. Poster at the 7th Annual Spotlight on Research in Aging, University of Florida, Gainesville, FL, USA.
7. **Ebner, N. C.** (August, 2016). *The role of the neuropeptide oxytocin in cognitive, social, and affective aging*. Poster at the 6th Indonesian-American Kavli Frontiers of Science symposium, sponsored by the U.S. Agency for International Development and supported by the U.S. National Academy of Sciences and the Kavli Foundation, in Malang, East Java, Indonesia.
8. Lai, S., *Ma, J., *Penman, C., **Ebner, N. C.**, Tisher, C., Nixon, S. J., & Guy, C. (July, 2016). *Effects of gardening on the brain: A preliminary fMRI study*. Poster at the 22nd Annual Meeting of the Organization for Human Brain Mapping, Geneva, Switzerland.
9. **Ebner, N. C.**, Chen, H., *Porges, E., *Lin, T., *Horta, M., Fischer, H., Feifel, D., & Cohen, R. A. (April, 2016). *Oxytocin's effect on resting-state functional connectivity varies by age and sex*. Poster at the Cognitive Aging Conference, Atlanta, GA, USA.

10. **Ebner, N. C.**, *Frazier, I., Duezel, S., Bertram, L., Kuehn, S., Gerstorff, D., Liu, T., & Lindenberger, U. (April, 2016). *Associations of oxytocin receptor gene (OXTR) single-nucleotide polymorphisms with cognition, socioemotional functioning, and brain volumes in the Berlin Aging Study II*. Poster at the Cognitive Aging Conference, Atlanta, GA, USA.
11. **Ebner, N. C.** (April, 2016). *An aging perspective to the science of decision making*. Colloquium, Applied Experimental and Human Factors, University of Central Florida, Orlando, FL, USA.
12. *Ellis, D., *Rocha, H., *Weir, D. H., *Dommaraju, S., *Yang, H., *Muradoglu, M., Oliveira, D., & **Ebner, N. C.** (April, 2016). *Age-related vulnerabilities to social-engineering attacks*. Poster at the Cognitive Aging Conference, Atlanta, GA, USA.
13. *Horta, M., *Lin, T., *Gulliford, D., Cohen, R. A., & **Ebner, N. C.** (April, 2016). *Dynamic emotion identification: Effects of age and oxytocin*. Poster at the Meeting of the Social and Affective Neuroscience Society, New York City, NY, USA.
14. *Strickland-Hughes, C. M., *Tasdemir-Ozdes, A., Bluck, B., & **Ebner, N. C.** (April, 2016). *Now or never: The effect of future perspective on healthy lifestyle choices in younger and older adults*. Poster at the Cognitive Aging Conference, Atlanta, GA, USA.
15. *Hofman, B., *Frazier, I., & **Ebner, N. C.** (March, 2016). *The predictability of blood oxytocin on several health factors in young and old adults*. Poster at the 17th Annual Undergraduate Research Symposium, University of Florida, Gainesville, FL, USA.
16. *Muradoglu, M., & **Ebner, N. C.** (March, 2016) *OXTR methylation as a predictor of variations in personality and attachment*. Poster at the 17th Annual Undergraduate Research Symposium, University of Florida, Gainesville, FL, USA.
17. *Weir, D. H., *Rocha, H., *Dommaraj, S., *Yang, H., Oliveira, D., & **Ebner, N. C.** (March, 2016). *Internet spam: User age influences the type of social engineering attacks*. Poster at the 6th Annual Student Research on Aging Exposition and Awards sponsored by the Institute for Learning in Retirement at Oak Hammock, Gainesville, FL, USA.
18. *Strickland-Hughes, C. M., *Tasdemir-Ozdes A., Bluck, S., & **Ebner, N. C.** (February, 2016). *Now or never: The effect of global and specific future time perspective on healthy lifestyle choices in younger and older adults*. Poster at the 6th Annual ILR Student Research on Aging Exposition and Awards, Gainesville, FL, USA.

Damon Geoffrey Lamb, PhD

Invited Talks:

Attention in aging. Florida Society for Neurology 2016 Annual Meeting.

Posters & Abstracts:

1. **Lamb DG**, Porges EC, Williamson JB. *Modulation of the signs and symptoms of posttraumatic stress disorder and traumatic brain injury by peripheral nerve stimulation*. Society for Neuroscience Annual Meeting, San Diego, CA, 2016.
2. **Lamb DG**, Correa L, Seider TR, Mosquera DM, Rodriguez JA, Salazar Bejarano L, Schwartz ZJ, Cohen RA, Falchhook AD, Heilman KM. *Aging and Motor Control: Movement Speed and Spatial Control Tradeoffs with Age*, MBRF Satellite meeting, San Diego, CA, 2016.
3. Gunay C, Doloc-Mihu A, **Lamb DG**, Calabrese RL. *Intrinsic conductances need not vary across animals*. Society for Neuroscience Annual Meeting, San Diego, CA, 2016.
4. Ashley D Harris, Eric Porges, Adam J Woods, **Damon G Lamb**, Ronald A Cohen, John B Williamson, Nicolaas AJ Puts, and Richard AE Edden. *Tissue correction strategy impacts GABA quantification: a study in healthy aging*. International Society for Magnetic Resonance in Medicine Annual Meeting, Singapore, 2016.
5. Tigran Kesayan, **Damon Lamb**, John Williamson, Adam Falchhook, Kenneth Heilman. *Perceptual Pseudoneglect: Laterality and the Perception of Tactile Pressure*. American Academy of Neurology Annual Meeting, Vancouver BC, Canada, 2016
6. Tigran Kesayan, **Damon Lamb**, John Williamson, Adam Falchhook, Kenneth Heilman. *Reduced Tactile Pressure Perception with Age*. American Academy of Neurology Annual Meeting, Vancouver BC, Canada, 2016
7. Nicholas Milano, Annika Goldman, Adam Woods, John Williamson, Lealani Acosta, **Damon Lamb**, Han Zhang, Kenneth Heilman. *The Influence of Right and Left Frontotemporal Stimulation on Visuospatial Creativity*. American Academy of Neurology 2016 Annual Meeting, Vancouver BC, Canada

8. Balavage KT, **Lamb D**, Knight L, Bielick D, Kincaid KJ, Heilman KM. *The Effects of the Allocation of Focal Attention and Habituation on the Line Bisection Task*. International Neuropsychological Society Annual Meeting, Boston, MA, 2016
9. Bielick D, **Lamb D**, Kincaid KJ, Balavage KT, Knight L, Heilman KM. *Hemispheric Lateralization of Attentional Background Distraction*. International Neuropsychological Society Annual Meeting, Boston, MA, 2016
10. Kincaid KJ, **Lamb D**, Bielick D, Balavage KT, Knight L, Heilman KM. *Influence of Viewing Eye on Altitudinal Attentional Bias*. International Neuropsychological Society Annual Meeting, Boston, MA, 2016
11. Knight L, **Lamb D**, Balavage KT, Bielick D, Kincaid KJ, Heilman KM. *Effects of Focal and Global Spatial Attention on Compound Line Bisection Tasks*. International Neuropsychological Society Annual Meeting, Boston, MA, 2016

Eric Porges, PhD

Talks:

1. *Neuroimaging and Cognitive Performance in Older Adults*. Presented at Northeastern University's Department of Psychology colloquium series. Boston, MA. 2016
2. *Neurotransmitter Alterations in the Aging Brain*. Presented at the "41st Annual Course in Cognitive & Behavioral Neurology: The Aging Brain" session of the "Florida Society of Neurology Annual Meeting." Orlando, FL. 2016
3. *GABA concentrations, Age, & Cognition*. Presented at the "McKnight Brain Research Foundation University of Florida Site Visit." Gainesville, FL. 2016
4. *Frontal GABA concentrations predict cognitive function beyond age-related decline and the extension of this research into HIV and heavy drinking*. Presented at the Twelfth GATOR pre-International Neuropsychological Society meeting. Waterville Valley, NH. 2016

Abstracts:

1. Clark DJ, Wratchford C, Chatterjee SA, **Porges EC**, Fox EJ, Balasubramanian B. *Sympathetic nervous system activity to assess the perceived challenge of walking adaptability after stroke*. Poster presented at the Brooks Rehabilitation annual research day, Gainesville, FL, 2016.
2. Bryant VE, Woods AJ, **Porges EC**, Cook RL, Kahler CW, O'Shea A, Tashima K, Cohen RA. *Frontal Neural Correlates of Working Memory Decline in Hazardous Drinkers Living With HIV*. Poster presented at the Research Society on Alcoholism, New Orleans, LA. 2016.
3. Chatterjee SA, Rose DK, **Porges EC**, Fox EJ, Daly JD, Christou EA, Otzel DM, Butera KA, Clark DJ. *Quantifying the perceived challenge of walking after stroke by measuring sympathetic activation: a pilot study* Poster presented to be Annual CSM meeting. San Antonio. 2016.
4. Bryant VE, Woods AJ, **Porges EC**, Cook RL, Kahler CW, O'Shea A, Tashima K, Cohen RA. *Frontal Neural Correlates of Working Memory Decline in Hazardous Drinkers Living With HIV*. Poster presented to be Annual SHARC meeting, Poster Prize awarded, Miami, FL. 2016.
5. Seider TR, Garcia AM, **Porges EC**, Woods AJ, & Cohen RA. *Default Mode Network Control, Social Engagement, and Memory in Older Adults*. Poster presented at the American Academy of Clinical Neuropsychology, Chicago, IL, 2016.
6. Bryant VE, Woods AJ, **Porges EC**, Cook RL, Kahler CW, O'Shea A, Tashima K, Cohen RA. Poster presented at the Frontal Neural Correlates of Working Memory Decline in Hazardous Drinkers Living With HIV. CHAART Conference, 2016.
7. Nissim NR, O'Shea A, Bryant V, **Porges EC**, Cohen, RA, & Woods AJ. *Neural Correlates of Working Memory in Healthy Older Adults*. Poster presentation at the Cognitive Aging Conference, Atlanta, GA. 2016.

John B. Williamson, PhD

1. Harris, A.D., Porges, E., Woods, A.J., Lamb, D.G., Cohen, R., **Williamson, J.B.**, Puts, N., and Edden, R. *Tissue correction strategy impacts GABA quantification: a study in healthy aging*. Poster presented at the 2016 annual meeting International Society for Magnetic Resonance in Medicine, Singapore.

2. Harciarek, **Williamson** et al. *Normalization of attentional and intentional behavior subsequent to kidney transplant*. Poster presented at the 2016 annual meeting of the International Neuropsychological Society, London.
3. Milano, N., Goldman, A., Woods, A., **Williamson, J.**, Acosta, L., Lamb, D., Zhang, H., Heilman, K. *The influence of right and left frontotemporal stimulation on visuospatial creativity*. Poster presented at the 2016 annual meeting of the American Academy of Neurology, Vancouver.
4. Kesayan, T. Lamb, D., **Williamson, J.B.**, Heilman, K.M. *Perceptual pseudoneglect: Laterality and the perception of tactile pressure*. Poster presented at the 2016 annual meeting of the American Academy of Neurology, Vancouver.
5. Kesayan, T. Lamb, D., **Williamson, J.B.**, Heilman, K.M. *Reduced tactile pressure perception with age*. Poster presented at the 2016 annual meeting of the American Academy of Neurology, Vancouver.

Adam Joshua Woods, PhD

1. **Woods AJ.** Lecture. Technical Aspects of tES: *Hardware, Devices, and Procedures*. NIMH Transcranial Electrical Stimulation (tES): *Mechanisms, Technology and Therapeutic Applications*. Bethesda, MD. September 29, 2016.
2. **Woods AJ.** Symposium. *Neural correlates of tDCS effects on working memory: implications for adjunctive cognitive therapies*. 6th International Conference on Transcranial Brain Stimulation. Gottingen, Germany. September 9, 2016.
3. **Woods AJ.** Lecture. *Expertise, Decision-Making, and Spatial Bias in American Football: an aging and expertise story*. GATOR Pre-INS Conference. Water Valley, NH. Feb 1, 2016.
4. **Woods AJ.** Lecture. *The role of neuroinflammation in cognitive aging*. University of Florida Clinical Translational Science Institute Research Day. Gainesville, FL., June 24, 2016.
5. **Woods AJ.** Lecture. *The impact of neuroinflammation on human cognitive aging*. The McKnight Brain Institute Site Visit. UF, Gainesville, FL. Feb 17, 2016.

PRESENTATIONS AT PUBLIC (NON-SCIENTIFIC) MEETINGS OR EVENTS:

Robert A. Fieo, PhD

1. A talk given at the University of Florida, Diabetes Institute (August, 2016)
Title: *Diabetes and non-vascular dementia, time to Alzheimer's*.
2. Conference talk given at Medicaid Managed Care Quality Forum (December, 2016)
Title: *Measuring Quality in Long-Term Services: Best Practices & Future Directions*

John B. Williamson, PhD

1. 2016 **Williamson J.B.**, *Emotional Autobiographical Memories*. Annual Florida Society of Neurology Meeting: 37th Annual Course in Neuropsychology and Behavioral Neurology: Cognitive Assessment: CE credit course.
2. 2016 **Williamson, J.B.** *Aging and Post Traumatic Stress Disorder* North Florida VAMC: CE credit course.

AWARDS (OTHER):

Ronald A. Cohen, PhD

1. Evelyn F. McKnight Chair for Clinical Translation Research in Cognitive Aging

2. COHEN, R. P01 AA019072 Monti (PI) 09/01/15 - 05/31/20 1.20 CM NIAAA 110,695
Alcohol and HIV: Biobehavioral Interactions and Intervention
 One of two primary R01 projects in the Brown University HIV-Alcohol center grant, this study focuses on the interactive effects of HIV and alcohol use on metabolic-vascular disturbances underlying brain dysfunction. We are longitudinally assessing HIV infected and seronegative controls who are stratified by alcohol use into three groups (heavy, moderate, none use) over a 36-month period. The study examines MRI-based neuroimaging biomarkers of brain dysfunction including diffusion tensor imaging (DTI), morphometry, and magnetic resonance spectroscopy (MRS) to measure cerebral metabolite abnormalities. Dr. Cohen is the principal investigator of this R01 project overseeing all aspects of the study.
Role: Co-Investigator

3. R56 HL127175-01 (Williamson, PI) 09/01/15-08/30/20 1.8 CM NHLBI \$31,989
The effects of heart failure and cardiac resynchronization on the brain and cognition
 The goal of this study is to determine the influence of increased blood flow through cardiac resynchronization on the brain and cognition.
Role: Co-I

4. U24 AA022002 Cook (PI) 09/01/2013-08/31/2017 .36 CM NIAAA \$6,313
 Southern HIV Alcohol Research Consortium (SHARC) Admin and Research Support Cure. The objective of this proposal is to establish the administrative structure and to provide research support for the SHARC, a collaboration that links several universities and investigators with a common goal of supporting new research related to HIV and alcohol consumption.
Role: Co-I

5. 2U01AA010797-06 (Cook, PI; Cohen MPI) 09/01/2016-08/30/2021 1.8CM NIAAA \$4,718,864
Effects of experimentally-induced reductions in alcohol consumption on brain cognitive, and clinical outcomes and motivation for changing drinking in older persons with HIV infection. This proposed U01 study will build on our past findings to determine the extent to which marked reductions in alcohol consumption over 4-weeks via contingency management (CM) improves cognitive performance, brain functions and pathophysiology, and HIV-associated health outcomes. We will conduct state-of-the-art neuroimaging, cognitive, and behavioral assessments at each time point and then continue to track long-term drinking and HIV outcomes in our companion Cohort (U24). The Specific Aims of this proposal are: 1) to demonstrate improved cognitive performance and brain function (fMRI) after 4-weeks of CM-induced alcohol reduction among HIV+ adults, followed by worsening of these effects 1-year later if heavy drinking resumes; 2) to demonstrate that cerebral metabolic (MRS) and neuroinflammatory (DTI-free water) markers will also improve with CM-induced alcohol reduction and worsen if drinking resumes post-CM; and 3) Determine whether perceived benefits and challenges to drinking reduction identified during motivational interviewing (MI) predict drinking reductions or relapse one-year post-CM. We will also determine whether changes in cerebral pathophysiology (MRS, DTI-FW) correspond with changes in cognition, brain function (fMRI) and serum inflammatory and liver biomarkers.
Role: MPI

6. AA022002 Cohen (MPI) 09/01/2013-08/31/2017 .36 CM NIAAA \$6,313
Effects of experimentally-induced reductions in alcohol consumption on brain cognitive, and clinical outcomes and motivation for changing drinking in older persons with HIV infection Southern HIV Alcohol Research Consortium (SHARC) Admin and Research Support Cure. The objective of this proposal is to establish the administrative structure and to provide research support for the SHARC, a collaboration that links several universities and investigators with a common goal of supporting new research related to HIV and alcohol consumption.
Role: MPI

7. 1R01DK099334 06/25/2014-05/31/2019 3.60 CM NIH \$1,826,328
Obesity and type-2 diabetes: Bariatric surgery effects of brain function
 The proposed prospective longitudinal study will examine whether cerebral metabolic and vascular dysfunction, including glucose/insulin disturbances (co-morbid diabetes) underlie obesity-associated cognitive dysfunction, and whether significant weight loss and diabetes remission following bariatric surgery reduces these disturbances.
Role: PI

8. NIH 1U54 EB020403 Thompson (PI) 07/01/2014-06/30/2018 .24 CM
ENIGMA Center for Worldwide Medicine, Imaging & Genomics \$180,000
 ENIGMA is not a project; it is a scientific movement of rapidly and constantly interacting collaborations that support each other. ENIGMA cohorts boost each other's power with gigantic datasets, and the tools and expertise to maximally exploit each

other's data, performing some of the world's largest disease studies, beyond what any one site could perform on its own.

Role: Co-I Sub Award PI

9. NIH/NIA 1R01AG054077-01 (Woods/Cohen) 7/1/2016-6/30/2021 1.2 cal months
Augmenting Cognitive Training in Older Adults – The ACT Grant \$21,538
This randomized clinical trial examines the effect of augmenting cognitive training with transcranial direct current stimulation to maximize cognitive and functional outcomes older adults experiencing age-related cognitive decline. Change in well-validated measures of everyday abilities and neurocognitive function will serve as outcome measures. Functional and structural neuroimaging biomarkers of neural plasticity and learning (fMRI, GABA MRS, etc.) will measure intervention-associated alterations in specific brain regions impacted by cognitive aging.
10. NIA K01AG050707-A1 (Woods, PI) 07/01/2016-06/30/21 .0 cal months
Neuromodulation of Cognition in Older Adults \$0
The goal of this study will be to investigate the ability of transcranial direct current stimulation to enhance the effectiveness of cognitive training targeting attention, speed of processing, and working memory function in older adults.
Role: Mentor

Overlap

None

Yenisei Cruz-Almeida, PhD

1. Project Number: K01 AG048259-01A1 (PI: Cruz-Almeida)
Source: National Institute on Aging
Title: *Neuroimaging Age-Related Changes in Pain Modulation*
Dates of Approved Project: 05/2015 - 04/2020
Role: PI
2. Project Number: UL1TR001427 (PIs: Cruz-Almeida, Ebner)
Source: University of Florida Clinical Translational Sciences Institute
Title: *Neurobiological Mechanisms of Oxytocin's Pain-Modulatory Role in Aging*
Dates of Approved Project: 08/2016 – 07/2018
Role: Pilot Project Co-PI
3. Project Number: UL1TR001427 (PIs: Cruz-Almeida, Woods)
Source: University of Florida Clinical Translational Sciences Institute
Title: *tDCS in Older Adults with Multi-Site Pain*
Dates of Approved Project: 08/2016 – 07/2018
Role: Pilot Project Co-PI
4. Project Number: P30AG028740-07 (PI: Cruz-Almeida)
Source: National Institute on Aging OAIC Pilot Project
Title: *Pain and Mobility Function in Older Adults*
Dates of Approved Project: 05/2015 – 04/2017
Role: Pilot Project PI
5. Project Number: N/A (PI: Cruz-Almeida)
Source: University of Florida Clinical Translational Sciences Institute
Title: *Cortico-striatal connectivity predicting pain and physical function in older adults*
Dates of Approved Project: 06/2014 – 05/2017
Role: PI

Natalie C. Ebner, PhD

1. 2016 CLAS International Educator of the Year Award
2. 2016 University of Florida Excellence Award – Assistant Professors

3. 2016 Conference Travel Award: *Opportunities for Advancing Behavioral and Social Research on Aging* (Preconference workshop at the Convention of the Association for Psychological Science)
NIH/NIA
4. 2016 Member of the Organizing Committee for the 6th Indonesian-American Kavli Frontiers of Science symposium, National Academy of Sciences, in Malang, East Java, Indonesia
5. 08/01/2016-07/31/2017
University of Florida Cognitive Aging and Memory Clinical Translational Research Program (CAM-CTRP) F016327 & NIH/NIA
University of Florida Claude D. Pepper Older Americans Independence Center P30 AG028740
Role of Oxytocin in Inflammation
PI: Ebner
Total Award: \$8,567; Ebner TDC: \$8,567
6. 07/01/2016-12/31/2017
PRICE-CTSI-IOA ARG DTD 03-26-2008
Neurobiological Mechanisms of Oxytocin's Pain-Modulatory Role in Aging
MPIs: Ebner, Cruz-Almeida
Total Award: \$24,907; Ebner TDC: \$24,907

Eric Porges, PhD

1. CTSA Institutional K-Scholar
2. KL2 Scholar

John B. Williamson, PhD

Invited Research Presentations and Lectures

1. 2016-2017 NIH 1R56HL127175-01
Brain and cognition effects of cardio resynchronization therapy in heart failure.
The goal of this funding is to characterize brain changes as a result of improved cardiac output in heart failure including hemodynamic, task dependent regional brain activation and brain metabolic changes associated with improved cognitive performance and brain health.
PI: Williamson
\$544,000
2. 2012-2017 1 LK2RX000707-01 CDA-2 (VA-K)
White matter changes and mild TBI: Emotional and autonomic consequences.
Funded by the Department of Veterans Affairs: Williamson, Principal Investigator
PI: Williamson
\$898,188
3. 2012-2016 Merit Review Award.
Vertical Spatial Neglect.
Funded by the Department of Veterans Affairs: Heilman,
PI: Heilman
Co-I: Williamson
~\$500,000

Adam Joshua Woods, PhD

NIH-Funded Studies

1. NIA K01AG050707-A1 (Woods; PI) 09/30/16-05/31/21 9.00 calendar
NIH \$612,715
Neuromodulation of Cognition in Older Adults
The goal of this study will be to investigate the ability of transcranial direct current stimulation to enhance the effectiveness of cognitive training targeting attention, speed of processing, and working memory function in older adults. Training will focus on cognitive aging interventions and advanced magnetic resonance imaging and spectroscopy methods.
Role: PI
2. NIA R01AG054077 (Woods/Cohen/Marsiske; MPIs) 09/01/16-08/31/21 0.00 calendar
National Institutes of Health \$5,778,764
Augmenting Cognitive Training in Older Adults (ACT)
This study is a Phase III definitive multi-site randomized clinical trial with an adaptive design that will establish the benefit of delivering adjunctive transcranial direct current stimulation (tDCS) with cognitive training in older adults to combat cognitive aging. This trial measures both trial success and intervention mechanisms using multimodal neuroimaging and magnetic resonance spectroscopy, as well as comprehensive neurocognitive and functional assessment.
Role: Contact PI (Overlap covered by K01AG050707-A1)

Other Funded Studies

1. Industry Sponsored Trial (Woods; PI) 06/15/16-06/15/18 1.20 calendar
Osato Research Institute \$268,360
Impact of Fermented Papaya Product on brain energetics, neuroinflammation, and cognition: The Efficient Brain Study
The goal of this study is to perform a pilot clinical trial investigating the influence of Fermented Papaya Product on brain energetics, neuroinflammation, and cognition in older adults with elevated systemic inflammation using multimodal neuroimaging (fMRI, DWI) and spectroscopy (31P, 1H-MRS), as well as assessment of systemic inflammation and cognition.
Role: PI
2. 2015-2017 NIH Loan Repayment Program Recipient, Funding Agency: National Institute on Aging

FACULTY BIOGRAPHICAL SKETCHES: See page 82

TRAINEES

Ronald A. Cohen, PhD

- a. **Post-doctoral**
 1. Ellen Terry, PhD
 2. Lisa Delmonico, PhD
- b. **Pre-doctoral**
 1. Amanda Garcia
 2. Talia Seider
 3. Vaughn Bryant
 4. Emeka Okefor
 5. Nicole Nissan
- c. **Other: Faculty mentoring: K awards, etc.**
 1. Adam Woods, PhD
 2. Eric Porges, PhD
 3. Robert Fieo, PhD

4. Yenisel Cruz-Almeda, PhD
5. Vonetta Dotson, PhD
6. Natalie Ebner, PhD
7. David Clark, PhD

Yenisel Cruz-Almeida, PhD

- a. **Post-doctoral**
 1. Nathaniel Eckert, PhD
 2. Corey Simon, PhD
 3. Ellen Terry, PhD
 4. Duane Corbett, PhD
- b. **Pre-doctoral**
 1. Josue Cardoso
 2. Paige Lysne
 3. Lorraine Hoyos
 4. Brandon Apagueno
 5. Fariha Hasham

Natalie C. Ebner, PhD

- b. **Pre-doctoral**
 1. Ian Frazier
 2. Marilyn Horta
 3. Tian Lin
 4. Aylin Tasdemir-Ozdes

John B. Williamson, PhD

- c. **Other: Faculty mentoring: K awards, etc.**

Aaron Colverson, BA – Thesis committee 2015 - Present. *Ethnomusicology*. Topic: Neurological components of musical experience and social engagement.

Adam Joshua Woods, PhD

- b. **Pre-doctoral**
 1. Nicole Nissim
 2. Lindsey Richards

CLINICAL/TRANSLATIONAL PROGRAMS

Ronald A. Cohen, PhD

a. New programs:

1. ACT
2. SHARC-2
3. ARCH-2
4. ENIGMA
5. McKnight Brain Inter-Institute Clinical Translational Core initiated

b. Update on existing clinical studies

1. MBAR project is underway. We are beginning to collect data from 50 older adults (> 85 years). Database integration across MBI institutes
2. ARML pilot studies initiated as described earlier
3. CANE nanotechnology collaboration project initiated. NSF proposal to develop microstimulation approaches for hypothalamic stimulation received excellent score. We are awaiting reverse site visit and hopefully funding
4. HIV-alcohol-aging studies progressing
5. Study of bariatric surgery effects on brain function underway. Data collection occurring.
6. Heart failure project to examine effects of increasing cardiac output on cerebral perfusion and brain function in older adults underway.

Yenisei Cruz-Almeida, PhD

b. Update on existing clinical studies

My lab is currently analyzing processing neuroimaging data in order to prepare a number of manuscripts.

John B. Williamson, PhD

a. New programs:

At the BRRC, VA Center of Excellence, we have started a TBI registry program. It is IRB approved and recruits patients with history of mild, moderate and severe TBI and also screens for common comorbidities including post traumatic stress disorder.

We have submitted an Engineering Research Center application through the NSF, Center for Autonomic Neural Engineering. I am co-lead on a testbed we designed to gain control of specific hypothalamic nuclei to modify eating behavior. This is a fully translational research program design, going from rats, to mini-pigs using methodologies that are adaptable to human populations.

b. Update on existing clinical studies

On my VA funded CDA-2 award, we have enrolled 82 participants across four groups including mild TBI and PTSD, mild TBI, PTSD, and health combat exposed controls. We have submitted one manuscript from this dataset that is currently pending and we have another in preparation. Further, we have used pilot data from this and a BRRC funded pilot on 22 veterans using electroceutical methods to modify emotional experience in PTSD to apply for additional VA funding through the Merit Review mechanism (pending).

Our heart failure program is now IRB approved (NIH R56 funding) and we are actively recruiting and enrolling participants in the protocol.

Adam Joshua Woods, PhD

a. New programs:

1. *Augmenting Cognitive Training in Older Adults: ACT*

ACT is a multisite phase III randomized clinical trial testing the benefits of transcranial direct current stimulation for cognitive training gains in older adults (n=360). This study is a \$5.8 million R01 funded across 3 McKnight sites: UF, University of Arizona, and University of Miami. The trial began 9/1/16 and is currently in its initial 6 month startup period. This is the largest tDCS trial in history and the first multi-McKnight site clinical trial.

2. *Neuromodulation of Cognition in Older Adults: The Stimulated Brain Study*

This study is a funded off of a K01 awarded to Dr. Woods and builds on the prior Stimulated Brain study funded as a CAM pilot. This study serves as a dose response study building off of the ACT study. It will enroll 80 older adults into a four arm Phase II randomized clinical trial investigating an abbreviated intervention dose of tDCS and cognitive training, as compared to ACT.

b. Update on existing clinical studies

The Stimulated Brain Pilot Study

The Stim Brain Pilot Study was central to acquiring ACT and NIH funded Stimulated Brain Study, providing pilot data and research infrastructure for both studies. This study acquired a total of 10 participants across intervention arms and build considerable infrastructure for feasibility and execution of the aforementioned studies. This study is ongoing, with long-term follow-up visits in process, as well as additional infrastructure development for new tDCS studies in older adults to combat cognitive aging.

TECHNOLOGY TRANSFER:

Eric Porges, PhD

a. Patents applications

Williamson, JB, Lamb DG, Porges EC. 2016. *New and useful improvements in vagal nerve stimulation*. U.S. Patent Application PCT/US15/65524, filed January 2016. Patent Pending.

3 other patents disclosed to University of FL that they have exercised their right to support for submission to Patent Office (in progress)

John B. Williamson, PhD

b. Revenue generated from technology

My colleagues and I have disclosed three technological advancements to the University of Florida technology transfer office. We (Drs. Porges, Lamb and I) have one that has been submitted for a full patent that is pending. We have another one that has been optioned by UF and is in the process of preparation by patent attorneys for a provisional patent application. We (Drs. Porges, Lamb, Woods and I) have a third one that has been optioned by UF and is in the process of preparation by patent attorneys for a provisional patent application.

BUDGET UPDATE: See page 62

EDUCATIONAL PROGRAMS FOCUSING ON AGE-RELATED MEMORY LOSS:

Natalie C. Ebner, PhD

b. Public

Collaborative programs with other McKnight Institutes, institutions and research programs Aging and Deception White paper submission to Board of Trustees this November (together with Miami and Arizona).

Robert A. Fieo, PhD

a. Scientific

Development/preparation of UF course on cognitive aging
Course Title: *Clinical Neuroscience*

Eric Porges, PhD

a. Scientific

Instructed graduate student class "GMS 6893: Clinical and Translational Science Seminar Series." Significant focus of the class was age related functional changes.

COLLABORATIVE PROGRAMS WITH OTHER MCKNIGHT INSTITUTES, INSTITUTIONS AND RESEARCH PROGRAMS

Ronald A. Cohen, PhD

The CAM-CTRP has continued to make considerable progress in fulfilling the mission of the center and meeting our objectives from last year, including the extension of collaboration with investigators in the UF ARML program, other UF departments, and across the other McKnight institutes. These objectives are listed below along with...

Clinical translation: We have continued to emphasize: 1. translation of pre-clinical research on cognitive aging in humans to clinical applications; and 2. integrating basic neuroscience findings coming from the AMRL faculty into the human realm with a focus on clinical translation and developing new clinical outcomes and biomarkers for cognitive aging and also novel interventions. Several initiatives are underway related to the first of these objectives:

1. Collaboration with Dr. Bizon and her group to develop agents to enhance cognitive and behavioral function in the elderly;
2. Studies bridging high field in vitro and in vivo MR methods in laboratory animals with human MRI and MRS approaches to study neuroinflammation, blood-brain-barrier function, and epigenetic mechanisms contributing to age-associated cognitive decline (Febo, DeKosky, Woods, Cohen);
3. Genetic and epigenetic analyses of blood from the CAM ACTIVE brain study of older adults (Foster, Woods, Cohen); and d) Closed-loop microelectrode brain stimulation for the control of diabetes and obesity (Judy, Porges, Lamb, Febo, Setlow, Cohen).
4. The initiation of the ACT grant represents a major accomplishment related to clinical translation in humans. Other clinical translation accomplishments, include the WISE study, Papaya study and a number of other projects. The status of these initiatives are outlined later in this report.

Yenisei Cruz-Almeida, PhD

Collaborations are planned with Drs. Cohen, Porges and Woods.

Damon Geoffrey Lamb, PhD

Initiated expanded ARML & CAM collaboration through local events to foster cross-fertilization of research groups.

Eric Porges, PhD

MBAR study of neurocognitive function of those 85 and older. (UF, UAB, UA, UM)

Adam Joshua Woods, PhD

The ACT study involves collaboration across UF, University of Arizona, and University of Miami, with 120 participants collected at each site. This is the first clinical trial for combating cognitive aging across multiple McKnight sites.

Cognitive Aging and Memory Intervention Core. The MBRF approved creation of the CAMI Core a few months past. At present, we have used this time to collect data on investigators, candidate interventions for further support, and clinical trial resources from the recent McKnight Brain Institute annual reports and Inter-Institute Meeting programs. In addition, we have developed smart forms for additional acquisition of this information for collation and dissemination across all McKnight Brain Institute sites. We also hold bi-monthly calls with the PIs at each site and are currently reviewing ongoing and proposed intervention studies for further consideration and completion of potential funding applications to the MBRF. In this vein, a smart form fill application has also been developed. This core involves all four McKnight Brain Institutes.

COLLABORATIONS ACROSS UF AND MCKNIGHT INSTITUTES:

Ronald A. Cohen, PhD

CAM-CTRP has a number of collaborations that meet these objectives. These include:

1. CTSI investigators in epidemiology, biostatistics, and health outcomes (e.g., SHARC U-grant, ARCH-2);
2. Veterans Administration Hospital Brain Rehabilitation Research Center investigators (multiple projects including TBI and aging, heart failure, and HIV);
3. biomedical engineering and nanotechnology (closed end feedback brain stimulation grant with Jack Judy, PhD);
4. cardiology (cardiac resynchronization for heart failure)
5. Epidemiology and Infectious Medicine (SHARC, ARCH-2)

COLLABORATIVE PROGRAM WITH NON-MCKNIGHT INSTITUTES, INSTITUTIONS AND RESEARCH PROGRAMS:

Ronald A. Cohen, PhD

1. ARCH project: Involves Brown University and UF collaboration
2. SHARC: University of Miami, FIU collaboration with UF
3. ENERGISE study: Multi-site study of anti-inflammatory drug treatment effects in the elderly. Study involves: UF, Tufts, Yale, Pittsburgh, etc.

Robert A. Fieo, PhD

UF Department of Health Outcomes

NIH-funded PROMIS® network (Patient-Reported Outcomes Measurement Information System)

Damon Geoffrey Lamb, PhD

Coordinating with UAB and Arizona sites to unify neuroimaging pipelines and data management SOP.

Adam Joshua Woods, PhD

Dr. Woods has ongoing collaborations in his areas of expertise in tDCS and neuroinflammation brain imaging at University of Arkansas for Medical Sciences; University of Alabama at Birmingham), University of California-San Diego, University of New Mexico, University of Miami, University of Arizona, Arizona State University, and Brown University.

BRIEFLY DESCRIBE PLANS FOR FUTURE RESEARCH AND/OR CLINICAL INITIATIVES:

Ronald A. Cohen, PhD

1. Continued expansion and development of current lines of research
2. Clinical translational studies testing effects of novel drugs for enhancing cognitive function in the elderly
3. With respect to the above goal, establishing an inter-MBI clinical translational investigation
4. Longitudinal studies of the MBAR cohort
5. Obtain R01 funding for current heart failure research
6. Apply for funding to conduct neuroimaging studies of chemotherapy and cancer effects on brain function in women with breast cancer
7. Facilitate current work on pain effects in cognitive and brain aging in collaboration with Dr. Cruz and her collaborators
8. Collaborate with other CAM investigators on their lines of research, including tDCS and vagal nerve stimulation methods

Yenisel Cruz-Almeida, PhD

Recent funding will allow for the development of a line of research using neurostimulation techniques to improve pain and cognition in collaboration with Dr. Woods. In collaboration with Dr. Cohen, we are also planning a grant aimed at disentangling the complex relationship between pain and age-related cognitive decline.

Natalie C. Ebner, PhD

My future research program revolves around the following four research questions:

1. *What are the cognitive mechanisms underlying processing biases for social and emotional information in aging?*
In a series of studies I was able to extend the own-age bias in recognition memory for neutral faces to attentional processes and to emotional faces (e.g., Ebner & Johnson, 2009; Ebner et al., 2011; He et al., 2011). My neuroimaging work further supports prefrontal cortex and amygdala as key structures in processing of own-age vs. other-age faces (Ebner et al., 2011, 2012, 2013). I am currently extending this research by examining the role of facial features (e.g., attractiveness, likeability) on the own-age bias, thereby determining conditions under which older adults show less of a memory deficit (Ebner et al., in revision; Lin et al., 2015).
2. *Is the neuropeptide oxytocin associated with improved social and emotional functioning in aging?*
Oxytocin is a neuropeptide with beneficial effects in social and emotional domains. To date, these effects are almost exclusively studied in young adults, schizophrenia, autism, and mostly in animals. Close to nothing is known about oxytocin in human aging. This is surprising given that oxytocin appears to benefit some capacities that decline with age. I have successfully established this novel line of investigation in the literature via publication of theoretical and review papers (Ebner et al., 2013, 2015a) as well as empirical data (Ebner et al., in press, 2016, 2015b), in support of my hypothesis that intranasal oxytocin intervention particularly benefits older men, given their increased deficits in social and emotional functioning. As a crucial milestone, I was able to put in place compounding of an FDA-approved formula of the oxytocin nasal spray in my lab. The front runner position of my lab in this field is also reflected in invitations I receive to serve as collaborator and consultant to labs in the US as well as abroad. Moving forward, in a series of studies using behavioral and neuroimaging techniques, I plan to determine the clinical potential of acute and chronic administration of intranasal oxytocin towards functional improvement in healthy aging as well as in the context of affective disorders with relevance in aging and in chronic pain patients.

3. *Can neurofeedback training promote social and emotional functioning in aging?*

In my previous work I had found evidence of age-related deficits in older adults' ability to correctly read emotions in other people's faces (emotion perception; Ebner & Johnson, 2009; Ebner et al., 2010, 2013; Riediger et al., 2011) as well as about older adults' difficulties with some forms of emotion regulation (positive reappraisal of a negative event; Voelkle et al., 2013, 2014). These capacities have been shown to be crucial for leading an emotionally and socially fulfilling life in aging. Directly following up on this previous work, I have started to use novel neurofeedback training towards improvement of emotion perception and emotion-regulatory capacities in healthy older adults. In particular, together with colleagues in biomedical engineering we have expanded existing infrastructure to allow for use of the highly innovative technique of real-time functional magnetic resonance imaging (rtfMRI). Neurofeedback is an operant conditioning procedure by which humans can learn to modulate neural activity. The learned volitional control of brain activity has been shown to lead to behavioral modifications, thus offering a direct test of brain-behavior links. We are now among the first groups to have established feasibility of the rtfMRI approach in aging (Rana et al., in press). I will continue this line of research with a comprehensive analysis of effects of rtfMRI neurofeedback on affective and social-cognitive plasticity and improved behavioral performance in healthy as well as pathological (e.g., mood disorders, Parkinson's Disease) aging.

4. *Can older adults' decision making capacities be improved to avoid cyberattacks?*

In a series of NSF- and MIT Lincoln Lab funded collaborative studies with colleagues in cyber security, we aim at identifying adult age differences in affective and social influences on decision making in the applied context of computer security. In particular, we use cognitive-behavioral and eye-tracking techniques towards development and validation of an open-source browser extension that provides visual security cues in an age-targeted fashion to protect older adults from web-based cyberattacks (e.g., phishing scams, links to malicious software) during their internet routines. Our work is based on the hypothesis that cyberattacks constitute a particular threat to older adults because (i) their decision making is generally characterized by less information seeking and more prone to use of false information; and (ii) they report higher levels of trust and show a reduced sensitivity to detect cues of untrustworthiness. Supporting our central hypothesis, older compared to young adults in their everyday internet use were particularly susceptible to phishing emails, combined with very low susceptibility awareness (Oliveira et al., under review a/b). Moving forward, we are conducting experiments to identify specific factors contributing to this particular vulnerability in aging (e.g., cognitive status, expertise, familiarity of sender).

Robert A. Fieo, PhD

1. Development of cognitive training paradigm that includes cognitive enrichment intervention for older adults with mild cognitive impairment.
2. Development of a performance-based functional status battery for older adults

Damon Geoffrey Lamb, PhD

Pursuing funding for non-invasive approaches to enhance memory and cognition as well as to prevent conversion from amnesic MCI to AD.

Eric Porges, PhD

My near term plans will include the extension of my work with GABA MRS in older adults, HIV+ and Heavy Drinkers to explore cognitive function in a number of domains. I am PI on a KL2 to investigate the relationship of GABA to cognitive flexibility. I am CO-I on studies investigating the relationship between GABA and motor function, GABA and pain and, GABA and social cognition. In the near future we plan to target GABAergic influences on these important domains via pharmacological intervention.

John B. Williamson, PhD

Follow up grants to my VA funded work on application of our mechanistic results in mild TBI and PTSD are pending.

I have pending initiatives using nerve stimulation to enhance cognitive performance. A DARPA submission that my team collaborated on earned partial funding (for the animal component) from which we will get critical data on mechanism of cognitive enhancement with this technology. We have preliminary data in humans that we have used for a pending R01 submission to enhance cognitive performance in people at risk for Alzheimer's disease.

A follow up to our R56 funded heart failure initiative will be submitted as soon as pilot data are sufficient from the current recruitment efforts.

Adam Joshua Woods, PhD

At present, Dr. Woods currently has two R01's planned for submission in the area of cognitive aging and memory. The first is a two-site study that leverages the ACT grant, but pairs with long-standing colleagues at the City College of New York to use advanced MRI-derived finite element modeling (a form of computational modeling) to predict individual subject based current flow models in our 360 ACT participants. This grant will use this modeling approach, which Dr. Woods has published on numerous times, to identify individualized treatment response models for participants and create a personalized medicine application approach for tDCS in the field of clinical trials. It will also explore mechanistic factors and genetic predictors impacting treatment response. The second R01 is a multisite neuroinflammation clinical trial that he previously submitted as an R01. This will involve scaling down the prior 5 site version to a smaller phase II trial focusing exclusively on Losartan treatment as a means of reducing neuroinflammation, improving cognitive function, and preventing dementia.

IF APPLICABLE, PLEASE PROVIDE ENDOWMENT INVESTMENT RESULTS FOR THE REPORT PERIOD: See page 71

WERE ANY FUNDS USED FOR A PROHIBITED PURPOSE DURING THE REPORT PERIOD? No

DO YOU RECOMMEND ANY MODIFICATION TO THE PURPOSE OR MANDATES IN THE GIFT AGREEMENT? No

DID ALL ACTIVITIES DURING THE REPORT PERIOD FURTHER THE PURPOSE? Yes

NEGATIVE EVENTS (LOSS OF PERSONNEL, SPACE, BUDGET ETC.): NA

ADDITIONAL COMMENTS: See letter on page 27

SIGNATURE, DATE AND TITLE OF PERSON SUBMITTING THE REPORT:

A handwritten signature in black ink, appearing to read 'R. Cohen', is positioned above the printed name.

Ronald A. Cohen, PhD, ABPP, ABCN
Professor, Aging, Neurology, and Psychiatry
Director, CAM-CTRP, and Evelyn McKnight Endowed Chair
for Clinical Translation in Cognitive Aging



William G. Luttge Lectureship in Neuroscience



November 28, 2016

Dear McKnight Brain Research Foundation Trustees,

On March 14, 2016 we hosted the 4th Annual William G. Luttge Lectureship in Neuroscience with Carol A. Barnes, Ph.D. as our lectureship speaker. Dr. Barnes' lecture titled "Aging is not a disease: normal lifespan changes in brain circuits critical for memory", was delivered to a full audience in the DeWeese Auditorium at the McKnight Brain Institute.

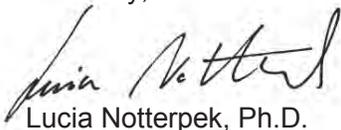
Dr. Barnes is the Regents' Professor in the Departments of Psychology, Neurology & Neuroscience, and is the Evelyn F. McKnight Endowed Chair for Learning and Memory in Aging, and the Director of the Evelyn F. McKnight Brain Institute, in Tucson, Arizona. Dr. Barnes received her BA in psychology from the University of California at Riverside, and her MA and PhD from Carleton University in Ottawa, Canada. She did postdoctoral training in neuropsychology and neurophysiology in the Department of Psychology at Dalhousie University, The Institute of Neurophysiology, University of Oslo, and in the Cerebral Functions Group at University College London. Dr. Barnes is past-president of the 42,000+ member Society for Neuroscience (2004-2005), an elected Fellow of the American Association for the Advancement of Science, and an Elected Foreign Member of the Royal Norwegian Society of Sciences and Letters. The central goal of Dr. Barnes' research program is to understand how the brain changes during the aging process and what the functional consequences of these changes are on information processing and memory. Her research program involves behavioral, electrophysiological and molecular biological approaches to the study of young and aged rodents and non-human primates.

Members of the Luttge Lectureship Committee are:

- Steven DeKosky, MD, Interim Director of the Evelyn F. and William L. McKnight Brain Institute at UF, and Aerts-Cosper Professor of Alzheimer's Research in the Department of Neurology
- Lucia Notterpek, PhD, Chair and Professor of the Department of Neuroscience
- Tom C. Foster, PhD, Professor of Neuroscience, and Evelyn F. McKnight Chair for Research on Age-related Memory Loss
- David R. Borchelt, PhD, Professor of Neuroscience, and Director of the Santa Fe Health Alzheimer's Disease Research Center
- Sara Jo Nixon, PhD, Professor of Psychiatry, and Addiction Research Division Chief, and Director of the Neurocognitive Laboratory

The committee is now organizing the 5th Luttge Lectureship which will be held during National Brain Awareness Week and is scheduled for Monday, March 13, 2017 with Dr. James L. McGaugh as speaker. Dr. McGaugh is a Research Professor of Neurobiology and Behavior in the School of Biological Sciences, and Fellow at the Center for the Neurobiology of Learning and Memory at the University of California, Irvine. He is recognized internationally for his pioneering studies of drug and stress-hormone influences on memory.

Sincerely,



Lucia Notterpek, Ph.D.
Professor and Chair Department of Neuroscience

Faculty Biographical Sketches



BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Bizon, Jennifer Lynn

eRA COMMONS USER NAME (agency login): jbizon

POSITION TITLE: Professor of Neuroscience and Psychiatry

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of North Carolina at Chapel Hill, Chapel Hill, North Carolina	BS	05/1993	Psychology
University of California, Irvine, Irvine, California	PhD	08/1998	Neurobiology and Behavior
John Hopkins University, Baltimore, MD	Postdoctoral Fellow	09/2002	Neuroscience and Psychological Sciences

A. Personal Statement

My NIH-funded research program is broadly focused on determining the neural processes that support memory, executive functions, and decision making, and that contribute to the decline of these functions in aging. Using rodent models, my laboratory employs an integrative approach that combines sensitive cognitive assessments with cellular, molecular, and behavioral pharmacological methodologies. My laboratory has uncovered disruptions in both glutamatergic and GABAergic signaling in the aged brain that contribute to impairments in cognitive flexibility, working memory, and decision making (ref a, b, c, d). Moreover, we have demonstrated that pharmacological targeting of these signaling alterations reverses several aspects of cognitive dysfunction in aged rats (ref a, b, c). Our long-term goal is to promote personal independence and quality of life in older adults by developing strategies to optimize cognitive function across the full lifespan.

- a. Bañuelos C, Beas BS, McQuail JA, Gilbert RJ, Frazier CJ, Setlow B, **Bizon JL**. Prefrontal cortical GABAergic dysfunction contributes to age-related working memory impairment. *J Neurosci*. 2014 Mar 5;34(10):3457-66. PMC3942567.
- b. McQuail JA, Beas BS, Simpson K, Kyle K, Frazier CJ, Setlow B, **Bizon JL** (2016) "NR2A-containing NMDA receptors in the prefrontal cortex are required for working memory and predict age-related cognitive decline." *J. Neurosci*. Epub ahead of print. PMC in process.
- c. McQuail JA, Frazier CJ, **Bizon JL** (2015) Molecular aspects of age-related cognitive decline: Role of GABA signaling. *Trends in Molecular Medicine*. Jul;21(7):450-60. doi: 10.1016/j.molmed.2015.05.002.
- d. Simon NW, LaSarge CL, Montgomery KS, Williams MT, Mendez IA, Setlow, B, **Bizon, JL**. Good things come to those who wait: attenuated discounting of delayed rewards in aged Fischer 344 rats. *Neurobiol Aging*. 2010 May;31(5):853-62. PMC2866647.

URL for full list of published work in My Bibliography: <http://www.ncbi.nlm.nih.gov/sites/myncbi/jennifer.bizon.1/bibliography/40679052/public/?sort=date&direction=descending>

Positions & Employment

1993	Research Assistant at University of North Carolina at Chapel Hill
1993-1998	Graduate Student Assistant, University of California, Irvine, Laboratory of Dr. Christine Gall
1998-2003	Postdoctoral Fellow, Johns Hopkins University, Laboratory of Dr. Michela Gallagher
2002-2004	Assistant Research Scientist, Dept. of Psychology, Johns Hopkins University
2004-2010	Assistant Professor of Psychology, Texas A&M University
2004-2010	Faculty of Neuroscience, Texas A&M University
2010-2016	Associate Professor of Neuroscience and Psychiatry, University of Florida College of Medicine
2011-2013	Co-director of Neuroscience Graduate Program, University of Florida College of Medicine

2013-2015 Director, Neuroscience Graduate Program, University of Florida College of Medicine
2016-present Professor of Neuroscience and Psychiatry, University of Florida College of Medicine

Other Experience and Professional Memberships

2009 Member, NIA Special Emphasis Panel (ZAG1 ZIJ-5), Mechanisms of Cognitive Aging
2010–present Advisory Board, Alzheimer’s Drug Discovery Foundation
2010–2012 Ad hoc member, NIH Clinical Neuroscience and Neurodegeneration Study Section
2010–present Member, Center for Smell and Taste, University of Florida
2011 Member, NSF Modulatory Brain Systems Review Panel
2012 Ad hoc member, NIH Chronic Dysfunction and Integrative Neurodegeneration
2013 Member, NIH Sensory and Motor Neuroscience, Cognition and Perception Fellowship Study Section (F02B)
2013–present Member, NIH Neurodevelopment, Synaptic Plasticity, Neurodegeneration Fellowship Study Section
2014 Member, NIEHS Special Emphasis Panel (ZES1 LWJ-K), Environmental Contributors to Neurodegeneration
2016–present Chair, NIH Neurodevelopment, Synaptic Plasticity, Neurodegeneration Fellowship Study Section
2014–present Section Editor, Cognition, Behavior and Physiology Section, Neurobiology of Aging
2015–present Ad hoc member, NIH National Institute on Aging Neuroscience Study Section (NIA-N)

Honors and Professional Activities

1995 Individual NRSA, F31 pre-doctoral award, National Institute of Mental Health
2001 Individual NRSA, F32 post-doctoral award, National Institute on Aging
2008 Montague Center for Teaching Excellence Award, College of Liberal Arts, Texas A&M University
2009 Leadership and Service Award, Faculty of Neuroscience, Texas A&M University
2011-2016 Exemplary Teaching Award, College of Medicine, University of Florida

B. Contribution to Science

1. A primary focus of my laboratory is to understand how GABAergic circuits and signaling are altered in the aged prefrontal cortex, and the contributions of shifts in excitatory/inhibitory (E/I) dynamics in this brain region to age-related cognitive decline. To date, much of our work has focused on GABA(B) receptors, which are broadly expressed in the brain. These receptors contribute to GABA signaling via both pre- and postsynaptic mechanisms. In prefrontal cortex (PFC), we have documented a number of biochemical and electrophysiological changes, which together suggest that pyramidal neurons in this brain region are subject to age-related increases in tonic inhibition (a, c). Potentially in response to this increased inhibition, GABA(B)R subunit expression is significantly reduced in the aged PFC (b, c). Indeed, we have found that lower PFC GABA(B)R subunit expression strongly predicts better working memory abilities among aged rats (c). We have further identified specific excitatory signaling alterations that contribute to E/I dysregulation and working memory impairments in aging, including reductions in presumptive synaptic NR2A-NMDARs (d). Based on these findings, my laboratory has explored the use of GABA(B)R antagonists and positive allosteric modulators for synaptic NMDARs for improving age-related cognitive decline.
 - a. McQuail JA, Bañuelos C, LaSarge CL, Nicolle MM, **Bizon JL**. GABA(B) receptor GTP-binding is decreased in the prefrontal cortex but not the hippocampus of aged rats. *Neurobiol Aging*. 2012 Jun;33(6):1124.e1-12. PMC3310948.
 - b. Bañuelos C, Beas BS, McQuail JA, Gilbert RJ, Frazier CJ, **Bizon, JL**. Prefrontal cortical GABAergic dysfunction contributes to age-related working memory impairment. *J Neurosci*. 2014 Mar 5;34(10):3457-66. PMC3942567.
 - c. McQuail JA, Beas BS, Simpson K, Kyle K, Frazier CJ, Setlow B, **Bizon JL** (2016) “NR2A-containing NMDA receptors in the prefrontal cortex are required for working memory and predict age-related cognitive decline.” *J. Neurosci*. Epub ahead of print. PMC in process.
 - d. McQuail JA, Frazier CJ, **Bizon JL**. (2015) Molecular aspects of age-related cognitive decline: Role of GABA signaling. *Trends in Molecular Medicine*. Jul;21(7):450-60. PMID 4500156.
2. My laboratory has developed sensitive behavioral methods to model hippocampal/medial temporal lobe-mediated deficits in aged rodents (refs a, b, c) and has used these behavioral models to investigate underlying neural mechanisms of cognitive decline in aging. Specifically, in the past several years, we have established sensitive behavioral tools for investigating how the perception and encoding of sensory stimuli is altered in aging and how such alterations contribute to mnemonic decline (refs a, d, and Yoder et al., under revision). We are now employing these same rigorous psychophysical methods (ref d) to better understand cognitive decline associated with Alzheimer’s disease, and are exploring whether sensory discrimination learning assessments have utility as a behavioral biomarker for disease pathology.

- a. LaSarge CL, Montgomery KS, Tucker C, Slaton GS, Griffith WH, Setlow B, **Bizon JL**. Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. *Neurobiol Aging*. 2007 Jun;28(6):928-36.
 - b. **Bizon JL**, LaSarge CL, Montgomery KS, McDermott AN, Setlow B, Griffith WH. Spatial reference and working memory across the lifespan of male Fischer 344 rats. *Neurobiol Aging*. 2009 Apr;30(4):646-55. PMC2703480.
 - c. Montgomery, KS, Edwards, G, Kumar, A, Levites, Y, Meyers CA, Gluck M, Setlow, B and **Bizon, JL** (2016) Deficits in hippocampal-dependent transfer generalization learning and synaptic function in mouse models of amyloidosis. *Hippocampus*. 26(4):455-71. doi: 10.1002/hipo.22535.
 - d. Yoder, WM, Setlow, B, **Bizon, JL**, & Smith, DW (2014) Characterizing olfactory perceptual similarity using carbon chain discrimination in behaviorally-trained Fischer 344 rats. *Chemical Senses*. 39(4):323-31. PMC4031640
3. Deciding among options that include both benefits and risks of adverse outcomes is fundamental to our ability to effectively navigate everyday life. As part of a long-standing collaboration with Dr. Barry Setlow, my laboratory has a strong interest in using animal models to better understand the neural processes that support decision making. One element of this work involves elucidation of the neural circuits and signaling mechanisms that mediate how individuals weigh rewards against putative costs such as punishment or delay to reward delivery (refs c, d). A second element of this research is to determine how cost-benefit decision making is altered across the lifespan (refs a, b). Our work was the first to show that aged rats have a strong preference for delayed over immediate rewards relative to young adult rats. These data are highly consistent with observations showing that aged individuals are better at delaying gratification, and suggest that age-related neurobiological alterations are not universally detrimental but can support some beneficial cognitive outcomes (ref a).
- a. Simon NW, LaSarge CL, Montgomery KS, Williams MT, Mendez IA, Setlow, B, **Bizon, JL**. Good things come to those who wait: attenuated discounting of delayed rewards in aged Fischer 344 rats. *Neurobiol Aging*. 2010 May;31(5):853-62. PMC2866647.
 - b. Gilbert RJ, Mitchell MR, Simon NW, Bañuelos C, Setlow B, **Bizon JL**. Risk, reward, and decision-making in a rodent model of cognitive aging. *Front Neurosci*. 2011;5:144. PMC3250056.
 - c. Simon NW, Montgomery KS, Beas BS, Mitchell MR, LaSarge CL, Mendez IA, Bañuelos C, Vokes CM, Taylor AB, Haberman RP, **Bizon JL**, Setlow, B. Dopaminergic modulation of risky decision-making. *J Neurosci*. 2011 Nov 30;31(48):17460-70. PMC3307370.
 - d. Orsini CA, Trotta RT, **Bizon JL**, Setlow B. Dissociable Roles for the Basolateral Amygdala and Orbitofrontal Cortex in Decision-Making under Risk of Punishment. *J Neurosci*. 2015 Jan 28;35(4):1368-79. PMC4308589.
4. My early research showed that memory loss is associated with impaired HPA axis function and protracted glucocorticoid release following a stressor, and that such changes occur in the absence of frank hippocampal neural loss. Instead, we found that these changes are likely attributable to attenuated GR/MR expression within both aged hippocampus and prefrontal cortex (ref a). Other findings in neuroscience during this time period highlighted the remarkable neurogenic capacity of the adult hippocampus (Kempermann and Gage, 1998; Gould and McEwen, 1993), and led to questions about whether age-related changes in this phenomenon could contribute to the decline of mnemonic abilities associated with aging. My postdoctoral studies examined hippocampal neurogenesis in relation to age-related memory loss and showed that while there is a marked attenuation of new neurons born in the aged hippocampus (>90%), new neuron production and differentiation did not predict the memory abilities of aged rats (refs b-d). Indeed, many aged rats were able to maintain spatial learning performance on par with young adults despite dramatic reductions in hippocampal neurogenesis. While these studies did not specifically address the role of hippocampal neurogenesis in normal learning and memory, they do indicate that reduced neurogenesis in normal aging is not sufficient to account for spatial memory dysfunction.
- a. **Bizon JL**, Helm KA, Han JS, Chun HJ, Pucilowska J, Lund, PK, Gallagher, M Hypothalamic-pituitary-adrenal axis function and corticosterone receptor expression in behaviourally characterized young and aged Long-Evans rats. *Eur J Neurosci*. 2001 Nov;14(10):1739-51.
 - b. **Bizon JL**, Gallagher M. Production of new cells in the rat dentate gyrus over the lifespan: relation to cognitive decline. *Eur J Neurosci*. 2003 Jul;18(1):215-9.
 - c. **Bizon JL**, Lee HJ, Gallagher M. Neurogenesis in a rat model of age-related cognitive decline. *Aging Cell*. 2004 Aug;3(4):227-34.
 - d. **Bizon JL**, Gallagher M. More is less: neurogenesis and age-related cognitive decline in Long-Evans rats. *Sci Aging Knowledge Environ*. 2005 Feb 16;2005(7):re2.

5. Throughout my career, I have had a long-standing interest in the role of basal forebrain and cholinergic signaling in the modulation of cortical circuits and memory function. Highlights of this work include several studies from my pre-doctoral training in the laboratory of Dr. Christine Gall, in which we identified several sources of local trophic support for basal forebrain and striatal cholinergic neurons (ref a, Bizon et al., 1996, Lauterborn et al., 1995). Subsequently, I used the selective neurotoxin, 192-IgG saporin to demonstrate that removal of cholinergic neurons influences spatial learning strategies (ref b) and HPA function (Han et al., 2002) in young rats. More recently, we have investigated both the number (ref d) and electrophysiological properties (ref c, Dubois et al., 2014) of cholinergic neurons in relation to age-related hippocampal-dependent spatial memory impairment. Our findings show that while there is modest cholinergic neuron loss with advanced aging, such changes cannot fully account for spatial learning deficits. Notably, our studies highlight a role for co-distributed basal forebrain GABAergic neurons in both cholinergic dysfunction (Dubois et al. 2014) and impaired memory (ref d).
 - a. **Bizon JL**, Lauterborn JC, Gall CM. Subpopulations of striatal interneurons can be distinguished on the basis of neurotrophic factor expression. *J Comp Neurol.* 1999 May;408(2):283-298.
 - b. **Bizon JL**, Han JS, Hudon C, Gallagher M. Effects of hippocampal cholinergic deafferentation on learning strategy selection in a visible platform version of the water maze. *Hippocampus.* 2003;13(6):676-84.
 - c. Murchison D, McDermott AN, LaSarge CL, Peebles KA, **Bizon JL**, Griffith, WH Enhanced calcium buffering in F344 rat cholinergic basal forebrain neurons is associated with age-related cognitive impairment. *J Neurophysiol.* 2009 Oct;102(4):2194-207. PMC2775378.
 - d. Bañuelos C, LaSarge CL, McQuail JA, Hartman JJ, Gilbert RJ, Ormerod, B, **Bizon, JL**. Age-related changes in rostral basal forebrain cholinergic and GABAergic projection neurons: relationship with spatial impairment. *Neurobiol Aging.* 2013 Mar;34(3):845-62. PMC3632262.

D. Current Research Support

Neural mechanisms of age-related cognitive decline

Principal Investigator: Jennifer L Bizon

R01 AG02942 (years 6-11), 2014/05/15-2019/05/19

National Institute on Aging

The goal of this project is to determine how age-related alterations in GABAergic signaling mechanisms in prefrontal cortex contribute to impairments in executive functions, including working memory, behavioral flexibility and decision making.

Neural mechanisms of age-related cognitive decline (Supplement)

Principal Investigator: Jennifer L Bizon

R01 AG02942-07S1, 2014/05/15-2019/05/17

National Institute on Aging

This supplement is for student training under the parent R01.

Risk taking and cocaine use: interactions, mechanisms, and therapeutic targets

Principal Investigator: Barry Setlow, Co-Investigator: Jennifer L Bizon

R01 DA036534, 2015/03/15-2020/03/31

National Institute on Drug Abuse

The goal of this project is to determine neural mechanisms underlying relationships between risk taking behavior and cocaine self-administration.

The contribution of declines in functional connectivity to cognitive aging

Principal Investigator: Sara Burke, Co-Investigator: Jennifer L Bizon

R01 R01AG049722, 2016/01/05-2021/01/15

National Institute on Aging

The goal of this project is investigate how disrupted communication between the prefrontal cortex and hippocampus contributes to age-associated cognitive decline.

Testing and forecasting hippocampal theta wave propagation in learning and memory

Principal Investigator: Andrew Maurer, Co-Investigator: Jennifer L Bizon

R01MH109548, 2016/11/01-2021/10/15

Agency: National Institute on Mental Health

The goal of this project is to investigate how the role of basal forebrain afferents in regulating theta during behavior.

Cognitive Augmentation through Neuroplasticity

Principal Investigator: Kevin Otto, Project Leader: Jennifer L Bizon

2017/1/15-2021/01/15

Agency: DARPA

The goal of this project is investigate vagal nerve stimulation as a means to enhance cognition.

Current Mentored Support

Molecular and physiological determinants of age-related working memory decline

Principal Investigator: Joseph A McQuail, Sponsor: Jennifer L. Bizon

F32AG051371, 6/1/15-5/31/18

Agency: National Institute on Aging

Neural circuits and mechanisms underlying maladaptive risk-taking following cocaine self-administration

Principal Investigator: Caitlin Orsini, co-Sponsor: Jennifer L. Bizon

K99 DA041493 Pending Award

Agency: National Institute on Drug Abuse

Impact score = 10

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: BURKE, SARA

eRA COMMONS USER NAME (agency login): sburke

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Oregon, Eugene, OR	BS	08/1999	Psychology, Chemistry
University of Oregon, Eugene, OR	MS	12/2000	Psychology
University of Arizona, Tucson, AZ	PHD	05/2009	Neuroscience, pharmacology
University of Arizona, Tucson, AZ	Postdoctoral Fellow	09/2013	Non-human primate and rodent models of cognitive aging

A. Personal Statement

My NIH/NIA funded research program is broadly focused on improving health outcomes in the elderly by determining the biological mechanisms that are responsible for the cognitive decline that occurs in later stages of life. Even in the absence of pathology, a large proportion of elderly people experience memory decline that interferes with their quality of life. Thus, understanding the neurobiology of memory impairments in advanced age is paramount both improving health outcomes in the elderly as well as distinguishing normal aging from dementia. A significant barrier to uncovering the neurobiology of age-related cognitive decline is that memory processes are distributed throughout the brain and a fundamental gap exists in our understanding of how different brain structures interact over the lifespan. The long-term goal of my laboratory is to determine the alterations in network-level interactions that underlie cognitive impairment in advanced age and dementia. Current projects are focused on uncovering mechanisms of age-related impairments in sensory discrimination across modalities, identifying age-associated changes in medial temporal lobe-prefrontal functional connectivity that contribute to memory deficits, and testing whether diet can globally improve neural network function in old animals. To answer these questions, my lab integrates neurophysiology and anatomy with behavioral analysis in order to determine the extent that age-related memory impairments manifest from dysfunction in inter-regional communication. Our rationale is that by elucidating how aging influences systems-level dynamics, we will be better positioned to develop interventions that broadly improve cognition.

B. Positions and Honors

Positions & Employment

- 1997 - 1999 Undergraduate Research Assistant, Dr. Richard Marrocco's Visual-Attention laboratory, University of Oregon, Eugene, OR
- 1999 - 2000 Graduate Research Associate, Dr. Richard Marrocco's Visual-Attention laboratory, University of Oregon, Eugene, OR
- 2000 - 2002 Research Associate, Dr. Alvin Eisner's Visual Adaptation laboratory, Oregon Health & Science University, Portland, OR
- 2003 - 2004 Graduate Teaching Assistant for MSB407: Cellular, Molecular Neuroscience, University of Arizona, Tucson, AZ
- 2006 - 2011 Teaching Assistant for NRSC4/524: Gerontology, University of Arizona, Tucson, AZ
- 2013 - Assistant Professor, Department of Neuroscience, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

- 2002 - Member, Society for Neuroscience
- 2008 - 2009 Mentor and small group leader, Undergraduate Biology Research Program, University of Arizona
- 2010 - 2012 Membership Enhancement Plan Working Group, Society for Neuroscience
- 2010 - 2011 Mentor, University of Arizona Assurance Program
- 2014 - Mentor, HHMI Science for Life
- 2014 - Member, North Central Florida Chapter of the Society for Neuroscience
- 2014 - Mentor, University of Florida Scholar Award

- 2015 - Judge for speaker competition, Junior Science, Engineering and Humanities Symposium
- 2015 - Member Faculty for Undergraduate Neuroscience
- 2015 - Director of the UF Summer Neuroscience Internship Program

Honors

- 1999 Departmental Honor's in Psychology, University of Oregon
- 1999 Magna Cum Laude, University of Oregon
- 1999 Inducted, Phi Beta Kappa
- 2002 National Institute of Health Training Grant Recipient, University of Arizona
- 2005 Society for Neuroscience, Travel Award Recipient
- 2006 Recipient of the Ruth L. Kirschstein National Research Service Award, National Institute of Health
- 2008 D.G. Marquis Behavioral Neuroscience Award, American Psychology Association
- 2009 Mentor of the Year Award, Undergraduate Biology Research Program, University of Arizona
- 2010 D.G. Marquis Behavioral Neuroscience Award , American Psychology Association
- 2012 Honorable Mention, Mentor of the Year, Undergraduate Biology Research Program, University of Arizona
- 2014 Best Talk, Department Data Blitz, Department of Neuroscience, University of Florida
- 2015 Claude D. Pepper Older Americans Independence Center Scholar Awardee
- 2015 Exemplary Teaching Award, University of Florida College of Medicine
- 2016 Nominated for Excellence Awards for Assistant Professors

C. Contribution to Science

1. My prior publications were the first to demonstrate that age-related deficits in object recognition memory are mediated by perirhinal cortical dysfunction. The perirhinal cortex is an area of the brain that receives sensory information from all modalities and is interconnected with the hippocampus to support memory. Using neurophysiological approaches (ref a) and activity-induced gene expression (ref b), my work showed that perirhinal activity is blunted in aged rats during an object exploration task and that this decline in perirhinal activity is tightly related to behavioral performance. This work demonstrates my experience is linking neural activity to behavioral performance, which is a central feature of the current proposal.
 - a. **Burke SN, Hartzell AL, Lister JP, Hoang LT, Barnes CA.** Layer V perirhinal cortical ensemble activity during object exploration: a comparison between young and aged rats. *Hippocampus*. 2012 Oct;22(10):2080-93. **PubMed PMID: 22987683; PubMed Central PMCID: PMC3523702.**
 - b. **Burke SN, Maurer AP, Nematollahi S, Uprety A, Wallace JL, et al.** Advanced age dissociates dual functions of the perirhinal cortex. *J Neurosci*. 2014 Jan 8;34(2):467-80. **PubMed PMID: 24403147; PubMed Central PMCID: PMC3870932.**
2. A long-standing presumption in the field of cognitive aging had been that aged animals have difficulty recognizing stimuli because they "forget" items that have been previously experienced. This idea, however, was difficult to reconcile with other data showing that aged subjects have an increase in false memories. I designed a series of experiments to elucidate the origins of age-associated recognition memory impairments that led to the novel observation that old animals have recognition memory deficits because they have a reduced ability to discriminate novel stimuli from those that are familiar, which manifests as a false memory (ref a). This work led to foundational insights regarding age-associated declines in recognition memory, which presumably arise from perirhinal cortical dysfunction, and was later replicated in monkeys (ref b) and humans (ref c). Moreover the paper published in 2010, of which I designed and implemented the experimental procedures, analyzed the data, and prepared the manuscript earned the D.G. Marquis Behavioral Neuroscience Award in 2010, which is indicative of my expertise in the cognitive assessment of rodent memory.
 - a. **Burke SN, Wallace JL, Nematollahi S, Uprety AR, Barnes CA.** Pattern separation deficits may contribute to age-associated recognition impairments. *Behav Neurosci*. 2010 Oct;124(5):559-73. **PubMed PMID: 20939657; PubMed Central PMCID: PMC3071152.**
 - b. **Burke SN, Wallace JL, Hartzell AL, Nematollahi S, Plange K, et al.** Age-associated deficits in pattern separation functions of the perirhinal cortex: a cross-species consensus. *Behav Neurosci*. 2011 Dec;125(6):836-47. **PubMed PMID: 22122147; PubMed Central PMCID: PMC3255096.**
 - c. **Burke SN, Ryan L, Barnes CA.** Characterizing cognitive aging of recognition memory and related processes in animal models and in humans. *Front Aging Neurosci*. 2012;4:15. **PubMed PMID: 22988437; PubMed Central PMCID: PMC3439640.**
3. Although the spatial correlates of hippocampal firing properties have been extensively described, less is known regarding the influence on non-spatial sensory information (e.g., 3-dimensional objects) on the activity patterns of these neurons.

The perirhinal cortex is extensively interconnected with the hippocampus and receives sensory input related to non-spatial information. Prior to my research it was believed that this structure supported recognition memory with changes in firing rate as a stimulus goes from novel to familiar. My work produced two foundational insights regarding the perirhinal cortex and its interactions with the hippocampus. First, we showed the perirhinal cortical neurons selectively respond to objects, but that firing rates do not change as a function of novelty (ref a). This observation called for a refinement of standard models of recognition memory. Second, we found that the neurons in the hippocampal subregion receiving direct perirhinal input are robustly modulated by objects (ref b).

- a. **Burke SN**, Maurer AP, Hartzell AL, Nematollahi S, Uprety A, et al. Representation of three-dimensional objects by the rat perirhinal cortex. *Hippocampus*. 2012 Oct;22(10):2032-44. **PubMed PMID: 22987680; PubMed Central PMCID: PMC3447635.**
 - b. **Burke SN**, Hartzell AL, Lister JP, Hoang LT, Barnes CA. Layer V perirhinal cortical ensemble activity during object exploration: a comparison between young and aged rats. *Hippocampus*. 2012 Oct;22(10):2080-93. **PubMed PMID: 22987683; PubMed Central PMCID: PMC3523702.**
 - c. **Burke SN**, Barnes CA. The neural representation of 3-dimensional objects in rodent memory circuits. *Behav Brain Res*. 2014 Sep 6; **PubMed PMID: 25205370.**
4. In young animals, dynamic hippocampal activity patterns support learning and memory. I have been involved in a series of papers that show how behavior-dependent modulation of hippocampal activity is compromised in aged animals to produce memory deficits. Moreover, we have shown that altering NMDA receptor currents with the Alzheimer's disease therapeutic memantine can restore experience-dependent plasticity in aged memory-impaired rats (ref a). This paper, on which I was first author, received the D.G. Marquis Behavioral Neuroscience Award from the American Psychological Association for the best paper published in Behavioral Neuroscience in 2008. These papers demonstrate my expertise regarding the *in vivo* physiological signatures of hippocampal dysfunction that are a component of the current proposal (Aims 1 and 2).
- a. **Burke SN**, Maurer AP, Yang Z, Navratilova Z, Barnes CA. Glutamate receptor-mediated restoration of experience-dependent place field expansion plasticity in aged rats. *Behav Neurosci*. 2008 Jun;122(3):535-48. **PubMed PMID: 18513124; PubMed Central PMCID: PMC2773228.**
 - b. Gerrard JL, **Burke SN**, McNaughton BL, Barnes CA. Sequence reactivation in the hippocampus is impaired in aged rats. *J Neurosci*. 2008 Jul 30;28(31):7883-90. **PubMed PMID: 18667620; PubMed Central PMCID: PMC2703197.**
 - c. Hartzell AL, **Burke SN**, Hoang LT, Lister JP, Rodriguez CN, et al. Transcription of the immediate-early gene Arc in CA1 of the hippocampus reveals activity differences along the proximodistal axis that are attenuated by advanced age. *J Neurosci*. 2013 Feb 20;33(8):3424-33. **PubMed PMID: 23426670; PubMed Central PMCID: PMC3711759.**
5. The size of hippocampal spatial receptive fields increases along the dorsal to ventral longitudinal axis. Working with my longtime collaborator Dr. Andrew Maurer (co-I on current proposal), we elaborated on the differences in the firing properties between neurons in the dorsal versus ventral hippocampus (ref a,b) and showed that the spatial metric of hippocampal receptive fields is changed when objects are added to an environment (ref c). This work produced new insights regarding the impact of sensory information along the hippocampal longitudinal axis and highlights the productive collaborative efforts of Dr. Maurer and myself.
- a. Maurer AP, Cowen SL, **Burke SN**, Barnes CA, McNaughton BL. Organization of hippocampal cell assemblies based on theta phase precession. *Hippocampus*. 2006;16(9):785-94. **PubMed PMID: 16921501.**
 - b. Maurer AP, Cowen SL, **Burke SN**, Barnes CA, McNaughton BL. Phase precession in hippocampal interneurons showing strong functional coupling to individual pyramidal cells. *J Neurosci*. 2006 Dec 27;26(52):13485-92. **PubMed PMID: 17192431.**
 - c. **Burke SN**, Maurer AP, Nematollahi S, Uprety AR, Wallace JL, et al. The influence of objects on place field expression and size in distal hippocampal CA1. *Hippocampus*. 2011 Jul;21(7):783-801. **PubMed PMID: 21365714; PubMed Central PMCID: PMC3314262.**

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/sara.burke.1/bibliography/47433007/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

01/01/16-11/30/20

1R01AG049722, National Institute on Aging

Sara N. Burke (PI)

Title: The Contribution of Declines in Functional Connectivity to Cognitive Aging

The major goal of this proposal is to determine how alterations in systems-level neural coordination in old animals produce cognitive impairments.

Role: PI (35% effort)

08/15/16-08/14/18

1R21AG051004 (**Burke, PI**)

Title: Single-Cell Imaging of Functional Connectivity as a Window into Cognitive Aging

The major goal of this award is to develop novel methods for quantifying functional connectivity between memory-associated brain structures in young and aged rats.

Role: PI (15% effort)

08/15/15-08/14/17

1R03AG049411-01A1, National Institute on Aging (Primary)

Sara N. Burke (Contact - mPI)

Neurogenesis and Memory Network Dynamics during Normal Aging

This proposal seeks to determine the integrity of dentate function in the aged animal.

Role: contract-PI (5% effort)

2015/08/01-2017/3/31

Claude D. Pepper Older Americans Independence Center Junior Scholar Award and Pilot Grant

Sara N. Burke (PI)

A Novel Rodent Model of Age-related Motor-Cognition Dual-Task Deficits

The goal of this award is to development a rodent model of the association between motor and cognitive frailty in order to test potential interventions for maintaining positive health outcomes in the elderly.

2013/10/01-2018/09/30

0011249, McKnight Brain Research Foundation

Sara N. Burke (PI)

Neural system dysfunction and cognitive aging

This goal of this award is to provide institutional support in order to establish a rigorous research program aimed and determining the neurobiological basis of cognitive impairments in the elderly and to identify potential therapeutic strategies.

2016/01/01-2016/12/31

AG047266, sub-Award 1Florida Alzheimer's Disease Research Center Pilot Grant

Sara N. Burke (contact mPI)

Age-associated functional connectivity declines in the anterior network and memory dysfunction

The goal of this pilot grant is to collect comparable data in rodents and humans that points to the mechanisms of age-related cognitive decline.

NIH GRANT11924071 (Maurer, P.I.)

Title: Testing and forecasting hippocampal theta wave propagation in learning and memory

The goal of this award is to understand the relationship between hippocampal oscillatory dynamics and memory.

Role: co-I (12% effort)

Pending

2017/01/01-2020/12/31

DARPA Targeted Neuroplasticity Training

Sara N. Burke (co-PI)

Cognitive Augmentation through Neuroplasticity (under contract negotiation)

The major goal of this award is to define the mechanisms by which peripheral stimulation of the vagus nerve improves behavioral performance.

Completed Research Support

2014/06/01-2016/05/30

00115480, University of Florida Research Seed Opportunity Fund

Sara N. Burke (PI)

Neurogenesis and Memory Network Dynamics during Normal Aging

The major goal of this award is to collect pilot data regarding the impact of reduced neurogenesis with age on the changes in activity pattern dynamics within the hippocampus. These data will be used to generate future NIH proposal.

2006/01/09-2009/01/08

F31 NS054465-03, National Institute of Neurological Disorders and Stroke (NINDS)

Sara N. Burke (PI)

Aging and Neural Ensembles in the Perirhinal Cortex

Role: PI

Huaihou Chen, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Huaihou Chen

eRA COMMONS USER NAME: chenh13

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Anhui University, Hefei, CHINA	B.S.	06/2004	Statistics
University of Science and Technology of China, Hefei, CHINA	M.S.	06/2007	Statistics
Columbia University, New York, NY	Ph.D.	05/2012	Biostatistics
New York University, New York, NY	Postdoctoral	06/2014	Biostatistics

A. Personal Statement

I have a broad background in biostatistics, with specific training and expertise in longitudinal and functional data analysis, and predictive modelling. Besides statistical methodological research, I have broad collaborative research experience in the areas of aging, HIV, psychiatry, neurology, chronic pain and etc. I apply standard and cutting edge statistical methods to solve scientific problems in collaborative research. I have a demonstrated record of accomplished and productive research projects on developing novel statistical methods as well as collaborative research. I have been working with Dr. Ronald Cohen on several projects on cognitive aging and HIV using multimodal neuroimaging since joining University of Florida. My expertise and experience have prepared me for the proposed study.

B. Positions and Honors

Positions and Employment

2012-2014 Post-Doctoral Fellow, Department of Child and Adolescent Psychiatry, New York University School of Medicine, New York, NY

2014 - Assistant Professor, Department of Biostatistics, Colleges of Medicine and Public Health & Health Professions, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

2010- American Statistical Association

2013- ASA Mental Health Statistics Section

Honors

2007-2009 Fellowship, Columbia University

2012 ASA Biometrics Section Travel Award

C. Contribution to Science

1. My research has focused primarily on longitudinal and functional data analysis, and predictive modelling. 1) I develop novel statistical methods for flexible modelling the nonlinear time trends, which may be misspecified by a polynomial model. 2) Classification and clustering of functional data. 3) Novel predictive model that account for the complex structure of the data. Those developed methods can reduce estimation bias, increase power in testing group difference, and improve prediction accuracy. Developed methods are useful for characterizing biomarkers' changes over time, disease dynamic progression, treatment response, and have been applied to neuroimaging, psychiatric, and neurological studies. The developed methods are published in top statistical journals.

- a. **Chen, H.**, Wang, Y. (2011). A penalized spline approach to functional mixed effects model analysis. *Biometrics*. 67, 861-870. PMID: 21155747.
 - b. **Chen, H.**, Wang, Y., Paik, M. C., Choi, H. (2013). A marginal approach to reduced-rank penalized spline smoothing for multilevel data. *Journal of the American Statistical Association*. 108, 1216-1229. PMID: 24497670.
 - c. **Chen, H.**, Reiss, P. T., Tarpey, T. (2014). Optimally Weighted L2 Distance for Functional Data. *Biometrics*. 70, 516-525. PMID: 26228660.
 - d. Wang, Y., **Chen, H.**, Zeng, D., Mauro, C., Duan, N., & Shear, M. K. (2013). Auxiliary marker-assisted classification in the absence of class identifiers. *Journal of the American Statistical Association*. 108, 553-565. PMID: 24039320.
2. As a biostatistician, I conduct collaborative research in the areas of neuroimaging, psychiatry, neurological, chronic pain and etc. I develop and apply cutting edge or standard statistical methods to neuroimaging, psychiatric, neurological studies. Applying the appropriate model and methods, I help my collaborators to predict food intake in anorexia nervosa patients, discover acute effects of Nimodipine for subarachnoid hemorrhage patients, building screening tools for psychosis in low-income minority. I develop methods for obtaining individual neurodevelopmental quantities and correlating the individual brain quantities with clinical outcomes.
- a. Steinglass, J., Sysko, R., Mayer, L., Berner, L., Schebendach, J., Wang, Y., **Chen, H.**, Albano, A., Simpson, B., and Walsh, T. (2010). Pre-meal anxiety and food intake in Anorexia Nervosa. *Appetite*. 55, 214-218. PMID: 20570701.
 - b. Choi, H. A., Ko, S. B., **Chen, H.**, Gilmore, E., Carpenter, A. M., Lee, D., Claassen, J., Mayer, S. A., Schmidt, J. M., Lee, K., Connelly, E. S., Paik, M., Badjatia, N. (2012). Acute Effects of Nimodipine on Cerebral Vasculature and Brain Metabolism in High Grade Subarachnoid Hemorrhage Patients. *Neurocritical Care*. 2012 Jun;16(3): 363-367.
 - c. **Chen, H.**, Kelly, C., F. Xavier Castellanos, Xi-Nian Zuo, Ye He, Reiss, P. T. (2015). Quantile rank maps: a new tool for understanding individual brain development. *NeuroImage*. 111, 454-463. PMID: 25585020.
 - d. Kantrowitz, Joshua T., Scott W. Woods, Eva Petkova, Barbara Cornblatt, Cheryl M. Corcoran, **Huaihou Chen**, Gail Silipo, and Daniel C. Javitt. D-serine for the treatment of negative symptoms in individuals at clinical high risk of schizophrenia: a pilot, double-blind, placebo-controlled, randomised parallel group mechanistic proof-of-concept trial. *The Lancet Psychiatry* 2, no. 5 (2015): 403-412. PMID: 26360284.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/pubmed/?term=Huaihou+Chen>

D. Research Support

OAIC Pepper Scholar (**Chen; PI**) 8/1/2015-3/31/2017

UF Claude D. Pepper Center (OAIC)

4.2 calendar M

Statistical learning methods for incorporating multimodal imaging biomarkers to advance aging research

Role: PI

Publications for 2016

- a. **Chen H**, Zhao B, Porges EC, Cohen RA, Ebner NC. (2016). Edgewise and subgraph-level tests for brain networks. *Stat Med*. 2016 Nov 30;35(27):4994-5008. doi: 10.1002/sim.7039. PMID: 27397632
- b. **Chen H**, Zhao B, Cao G, Proges EC, O'Shea A, Woods AJ, Cohen RA. (2016) Statistical Approaches for the Study of Cognitive and Brain Aging. *Front Aging Neurosci*. 2016 Jul 19;8:176. doi: 10.3389/fnagi.2016.00176. PMID: 27486400
- c. Sibille KT, Steingrimsdóttir ÓA, Fillingim RB, Stubhaug A, Schirmer H, **Chen H**, McEwen BS, Nielsen CS. (2016) Investigating the Burden of Chronic Pain: An Inflammatory and Metabolic Composite. *Pain Res Manag*. 2016;2016:7657329. doi: 10.1155/2016/7657329. PMID: 27445627
- d. Lyon DE, Cohen R, **Chen H**, Kelly DL, Starkweather A, Ahn HC, Jackson-Cook CK. (2016) The relationship of cognitive performance to concurrent symptoms, cancer- and cancer-treatment-related variables in women with early-stage breast cancer: a 2-year longitudinal study. *J Cancer Res Clin Oncol*. 2016 Jul;142(7):1461-74. doi: 10.1007/s00432-016-2163-y. PMID: 27102492
- e. Ebner NC, **Chen H**, Porges E, Lin T, Fischer H, Feifel D, Cohen RA. (2016) Oxytocin's effect on resting-state functional connectivity varies by age and sex. *Psychoneuroendocrinology*. 2016 Jul;69:50-9. doi: 10.1016/j.psyneuen.2016.03.013. PMID: 27032063

- f. Seider TR, Gongvatana A, Woods AJ, **Chen H**, Porges EC, Cummings T, Correia S, Tashima K, Cohen RA. (2016) Age exacerbates HIV-associated white matter abnormalities. *J Neurovirol.* 2016 Apr;22(2):201-12. doi: 10.1007/s13365-0150386-3. **PMID: 26446690**
- g. Lyon DE, Cohen R, **Chen H**, Kelly DL, McCain NL, Starkweather A, Ahn H, Sturgill J, Jackson-Cook CK. (2016) Relationship of systemic cytokine concentrations to cognitive function over two years in women with early stage breast cancer. *J Neuroimmunol.* 2016 Dec 15;301:74-82. doi: 10.1016/j.jneuroim.2016.11.002. **PMID: 27890459**

Presentations for 2016

- a. **Chen, H.** Age-related multimodal neuroimaging biomarker changes. Institute on Aging, University of Florida. April, 2016.
- b. **Chen, H.,** Zeng, D., Wang, Y. Penalized Nonlinear Mixed Effects Model for Identifying Disease Progression Biomarkers. Department of Biostatistics and Epidemiology, University of Pennsylvania. March, 2016.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Cohen, Ronald

eRA COMMONS USER NAME rcohen1

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Tulane University	BS	05/1976	Psychology
Louisiana State University	PHD	12/1982	Clinical Psychology, Neuropsychology
UCLA Neuropsychiatric Institute, Westwood, CA	Resident	07/1982	Clinical Psychology Internship
University of Florida, Gainesville, FL	Postdoctoral Fellow	09/1983	Neuropsychology

A. Personal Statement

Dr. Cohen is director of the UF center for Cognitive Aging and Memory-Clinical Translational Program. He is a professor of Clinical and Health Psychology with joint appointments in the departments of Neurology, Psychiatry, and Aging in the College of Medicine. Dr. Cohen also is the Evelyn McKnight Chair for Cognitive and Memory at UF. The CAM-CTRP is a multidisciplinary research program focused on factors that influence cognitive aging that will integrate neurocognitive, neuroimaging, and laboratory biomarker methods. A primary goal of this center is clinical translational in nature with a focus on translating neuroscience findings from the laboratory to clinical application for both improvement assessment and intervention. He has extensive background in neuroimaging and the neuroscience of attention-executive functions, and strong record of research involving the use of functional and structural neuroimaging methods in studies of age-associated brain disorders and neurodegenerative brain disorders. He has published over 250 peer-reviewed articles, and numerous book chapters on topics of relevance to this project. Besides co-editing several books on topics related to areas of clinical neuropsychological research, Dr. Cohen authored "Neuropsychology of Attention" in 1993 which was the first book on this topic in the field, which was recently updated and published as a second edition this year. He authored a book "Brain Imaging in Behavioral Medicine and Clinical Neuroscience", which will be the first to address the use of neuroimaging methods for studying various problems in clinical neuroscience and to lead the current project. Specifically, Dr. Cohen's CAM-CTRP laboratory has been conducting human studies employing multimodal neuroimaging in conjunction with MRS to examine pathophysiological changes occurring in normal and pathological brain aging, and also secondary to risk factors including obesity, diabetes, heart disease, viral infections (e.g., HIV), and neurodegenerative disease such as AD. He has assembled an outstanding team of researchers with specific areas of expertise that will enable the success of the CAM-CTRP.

B. Positions and Honors

Positions and Employment

- 1983 - 1990 Assistant Professor, Department of Neurology, University of Massachusetts Medical School
- 1990 - 1993 Associate Professor, Department of Neurology, University of Massachusetts Medical School
- 1993 - 1996 Assistant Professor, Department of Psychiatry-Human Behavior, Brown University
- 1993 - 2008 Director of Neuropsychology, The Miriam Hospital, Warren Alpert School of Medicine, Brown University
- 2004 - 2012 Professor, Department of Psychiatry-Human Behavior, Brown University
- 2004 - 2012 Professor, Brain Sciences Program, Brown University
- 2012 - Professor, Departments of Aging, Neurology and Psychiatry, University of Florida
- 2012 - Director, Center for Cognitive Aging and Memory, University of Florida

Other Experience and Professional Memberships

- 1983 - Member, International Neuropsychological Society

Honors

- 2012 Endowment in Support of the Center for Cognitive Aging and Memory, McKnight Brain Research Foundation
- 2015 Evelyn McKnight Chair, Cognitive Aging and Memory

C. Contribution to Science

1. My research was an outgrowth of interest and expertise in neuropsychology and cognitive neuroscience. My early research focused on attentional influences on cognitive functions, including studies of the effects of particular neurological brain disorders and psychiatric disturbances on effort and attentional control. This led to a number of publications focusing on the cingulate cortex, intentional behavior and also emotional processing, with much of this work culminating in the publication of his book "*Neuropsychology of Attention*." These studies present major contributions to neuropsychology and cognitive neuroscience. A few examples of these studies are listed above.

My early clinical research focused on neurodegenerative disease in the elderly (AD). This evolved into investigations focusing on vascular dementia, as shown in a sample of my publications below, which employed neuroimaging methods to examine white matter abnormalities (FLAIR), cortical and subcortical morphometry, and functional imaging.

- a. **Cohen RA**, O'Donnell BF, Meadows ME, Moonis M, Stone WF, Drachman DA. ERP indices and neuropsychological performance as predictors of functional outcome in dementia. *J Geriatr Psychiatry Neurol*. 1995 Oct;8(4):217-25. **PubMed PMID: 8561835**.
 - b. **Cohen RA**, Paul RH, Zawacki TM, Sethi M, Ott BR, Moser DJ, Stone W, Noto R, Gordon N. Single photon emission computed tomography, magnetic resonance imaging hyperintensity, and cognitive impairments in patients with vascular dementia. *J Neuroimaging*. 2001 Jul;11(3):253-60. **PubMed PMID: 11462291**.
 - c. **Cohen RA**, Paul RH, Ott BR, Moser DJ, Zawacki TM, Stone W, Gordon N. The relationship of subcortical MRI hyperintensities and brain volume to cognitive function in vascular dementia. *J Int Neuropsychol Soc*. 2002 Sep;8(6):743-52. **PubMed PMID: 12240738**.
 - d. Sweet LH, Paul RH, **Cohen RA**, Moser D, Ott BR, Gordon N, Browndyke JN, Shah P, Garrett KD. Neuroimaging correlates of dementia rating scale performance at baseline and 12-month follow-up among patients with vascular dementia. *J Geriatr Psychiatry Neurol*. 2003 Dec;16(4):240-4. **PubMed PMID: 14653434**.
2. As my work on VaD progressed, it became clear that it was necessary to examine patients with vascular disease and risk factors before they developed dementia. This led to R01 funded studies focusing on cognitive and neuroimaging abnormalities associated with cardiovascular disease, including heart failure. This work incorporated systemic vascular indices in conjunction with structural and functional measures. We also began to examine vessel and blood-barrier disturbances that might link vascular factors with AD (Stopa et al.). To address these questions my research began to employ other neuroimaging methods, including ASL to assess CBF disturbances in relationship to fMRI alterations in HF and vascular cognitive impairment. My laboratory made significant contributions to characterizing the interaction between systolic problems linked to cardiac output and microvascular disease in the brain causing hemodynamic dysregulation and vulnerability to neuronal and white matter injury.
 - a. Haley AP, Sweet LH, Gunstad J, Forman DE, Poppas A, Paul RH, Tate DF, **Cohen RA**. Verbal working memory and atherosclerosis in patients with cardiovascular disease: an fMRI study. *J Neuroimaging*. 2007 Jul;17(3):227-33. **PubMed PMID: 17608908**.
 - b. Jefferson AL, Tate DF, Poppas A, Brickman AM, Paul RH, Gunstad J, **Cohen RA**. Lower cardiac output is associated with greater white matter hyperintensities in older adults with cardiovascular disease. *J Am Geriatr Soc*. 2007 Jul;55(7):1044-8. **PubMed PMID: 17608877; PubMed Central PMCID: PMC2721459**.
 - c. Stopa EG, Butala P, Salloway S, Johanson CE, Gonzalez L, Tavares R, Hovanesian V, Hulette CM, Vitek MP, **Cohen RA**. Cerebral cortical arteriolar angiopathy, vascular beta-amyloid, smooth muscle actin, Braak stage, and APOE genotype. *Stroke*. 2008 Mar;39(3):814-21. **PubMed PMID: 18258839**.
 - d. **Cohen RA**, Poppas A, Forman DE, Hoth KF, Haley AP, Gunstad J, Jefferson AL, Tate DF, Paul RH, Sweet LH, Ono M, Jerskey BA, Gerhard-Herman M. Vascular and cognitive functions associated with cardiovascular disease in the elderly. *J Clin Exp Neuropsychol*. 2009 Jan;31(1):96-110. **PubMed PMID: 18608677; PubMed Central PMCID: PMC2739675**.
 3. My research on vascular and metabolic factors affecting the aging brain led to R01 funding focusing on HIV. I was a co-PI of HIV Neuroimaging Initiative to investigate longitudinal changes in brain function, structure and cerebral metabolite abnormalities. This work employed MRS, DTI, and more recently fMRI. Subsequent R01 grants awarded to me examined HIV and aging, and HIV in the context of alcohol and other drug use. Neuroimaging methods continue to play a major role in this area of my research, with current funded projects employing fMRI to examine functional connectivity in relationship to white matter connectivity and regional cerebral metabolite disturbance.
 - a. Paul RH, Ernst T, Brickman AM, Yiannoutsos CT, Tate DF, **Cohen RA**, Navia BA. Relative sensitivity of magnetic resonance spectroscopy and quantitative magnetic resonance imaging to cognitive function among nondemented individuals infected with HIV. *J Int Neuropsychol Soc*. 2008 Sep;14(5):725-33. **PubMed PMID: 18764968**.

- b. Bunea F, She Y, Ombao H, Gongvatana A, Devlin K, **Cohen RA**. Penalized least squares regression methods and applications to neuroimaging. *Neuroimage*. 2011 Apr 15;55(4):1519-27. **PubMed PMID: 21167288**.
 - c. Gongvatana A, Harezlak J, Buchthal S, Daar E, Schifitto G, Campbell T, Taylor M, Singer E, Algers J, Zhong J, Brown M, McMahan D, So YT, Mi D, Heaton R, Robertson K, Yiannoutsos C, **Cohen RA**, Navia B. Progressive cerebral injury in the setting of chronic HIV infection and antiretroviral therapy. *J Neurovirol*. 2013 Jun;19(3):209-18. **PubMed PMID: 23613008; PubMed Central PMCID: PMC3740160**.
 - d. Caldwell JZ, Gongvatana A, Navia BA, Sweet LH, Tashima K, Ding M, **Cohen RA**. Neural dysregulation during a working memory task in human immunodeficiency virus-seropositive and hepatitis C coinfecting individuals. *J Neurovirol*. 2014 Aug;20(4):398-411. **PubMed PMID: 24867610; PubMed Central PMCID: PMC4351737**.
4. In addition, to these specific areas of clinical focus, my laboratory continues to conduct studies that address more basic cognitive and behavioral neuroscience questions using neuroimaging as a core component. Some examples are listed below. Studies with Wing, McCaffery, Sweet and me focused on the role of brain reward and inhibitory control systems in obesity. This related to other work on obesity and metabolic effects on the brain and recent R01 funding to use neuroimaging to study bariatric surgery and weight loss effects on the brain. We continue to also conduct studies to better understand the neural bases of functional neuroimaging responses, including the temporal dynamics of the BOLD response of specific tasks (e.g., Paskavitz et al). I also continue to conduct studies that examine older adults with and without evidence of cognitive decline. For example, Ott et al. showed the relationship between ventricular volume increases and CSF biomarkers in AD, MCI and healthy controls. This represents a small sample of the areas of research that my center continues to explore.
- a. McCaffery JM, Haley AP, Sweet LH, Phelan S, Raynor HA, Del Parigi A, **Cohen RA**, Wing RR. Differential functional magnetic resonance imaging response to food pictures in successful weight-loss maintainers relative to normal-weight and obese controls. *Am J Clin Nutr*. 2009 Oct;90(4):928-34. **PubMed PMID: 19675107; PubMed Central PMCID: PMC2744621**.
 - b. Ott BR, **Cohen RA**, Gongvatana A, Okonkwo OC, Johanson CE, Stopa EG, Donahue JE, Silverberg GD, Alzheimer's Disease Neuroimaging Initiative. Brain ventricular volume and cerebrospinal fluid biomarkers of Alzheimer's disease. *J Alzheimers Dis*. 2010;20(2):647-57. **PubMed PMID: 20182051; PubMed Central PMCID: PMC3078034**.
 - c. Paskavitz JF, Sweet LH, Wellen J, Helmer KG, Rao SM, **Cohen RA**. Recruitment and stabilization of brain activation within a working memory task; an FMRI study. *Brain Imaging Behav*. 2010 Mar;4(1):5-21. **PubMed PMID: 20503110**.
 - d. Daiello LA, Gongvatana A, Dunsiger S, **Cohen RA**, Ott BR. Association of fish oil supplement use with preservation of brain volume and cognitive function. *Alzheimers Dement*. 2015 Feb;11(2):226-35. **PubMed PMID: 24954371**.
5. A major emphasis on my work over the past decade has been clinical translational research focused at factors that affect the brain and cognition in the context of normal aging. We have been conducting studies within the the CAM-CTRP of the UF Institute on Aging directed at the influence of systemic and neuroinflammation, endocrine changes, and other factors occurring with aging that may accelerate cognitive decline as people reach advanced age.
- a. Woods AJ, **Cohen RA**, Pahor M. Cognitive frailty: frontiers and challenges. *J Nutr Health Aging*. 2013 Sep;17(9):741-3. **PubMed PMID: 24154645; PubMed Central PMCID: PMC4471842**.
 - b. Szabo AJ, Alosco ML, Miller LA, McGeary JE, Poppas A, **Cohen RA**, Gunstad J. Brain-derived neurotrophic factor Val66Met polymorphism and cognitive function in persons with cardiovascular disease. *Psychogeriatrics*. 2013 Dec;13(4):206-12. **PubMed PMID: 24289461; PubMed Central PMCID: PMC3847660**.
 - c. **Cohen RA**, Seider TR, Navia B. HIV effects on age-associated neurocognitive dysfunction: premature cognitive aging or neurodegenerative disease? *Alzheimers Res Ther*. 2015;7(1):37. **PubMed PMID: 25848401; PubMed Central PMCID: PMC4386102**.
 - d. Hawkins MA, Alosco ML, Spitznagel MB, Strain G, Devlin M, **Cohen RA**, Crosby RD, Mitchell JE, Gunstad J. The Association Between Reduced Inflammation and Cognitive Gains After Bariatric Surgery. *Psychosom Med*. 2015 Jul-Aug;77(6):688-96. **PubMed PMID: 25478707; PubMed Central PMCID: PMC4456339**.

D. Research Support

Ongoing Research Support

1R01DK09933401A1 (Ronald Cohen, PI)

09/30/14-8/30/19

NIDDK

"Obesity and Type-2 Diabetes: Bariatric Surgery Effects on Brain Function"

The study will delineate mechanism underlying the effects of chronic obesity on brain functioning and determine if cognitive benefits of bariatric surgery and weight loss contribute to enhanced cerebral metabolic or hemodynamic function assessed using multimodal neuroimaging methods. **35% effort**

2 P01 AA019072 (Monti)

9/1/15-5/31/20

NIAAA

\$893,352

Alcohol and HIV: Biobehavioral Interactions and Intervention

The goals of this program project are to study the effects of alcohol use on HIV disease progression, the effects of interventions to reduce alcohol use in HIV-infected populations, and the effects of alcohol on sexual decision making. The project also fosters multidisciplinary collaborations and training in research on alcohol and HIV and dissemination of research findings to clinicians treating addictions and HIV. Research Component 1 (Cohen, PI) is a continuation of the study being conducted in the parent ARCH, but will now examine the effects of reducing alcohol consumption via a motivational interviewing approach in HIV-infected heavy drinkers, with a specific focus on changes in cognitive performance, functional brain response on fMRI, and cerebral metabolite abnormalities (MRS).

Role: **Co-1; Research Component-1: PI (20% effort)**

U24 AA022002 Cook (PI)

09/01/13-08/31/16

NIAAA

Southern HIV Alcohol Research Consortium (SHARC) Admin and Research Support Cure

The objective of this proposal is to establish the administrative structure and to provide research support for the SHARC, a collaboration that links several universities and investigators with a common goal of supporting new research related to HIV and alcohol consumption.

Role: **Co-I**

P01 AA019072 Monti (PI) 09/30/10 - 08/31/16

NIAAA

Alcohol and HIV: Biobehavioral Interactions and Intervention (ARCH)

One of two primary R01 projects in the Brown University HIV-Alcohol center grant, this study focuses on the interactive effects of HIV and alcohol use on metabolic-vascular disturbances underlying brain dysfunction. We are longitudinally assessing HIV infected and seronegative controls who are stratified by alcohol use into three groups (heavy, moderate, none use) over a 36-month period. The study examines MRI-based neuroimaging biomarkers of brain dysfunction including diffusion tensor imaging (DTI), morphometry, and magnetic resonance spectroscopy (MRS) to measure cerebral metabolite abnormalities. Dr. Cohen is the principal investigator of the R01 type project (RC1) overseeing all aspects of the study.

Role: **Co-Investigator; PI: Research Component 1**

P01 AA019072 Monti (PI) 08/30/10 - 08/31/16

NIAAA

Alcohol and HIV: Biobehavioral Interactions and Intervention

One of two primary R01 projects in the Brown University HIV-Alcohol center grant, this study focuses on the interactive effects of HIV and alcohol use on metabolic-vascular disturbances underlying brain dysfunction. We are longitudinally assessing HIV infected and seronegative controls who are stratified by alcohol use into three groups (heavy, moderate, none use) over a 36-month period. The study examines MRI-based neuroimaging biomarkers of brain dysfunction including diffusion tensor imaging (DTI), morphometry, and magnetic resonance spectroscopy (MRS) to measure cerebral metabolite abnormalities. Dr. Cohen is the principal investigator of the R01 type project (RC1) overseeing all aspects of the study.

Role: **Co-Investigator; PI: Research Component 1**

R01 NS080655 Thompson (PI) 8/1/2012-7/31/2016

NINDS

Predicting Brain Changes in HIV/AIDS

This project greatly advances the ability to map, and predict, brain changes in people living with HIV/AIDS. HIV/AIDS is perhaps the greatest threat to public health worldwide in the 21st century. 40 million people are HIV-infected - a shocking 1 out of every 100 people aged 18-45 - and 40% have some neurological or cognitive impairment. This work offers 3 immediate public health consequences: (1) new methods to predict whether a person with HIV/AIDS will show imminent brain decline; (2) enhancing basic neuroscience by identifying brain circuits disrupted by the virus, and (3) a clear method to boost power for clinical trials of drugs to treat the brain in the millions of people now living with HIV/AIDS.

Role: **Co-Investigator**

1U54EB020403-01 Thompson (PI)

9/29/14-9/30/18

ENIGMA: Center for Worldwide Medicine, Imaging and Genomics

The Enigma Center for Worldwide Medicine, Imaging and Genomics is an unprecedented global effort bringing together 287 scientists and all their vast biomedical datasets, to work on 9 major human brain diseases: schizophrenia, bipolar disorder, major depression, ADHD, OCD, autism, 22q deletion syndrome, HIV/AIDS and addictions. Enigma integrates images, genomes, connectomes and biomarkers on an unprecedented scale, with new kinds of computation for integration, clustering, and learning from complex biodata types. Responding to the BD2K RFA, ENIGMA'S Working Groups target key programmatic goals of BD2K funders across the NIH, including NIMH, NIBIB, NICHD, NIA, NINDS, NIDA, NIAAA, NHGRI and FIC. Enigma creates novel

computational algorithms and a new model for Consortium Science to revolutionize the way Big Data is handled, shared and optimized, creating new algorithms to handle Big Data from (1) Imaging Genomics, (2) Connectomics, and (3) Machine Learning & Clinical Prediction

Dr. Cohen is a co-I (10% effort) and director of HIV data initiative

Completed Research Support

R01 HL089311 Gunstad (PI) 09/15/08 - 05/31/12

NHLBI/Subcontract from Kent State

Cognitive Benefits of Cardiac Rehabilitation in Heart Failure

The main goal of this project will be to study CVD and its effects on the brain, and particularly how cardiac rehabilitation and the effects of vascular conditioning are influenced by the vascular CVD and systemic vascular disease factors.

Role: **PI of Subcontract**

R01 HL084178 Sweet (PI) 01/25/07 - 11/30/12

NHLBI/Subcontract from Butler Hospital

Hemodynamic and Cognitive Function in Cardiovascular Disease

This study aims at characterizing the relationship between cerebral hypoperfusion and abnormalities of BOLD on FMRI in association with working memory and attention performance among patients with heart failure.

Role: **PI of Subcontract**

R01 MH074368 Cohen (PI) 09/30/06 - 08/31/12

Age Effects on HIV-Associated Brain Dysfunction

The goal of this project was to achieve greater understanding of how HIV infection interacts with aging to cause brain abnormalities that affect neurocognitive functioning.

Dr. Cohen oversees this entire project.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Cruz-Almeida, Yenisel

eRA COMMONS USER NAME (credential, e.g., agency login): ycruzalmeida

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Florida, Gainesville, FL	BS	05/2001	Microbiology & Cell Science
University of Miami, Miami, FL	MSPH	08/2004	Epidemiology & Public Health
University of Miami, Miami, FL	PhD	12/2011	Neuroscience
University of Florida, Gainesville, FL	Postdoctoral Fellow	12/2012	Translational Pain Research

A. Personal Statement

My research interests are to elucidate the underlying nervous system mechanisms associated with increased clinical pain in older adults that impacts on cognitive and physical function. This area of interest stems from my previous research training in the fields of Neuroscience and Epidemiology. During my doctoral work, I studied brain metabolites associated with pain phenotype profiles in persons with spinal cord injury combining Magnetic Resonance Spectroscopy (MRS) and Quantitative Sensory Testing (QST). During my post-doctoral training, I became familiar with experimental methods to assess endogenous pain modulation, both behavioral inhibition and facilitation in older adults. In addition, my laboratory also studies the systemic inflammation commonly associated with pain and aging, which may be another potential mechanism of increased pain and reduced cognitive and physical function in older individuals. Currently, we have a manuscript under peer review highlighting the simultaneous contribution of pain to cognitive and physical decline:

Cruz-Almeida Y, Rosso AL, Marcum Z, Harris T, Satterfield S, Newman A, Yaffe K, Rosano C. Associations of musculoskeletal pain with mobility in older adults: potential cognitive mechanisms. *Journal of Gerontology: Medical Sciences*.

Cruz-Almeida Y, Aguirre M, Sorensen H, Wallet SM, Riley JL. Age-differences in salivary inflammatory biomarkers in response to experimental pain: Does venipuncture matter? *Journal of Pain*.

B. Positions and Honors

Positions and Employment

- 1997 - 1999 Clinical Research Coordinator, Florida Ophthalmic Institute, Gainesville, FL
- 1999 - 2001 Medical Laboratory Assistant, Shands Hospital, Gainesville, FL
- 2004 - 2006 Research Associate, University of Miami, Miami Project to Cure Paralysis, Miami, FL
- 2004 - 2006 Senior Research Associate, University of Miami, Miami Project to Cure Paralysis, Miami, FL
- 2006 - 2011 PhD Student, University of Miami, Neuroscience Graduate Program, Miami, FL
- 2011 - 2012 Post-doctoral Fellow, University of Florida, College of Dentistry, Gainesville, FL
- 2012 - 2014 Research Assistant Professor, University of Florida, College of Dentistry, Gainesville, FL
- 2014 - Assistant Professor, University of Florida, College of Medicine, Gainesville, FL

Other Experience and Professional Memberships

- 2003 - Member, American Pain Society
- 2004 - Member, International Association for the Study of Pain
- 2004 - 2012 Member, National Neurotrauma Society
- 2006 - Member, Society for Neuroscience
- 2009 - Ad-hoc Reviewer, Pain Medicine
- 2012 - Ad-hoc Reviewer, Journal of Pain
- 2013 - Member, Gerontological Society of America
- 2013 - Ad-hoc Reviewer, Clinical Journal of Pain
- 2013 - Editorial Board, Journal of Geriatrics & Palliative Care
- 2014 - Ad-hoc Reviewer, Experimental Gerontology

- 2014 - Chapter Faculty Advisor, Gamma Eta Sorority
- 2014 - 2018 Elected Chair, American Pain Society, Shared Interest Group: Measurement of Pain
- 2014 - 2016 Elected Member, American Pain Society, Early Career Forum Planning Committee
- 2015 - Executive Board Member, ElderCare of Alachua County UFHealth
- 2015 - Appointed Member, American Pain Society, Membership Committee
- 2015 - 2016 Appointed Member, American Pain Society, Early Career Advisory Group
- 2016 - 2018 Elected Chair, American Pain Society, Shared Interest Group: Geriatrics and Pain

Honors

- 1998 Leadership Award, University of Florida Hispanic Student Association
- 1999 Outstanding Student Award in Community Service, University of Florida
- 2004 Award for Academic Merit, University of Miami
- 2004 Young Investigator Travel Award, American Pain Society
- 2006 Lois Pope Life Fellowship, University of Miami Neuroscience Program
- 2006 Predoctoral Training Fellowship, NINDS/NIH
- 2006 Young Investigator Travel Award, American Pain Society
- 2007 Florida Graduate Academic Scholar Award, University of Miami
- 2008 Congress Scholarship, Congress of Spinal Cord Medicine and Rehabilitation
- 2010 Inductee, Alpha Epsilon Lambda Graduate Honor Society
- 2010 RR&D Predoctoral Fellowship Award, Department of Veteran Affairs
- 2011 Top Student Competition Finalist, National Neurotrauma Symposium
- 2011 Young Investigator Best Clinical Poster Presentation Award, Miami VA Medical Center
- 2011 Young Investigator Travel Award, American Pain Society
- 2011 Margaret Whelan Graduate Student Scholarship Award, University of Miami Medical Faculty Association
- 2012 Postdoctoral Training Fellowship, NIDCR/NIH
- 2013 Affiliated Junior Pepper Scholar, University of Florida
- 2013 Young Investigator Travel Award, American Pain Society
- 2014 Junior Pepper Scholar, University of Florida
- 2014 Junior Cognitive Aging & Memory Scholar, University of Florida
- 2014 Scientific Annual Meeting Faculty, Tampa, Florida, American Pain Society
- 2015 Annual Meeting Faculty, Palm Springs California, American Pain Society
- 2015 Annual Meeting Best Poster Presentation Award, Washington, DC, OAIC
- 2015 Annual Meeting Faculty, Orlando Florida, Gerontological Society of America

C. Contribution to Science

1. My research during graduate school focused on chronic pain after spinal cord injury. My publications during my training revolved around measuring pain from a biopsychosocial perspective. Specifically, we characterized the psychosocial profiles associated with clinical pain after SCI both cross-sectionally as well as longitudinally. My work has also included the characterization of somatosensory function after SCI and for the first time, how these various phenotypes are associated with brain metabolites.
 - a. **Cruz-Almeida Y**, Martinez-Arizala A, Widerström-Noga EG. Chronicity of pain associated with spinal cord injury: A longitudinal analysis. *J Rehabil Res Dev*. 2005 Sep-Oct;42(5):585-94. **PubMed PMID: 16586184**.
 - b. **Cruz-Almeida Y**, Felix ER, Martinez-Arizala A, Widerström-Noga EG. Pain symptom profiles in persons with spinal cord injury. *Pain Med*. 2009 Oct;10(7):1246-59. **PubMed PMID: 19818035**.
 - c. **Cruz-Almeida Y**, Felix ER, Martinez-Arizala A, Widerström-Noga EG. Decreased spinothalamic and dorsal column medial lemniscus-mediated function is associated with neuropathic pain after spinal cord injury. *J Neurotrauma*. 2012 Nov 20;29(17):2706-15. **PubMed PMID: 22845918; PubMed Central PMCID: PMC3510448**.
 - d. Widerström-Noga E, **Cruz-Almeida Y**, Felix ER, Pattany PM. Somatosensory phenotype is associated with thalamic metabolites and pain intensity after spinal cord injury. *Pain*. 2015 Jan;156(1):166-74. **PubMed PMID: 25599312; PubMed Central PMCID: PMC4423177**.
2. Ongoing research interests have been related to the appropriate measurement of pain and its impact across various populations. The examination of the psychometric properties of various self-report measures as well as of experimental pain measures is required in order to perform meaningful translational research that can be translated into effective therapies.

- a. **Cruz-Almeida Y**, Alameda G, Widerström-Noga EG. Differentiation between pain-related interference and interference caused by the functional impairments of spinal cord injury. *Spinal Cord*. 2009 May;47(5):390-5. **PubMed PMID: 19030010**.
 - b. **Cruz-Almeida Y**, Riley JL 3rd, Fillingim RB. Experimental pain phenotype profiles in a racially and ethnically diverse sample of healthy adults. *Pain Med*. 2013 Nov;14(11):1708-18. **PubMed PMID: 23889771; PubMed Central PMCID: PMC3983947**.
 - c. **Cruz-Almeida Y**, Fillingim RB. Can quantitative sensory testing move us closer to mechanism-based pain management?. *Pain Med*. 2014 Jan;15(1):61-72. **PubMed PMID: 24010588; PubMed Central PMCID: PMC3947088**.
 - d. **Cruz-Almeida Y**, Naugle KM, Vierck CJ, Fillingim RB, Riley JL. Reliability of pain intensity clamping using response-dependent thermal stimulation in healthy volunteers. *BMC Neurosci*. 2015 Apr 18;16:21. **PubMed PMID: 25909597; PubMed Central PMCID: PMC4409722**.
3. In another line of research, we have investigated factors contributing to individual differences in pain related to knee osteoarthritis. In collaboration with other researchers, we have used sophisticated psychophysical protocols to investigate age-related changes in pain modulation profiles, which may contribute to increased clinical pain among older adults. We have also examined the extent to which demographic factors contribute to individual differences in pain responses, including their interactions with psychosocial variables.
- a. **Cruz-Almeida Y**, King CD, Goodin BR, Sibille KT, Glover TL, Riley JL, Sotolongo A, Herbert MS, Schmidt J, Fessler BJ, Redden DT, Staud R, Bradley LA, Fillingim RB. Psychological profiles and pain characteristics of older adults with knee osteoarthritis. *Arthritis Care Res (Hoboken)*. 2013 Nov;65(11):1786-94. **PubMed PMID: 23861288; PubMed Central PMCID: PMC3922880**.
 - b. Riley JL 3rd, **Cruz-Almeida Y**, Glover TL, King CD, Goodin BR, Sibille KT, Bartley EJ, Herbert MS, Sotolongo A, Fessler BJ, Redden DT, Staud R, Bradley LA, Fillingim RB. Age and race effects on pain sensitivity and modulation among middle-aged and older adults. *J Pain*. 2014 Mar;15(3):272-82. **PubMed PMID: 24239561; PubMed Central PMCID: PMC4005289**.
 - c. **Cruz-Almeida Y**, Sibille KT, Goodin BR, Petrov ME, Bartley EJ, Riley JL 3rd, King CD, Glover TL, Sotolongo A, Herbert MS, Schmidt JK, Fessler BJ, Staud R, Redden D, Bradley LA, Fillingim RB. Racial and ethnic differences in older adults with knee osteoarthritis. *Arthritis Rheumatol*. 2014 Jul;66(7):1800-10. **PubMed PMID: 24729357; PubMed Central PMCID: PMC4077911**.
 - d. Petrov ME, Goodin BR, **Cruz-Almeida Y**, King C, Glover TL, Bulls HW, Herbert M, Sibille KT, Bartley EJ, Fessler BJ, Sotolongo A, Staud R, Redden D, Fillingim RB, Bradley LA. Disrupted sleep is associated with altered pain processing by sex and ethnicity in knee osteoarthritis. *J Pain*. 2015 May;16(5):478-90. **PubMed PMID: 25725172; PubMed Central PMCID: PMC4424160**.
4. An additional line of research addresses the utility of somatosensory function and associations with age as well as immune and mobility function.
- a. Sorond FA, **Cruz-Almeida Y**, Clark DJ, Viswanathan A, Scherzer CR, De Jager P, Csiszar A, Laurienti PJ, Hausdorff JM, Chen WG, Ferrucci L, Rosano C, Studenski SA, Black SE, Lipsitz LA. Aging, the Central Nervous System, and Mobility in Older Adults: Neural Mechanisms of Mobility Impairment. *J Gerontol A Biol Sci Med Sci*. 2015 Dec;70(12):1526-32. **PubMed PMID: 26386013; PubMed Central PMCID: PMC4643615**.
 - b. **Cruz-Almeida Y**, Aguirre M, Sorenson HL, Tighe P, Wallet SM, Riley JL 3rd. Age differences in cytokine expression under conditions of health using experimental pain models. *Exp Gerontol*. 2015 Dec;72:150-6. **PubMed PMID: 26456458; PubMed Central PMCID: PMC4664177**.
 - c. **Cruz-Almeida Y**, Black ML, Christou EA, Clark DJ. Site-specific differences in the association between plantar tactile perception and mobility function in older adults. *Front Aging Neurosci*. 2014 Apr 11;6:68. **PubMed PMID: 24782765; PubMed Central PMCID: PMC3990110**.
 - d. **Cruz-Almeida Y**, King CD, Wallet SM, Riley JL 3rd. Immune biomarker response depends on choice of experimental pain stimulus in healthy adults: a preliminary study. *Pain Res Treat*. 2012;2012:538739. **PubMed PMID: 23213513; PubMed Central PMCID: PMC3508574**.

Complete List of Published Work in My Bibliography:

<http://1.usa.gov/1mZlkpt>

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

K01AG048259, National Institute on Aging

Yenisel Cruz-Almeida (PI)

05/15/15-05/14/20

Neuroimaging age-related changes in pain modulation

The primary goal of this award is to provide the necessary training and mentoring for Dr. Cruz-Almeida to establish an independent neuroscience research program aimed at studying the neurobiological mechanisms underlying abnormal pain modulation in older adults that may account for increased clinical pain in this population.

Role: PI

R01AG039659, National Institute on Aging

Joseph L. Riley III (PI)

06/01/15-05/31/17

The effects of aging on experimental models of pain inhibition and facilitation

The overall aim of the research project is to characterize alterations in pain perception and endogenous pain modulation associated with aging.

Role: Co-Investigator

P30AG028740, National Institute on Aging

Yenisel Cruz-Almeida (PI)

04/01/15-03/31/17

Pain and mobility function in older adults

The overall aim of the present study is to examine the associations between pain-related brain network reorganization and complex walking function in older persons with musculoskeletal pain.

Role: PI

Completed Research Support

UL1TR000064, University of Florida CTSI

Yenisel Cruz-Almeida (PI)

06/01/14-05/31/16

Cortico-striatal connectivity in predicting clinical OA-related pain

The overall aim of the research project is to obtain pilot data to characterize cortico-striatal connectivity in older adults with and without widespread pain.

Role: PI

P30AG028740-07, National Institute on Aging

Thomas Buford (PI)

11/01/14-05/31/16

Development of clinical methods to evaluate neural function in aging

This research development project aims to develop and refine experimental methodology as well as to obtain preliminary data to support future grant proposals.

Role: Co-Investigator

UL1TR000064, University of Florida CTSI

Joseph L. Riley III (PI)

06/01/14-05/31/16

Neuro-immune mechanisms associated with pain modulation in older adults

The goal of this pilot study is to further understand the associations between neuro-immune plasma biomarkers and their association with experimental pain modulation in older adults.

Role: Co-Investigator

R01AG039659-02S1, National Institute on Aging

Joseph L. Riley III (PI)

11/21/12-11/20/14

The effects of aging on experimental models of pain inhibition and facilitation

The overall aim of the research project is to characterize alterations in pain perception and endogenous pain modulation

associated with aging. The specific goals of the funded supplement are to provide the training and environment for preparing the candidate (Dr. Cruz-Almeida) to transition into the field of aging.

Role: CPI

ULTR000064, University of Florida CTSI

Yenisel Cruz-Almeida (PI)

06/01/12-12/31/13

Saliva as an alternative to plasma to measure biomarkers associated with pain mechanisms

The goal of this pilot study is to delineate and quantify the standard basal and pain-evoked changes in saliva concentrations of relevant biomarkers and validate their analysis with plasma samples in the context of experimental pain stimulation across various age groups in a sample of healthy adults.

Role: PI

n/a, VA RR&D Office of Academic Affiliations

Yenisel Cruz-Almeida (PI)

09/01/10-08/31/11

Utility of thalamic metabolites and sensory testing in neuropathic pain conditions after spinal cord injury using Magnetic Resonance Spectroscopy and Quantitative Sensory Testing

The goal of the study was to assess the diagnostic utility of thalamic biomarkers in predicting clinical pain phenotypes in persons with pain after spinal cord injury used for Dr.Cruz-Almeida's dissertation.

Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Ebner, Natalie C

eRA COMMONS USER NAME (agency login): NATALIE.EBNER

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Free University Berlin, Berlin	BA	04/1998	Psychology
Free University Berlin, Berlin	MA	03/2001	Psychology
Free University Berlin, Berlin	PHD	05/2005	Psychology

A. Personal Statement

I am an expert in experimental research on cognition and affect in adulthood using behavioral and neuroimaging techniques, in particular functional magnetic resonance imaging (fMRI). My body of work is documented in 39 publications. As a pre- and postdoctoral fellow at the Free University Berlin and the Max Planck Institute for Human Development, I have supervised behavioral research on emotion-cognition interactions across adulthood. As a postdoctoral fellow and later as Associate Research Scientist at Yale University and as faculty at University of Florida (UF), I expanded my research to examine neuropsychological changes associated with cognition-emotion interactions across adulthood using neuroimaging and eye tracking techniques as well as pharmacological interventions. Currently, in addition to my primary appointment in the Department of Psychology at UF, I hold a joint appointment as faculty in the Center for Cognitive Aging and Memory (CAM) in the Aging Department at the College of Medicine at UF. I am also affiliated with the McKnight Brain Institute (MBI) on campus. My work has been funded by the NIH-sponsored Scientific Research Network on Decision Neuroscience and Aging, the National Science Foundation, the MIT Lincoln Lab, the UF Pepper Center, and the UF Clinical and Translational Science Institute. I have received awards such as the Young Research Scientist Award from the German Psychological Association, the International Max Planck Research School on the Life Course Outstanding Alumni Award sponsored by the APA Board of Educational Affairs Award to Advance Interdisciplinary Education and Training in Psychology, and recently the UF Assistant Professor Excellence Award and a College of Liberal Arts and Sciences Educator of the Year Award.

Representative Publications

1. **Ebner, NC**, Chen, H, Porges, E, Lin, T, Fischer, H, Feifel, D, & Cohen, RA. Oxytocin's effect on resting-state functional connectivity varies by age and sex. *Psychoneuroendocrinology*, 2016 Apr, 69:50-59. **PubMed PMID: 27032063**.
2. **Ebner NC**, Johnson MR, Rieckmann A, Durbin KA, Johnson MK, Fischer H. Processing own-age vs. other-age faces: neuro-behavioral correlates and effects of emotion. *Neuroimage*. 2013 Sep; 78:363-71. **PubMed PMID: 23602923; PubMed Central PMCID: PMC3684564**.

B. Positions and Honors

Positions and Employment

- 2001 - 2005 Predoctoral Fellow, Free University Berlin & Max Planck Institute for Human Development, Berlin
- 2005 - 2007 Postdoctoral Fellow, Max Planck Institute for Human Development, Berlin
- 2007 - 2010 Postdoctoral Fellow, Yale University, Department of Psychology, New Haven, FL
- 2010 - 2011 Associate Research Scientist, Yale University, Department of Psychology, New Haven, CT
- 2011 - Assistant Professor, University of Florida, Department of Psychology, Gainesville, FL
- 2013 - Adjunct Faculty, at Cognitive Aging and Memory Clinical Translational Research Program; CAM-CTRP, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

- 2000 - 2011 Member, German Psychological Association
- 2003 - Member, Society for Personality and Social Psychology
- 2003 - 2009 Member, American Psychological Association
- 2008 - Member, Association for Psychological Science

- 2009 - 2009 Reviewer, Retirement Research Foundation Doctoral Dissertation Award in the Psychology of Aging (American Psychological Association)
- 2010 - Member, Society for Social Neuroscience
- 2012 - Member, Cognitive Neuroscience Society
- 2012 - Reviewer, Swiss National Fund
- 2012 - Early Career Reviewer (ECR), National Institute of Health, Center for Scientific Review (CSR)
- 2014 - Member, Society for Affective Science

Honors

- 2003 Student Research Award, American Psychological Association (Division 20)
- 2004 Graduate Student Poster Award, Society for Personality and Social Psychology
- 2006 Heinz-Heckhausen-Jungwissenschaftlerpreis (Young Research Scientist Award), German Psychological Association
- 2014 International Max Planck Research School on the Life Course (LIFE) Outstanding Alumni Award, APA Board of Educational Affairs Award to Advance Interdisciplinary Education and Training in Psychology
- 2015 Kavli Fellow National Academy of Sciences
- 2016 University of Florida Excellence Award – Assistant Professors
- 2016 University of Florida College of Liberal Arts and Sciences Educator of the Year Award

Publications in Peer-Reviewed Journals: 2016

Dark-Freudeman, A. R., **Ebner, N. C.**, & West, R. L. (in press). Psychosocial aspects of aging. In K. E. Light (Ed.), *Geriatric Rehabilitation*. F.A. Davis.

Chen, A., Brahma, P., Wu, D. O., **Ebner, N. C.**, Matthews, B., Crandall, J., Wei, X., Faloutsos, M., & Oliveira, D. Cross-layer personalization as a first class citizen for situation awareness and computer infrastructure security. Accepted to the *ACM New Security Paradigms Workshop* (NSPW 2016). September 26-29, 2016. C Lazy U Ranch Colorado, USA. Acceptance rate (46%).

Chen, H., Zhao, B., *Porges, E. C., Cohen, R. A., & **Ebner, N. C.** (in press). Edgewise and subgraph level tests for brain networks. *Statistics in Medicine*.

Ebner, N. C., *Frazier, I., & *Ellis, D. (in press). Visual search and attention test. In J. S. Kreutzer, J. DeLuca, & B. Caplan (Eds.), *Encyclopedia of Clinical Neuropsychology*. New York, London: Springer.

Ebner, N. C., *Gulliford, D., & *Yumusak, S. (in press). Saccadic eye movements. In J. S. Kreutzer, J. DeLuca, & B. Caplan (Eds.), *Encyclopedia of Clinical Neuropsychology*. New York, London: Springer.

Ebner, N. C., *Weir, D., & *Rainer, R. (in press). Eye tracking. In J. S. Kreutzer, J. DeLuca, & B. Caplan (Eds.), *Encyclopedia of Clinical Neuropsychology*. New York, London: Springer.

Rana, M., Varan, A. Q., Davoudi, A., Cohen, R. A., Sitaram, R., & **Ebner, N. C.** (2016). Real-time fMRI in neuroscience research and its use in studying the aging brain. [Research topic] *Frontiers in Aging Neuroscience*, 8(239), 1-16. DOI: 10.3389/fnagi.2016.00239

*Strickland-Hughes, C. M., West, R. L., *Smith, K., A., & **Ebner, N. C.** (in press). False feedback and beliefs influence name recall in younger and older adults. *Memory*.

Westberg, L., *Henningson, S., *Zettergren, A., *Svärd, J., *Hovey, D., *Lin, T., **Ebner, N. C.**, & Fischer, H. (in press). Variation in the oxytocin receptor gene is associated with face recognition and its neural correlates. *Frontiers in Behavioral Neuroscience*.

*Ziaei, M., Burianová, H. A., von Hippel, W., **Ebner, N. C.**, Phillips, L. H., & Henry, J. D. (in press). Age-related differences in the neural networks involved in gaze and emotional processing. *Neurobiology of Aging*.

*Ziaei, M., **Ebner, N. C.**, & Burianová, H. A. (in press). Functional brain networks involved in gaze and emotional processing. *European Journal of Neuroscience*.

Ebner, N. C., Bailey, P. E., *Horta, M., *Joiner, J., & Chang, S. W. C. (2016). Multidisciplinary perspective on prosociality in aging. In J. A. Sommerville & J. Decety (Eds.), *Social Cognition: Developmental across the life span*, *Frontiers in Developmental Science Series*. (pp. 185–202). Psychology Press: Taylor and Francis Group.

*Szymkowicz, S. M., Persson, J., *Lin, T., Fischer, H., & **Ebner, N. C.** (2016). Hippocampal brain volume is associated with faster facial emotion identification in older adults: Preliminary results. *Frontiers in Aging Neuroscience*. DOI: 10.3389/fnagi.2016.00203

*Tasdemir-Ozdes, A., *Strickland-Hughes, C. M., Bluck, B., & Ebner, N. C. (2016). Future perspective and healthy lifestyle choices in adulthood. *Psychology and Aging*. Advance online

publication. <http://dx.doi.org/10.1037/pag0000089>

Ebner, N. C., Chen, H., *Porges, E., *Lin, T., Fischer, H., Feifel, D., & Cohen, R. A. (2016). Oxytocin's effect on resting-state functional connectivity varies by age and sex. *Psychoneuroendocrinology*, 69, 50-59. DOI: 10.1016/j.psyneuen.2016.03.013

D. Research Support

Ongoing Research Support

2015/09/01-2019/08/31

National Science Foundation SaTC Medium 1513572

Ebner, Natalie C. (MPI)

Developer Crowdsourcing: Capturing, Understanding, and Addressing Security-related Blind Spots in APIs

The goal of this project is to determine blind spots in programmer's attention when writing code.

2015/10/01-2017/09/30

Massachusetts Institute of Technology, Lincoln Laboratory & US Air Force 7000341318

Ebner, Natalie C. (MPI)

Enhanced Operating System Level User Profile Extraction

The goal of this project is to determine computer user profiles and to develop a tool to alert unusual use.

2013/01/01-2017/12/31

Swedish Research Council

Ebner, Natalie C. (MPI)

Effects of Oxytocin on Physical and Cognitive Functioning in the Elders

The goal of this project is to examine acute effects of intranasal oxytocin administration on cognition and social functioning in aging.

2013/10/01-2017/09/01

1R01AA022456-01, NIH/NIAAA

Nixon, Sara Jo (PI)

Neurobehavioral and Emotional Deficits in Male and Female Alcoholics

The goal of this project is to examine gender differences in deficits in cognitive and emotional functioning in alcoholics.

Role: Co-Investigator

2014/09/01-2017/08/31

SaTC EAGERs NSF 13-037, National Science Foundation

Ebner, Natalie C (MPI)

Age-Targeted Automated Security Cueing Against Web-Based Social Engineering Attacks

The goal of this project is to develop and validate an open-source browser extension that provides visual security cues in an age-targeted fashion to protect older adults from web-based social engineering attacks during their everyday internet use.

2013/08/01-2017/03/31

P30AG028740, University of Florida Center for Cognitive Aging and Memory & Claude D. Pepper Older Americans Independence Center (sponsor: NIH/NIA)

Ebner, Natalie C. (PI)

Effects of Oxytocin on Physical and Cognitive Functioning in the Elders

The goal of clinical trial is to examine the effects of intranasal oxytocin administration on cognition, health, and socioemotional functioning in aging over time.

Completed Research Support

2014/09/01-2015/03/31

Scientific Research Network on Decision Neuroscience and Aging (SRNDNA; sponsored by NIH/NIA)

Ebner, Natalie C. (MPI)

The Role of Oxytocin in Prosocial Decision Making in Aging Across Humans and Monkeys

The goal of this project is to compare the effects of the neuropeptide oxytocin on social preferences and altruism in young and older primates and humans.

2013/01/01-2013/12/31

UL1 TR000064, University of Florida Clinical and Translational Science Institute (CTSI) Pilot Project Award (sponsor: NIH/NCATS Clinical and Translational Science Award to the University of Florida)

Ebner, Natalie C. (PI)

Neuro-behavioral effects of oxytocin on decisions of trust in aging

The goal of this project was to determine neuroendocrine and socio-behavioral effects of oxytocin on decisions of trust in aging.

2011/08/15-2013/06/01

N/A, Department of Psychology 2011 Michael L. & Judith D. Woodruff Research Competition Grant

Ebner, Natalie C. (PI)

Neural mechanisms of social memory in young and older adults

The goal of this project was to determine the neural correlates for older adults' increased schema reliance and to examine whether self-relevance of information counteracts memory biases arising from schemas.

2007/07/01-2010/06/30

DFG EB 436/1-1, German Science Foundation

Ebner, Natalie C. (PI)

Motivational orientation in adulthood

The goal of this project was to assess behavioral and neural correlates of age-related differences in processing motivationally and socially relevant information.

Robert A. Fieo, PhD

BIOGRAPHICAL SKETCH

NAME: Robert A Fieo, PhD

eRA COMMONS USER NAME

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Drexel University, Philadelphia, PA	Bachelor of Science	06/1995	Psychology
University of Edinburgh, Scotland, UK	Doctor of Philosophy	06/2011	Psychology
University of Copenhagen, Denmark	Post-Doctoral	12/2012	Cognitive Aging
Columbia University, New York, NY	Post-Doctoral Fellow	10/2014	Cognitive Aging

RESEARCH INTERESTS:

Functional Impairment, Cognitive Aging, Enrichment Activities, Neural Compensation

RESEARCH METHODS:

Psychometrics, epidemiology and relative risk

A. Personal Statement

During my PhD within the Center of Cognitive Epidemiology I received rigorous training in statistical models, which included item response theory methodology that I applied to health outcomes research, e.g., functional status.

During my post doctoral fellowship at Columbia University in the Department of Neurology I was tasked with examining the construct of cognitive reserve (CR), and the well-studied CR proxy of cognitive enrichment activities. One product of this investigation was grouping together cognitive enrichment activities (i.e., advanced or complex ADLs) and examining how they relate to total functional status. During my postdoctoral training I constructed and validated (included cognitive associations) an instrument, Columbia IADL-extended (IADL-x; Fieo et al, 2013), intended to quantify functional status as a latent trait for the purpose of extending the range of standard instrumental activities of daily living (IADL) into the preclinical range of dementia.

In September of 2015, I was offered a junior faculty position as a Research Assistant Professor at the University of Florida at the Institute on Aging. The impetus for accepting this position directly relates to the expressed desire to translate epidemiological findings into clinical practice. This aim coincides with the larger agenda of the UF Clinical & Translational Science Institute. My current target population is vascular dementia, within the discipline of neuroepidemiology. Key variables under investigation include hypertension, diabetes, and functional impairment. Methodological types would include etiology, distribution, management, and reducing the impact of the disease.

B. Positions and Honors

Positions and Employment

2011	Post doctorate University of Copenhagen, School of Social Medicine
2012	Post doctorate fellow, Columbia University, Department of Neurology
2014	Associate Research Scientist, Columbia University, Mailman Scholl of Public Health
2016	Assistant Professor, University of Florida, College of Medicine
2009	United Kingdom Medical Research Council Scholarship Award
2011	National Institute of Health (NIH), Ruth L. Kirschstein National Research Service Award (NRSA), Institutional Research Training Grant (T32), and Columbia University, Taub Institute for Research on Alzheimer's disease and the Aging Brain, New York, NY. For my dissertation on 'Determinants of functional decline in community-dwelling older
2012	Award to participate in the NIA funded 2012 Advanced Psychometrics Methods in Cognitive Aging workshop
2014 -	Current Collaboration with NIH PROMIS (Patient reported outcomes measurement information system)

C. Contributions to Science

Contribution 1: During my postdoctoral study at the University of Copenhagen, I was working with Prof. Kirsten Avlund, an internationally recognized researcher for her work on fatigue in older adults. At this time there was a call from the Gerontological Society of America to enhance the measurement of subjective fatiguability. In response I developed a mobility fatigue scale; this was an early incarnation of the construct of fatiguability. The metric was published in the *Journal of the American Geriatric Society*^a. The scale was highly predictive of disability and mortality over a five-year period.

- a. **Fieo R**, Mortensen EL, Lund R, Avlund K. Examining the construct validity of the Multidimensional Fatigue Inventory (MFI-20): Moving beyond classical measures of dimensionality. *Assessment*. 2014; 21(6):706-12

Contribution 2: With particular relevance to my current research goals for this proposal, I acted as a consultant to Cornell University Rehabilitative Medicine. This project incorporated methods associated with dynamic performance analyses and IRT. This is relevant to the current proposal in that I am seeking to couple dynamic performance analyses and IRT to derive subtask for enrichment activities. This was also significant for my scientific growth in that it was the first time I employed Rash scaling, this a model that was developed in Europe and is gradually being employed in the U.S. with greater frequency. This new methodology is also relevant to his proposal in that acquiring this new skill I can perform IRT in the small sample size that is present in the intervention. This manuscript was published in 2013^b.

- b. O'Dell MW, Kim G, Rivera L, **Fieo R**, Christos P, Polistena C, Fitzgerald K, Gorga D. A psychometric evaluation of the Arm Motor Ability Test. *Journal of Rehabilitation Medicine*. 2013; 45(6):519-27

Contribution 3: In 2012, I was received a funding award to participate in the NIA 2012 Advanced Psychometric Methods in Cognitive Aging workshop at University of Washington, Friday Harbor. There the task was to employ Item Response Theory to detect item bias in the most widely used depression inventories in samples of clinical cognitive impairment (i.e., CES-D and GDS). Here we showed that there was evidence of MCI and dementia subjects interpreting and reporting the items differently than normal subjects. This could be considered particularly important given that the questionnaires often have cut points such that item bias could skew diagnosis. This work was published in the *International Journal of Geriatric Psychiatry* in 2015^c.

- c. **Fieo R**, Mukherjee S, Dmitrieva NO, Fyffe D, Gross A, Sanders RE, Potter GG, Manly JJ, Romero HR, Mungas D, Gibbons LE. Item bias due to cognitive status does not impact depression symptom measures in four heterogeneous samples of older adults. *Int J Geriatr Psychiatry*. 2015 Sep;30(9):911-8.

Contribution 4: I am including a manuscript that it is under revision because it is an indication that I can publish in high impact factor journals (i.e., *Neurology*). In this manuscript we set out to establish functional change in the presymptomatic range of functional status. This is particularly relevant to my current project in that I am attempting to describe subtle IADL impairment in subjects with vascular dementia. An important observation from this work was that baseline functional status predicted (using growth modelling) (change) in speed of processing. Perhaps more noteworthy is that we showed that change in function for those developing dementia was present in the presymptomatic phase^d.

- d. **Fieo R**, Manly J, Zahodne L, Stern Y. A continuum from cognitive leisure activities to IADL. Evidence for functional change in the pre-clinical range of dementia. *Neurology*. (resubmit stage).

Contribution 5: My first manuscript published at the University of Florida was entitled "*Cognitive engaging activity is associated with greater cortical and subcortical volumes*"^e. This has scientific merit that in that to date very few studies have shown brain volume associated with enrichment activities. I plan to follow-up this manuscript with a more targeted examination of specific regions of Interest i.e., hippocampal complex. In this further study we will describe the quality of specific enrichment activities

in terms of the strength of the relationship with regions of interest. Once these high quality items have been established (high discriminatory power), I will investigate their relationship with neocortical regions associated with neural compensation.

- e. Seider TR, **Fieo RA**, O'Shea A, Porges E, Woods AJ, Cohen RA. Cognitively Engaging Activity Is Associated with Greater Cortical and Subcortical Volumes. *Frontiers in Aging Neuroscience*. 5/2/2016, p1-10. 10p.

D. Research Support

January 1, 2016 to October 31, 2016 100% funded by the Cognitive Aging and Memory Program.
Commencing on November 1, 2016

- 1) University of Florida, Institute on Aging, **Fieo, role PI (75% effort)**.
- 2) 25% effort supported by University of Florida Department of Health Outcomes

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME:

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE:

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Arizona, Tucson AZ	BS	1981	Psychology
Bowman Gray, School of Medicine, W-S, NC	PhD	1987	Physio/Pharm
University of Colorado, Boulder CO	Postdoctoral	1991	Neurophysiology and behavior

A. Personal Statement

My research focuses on understanding the relationship of brain aging and age-related cognitive decline and neurodegenerative disease of aging. My long-term goal is the amelioration of memory deficits associated with aging. My research program utilizes a combination of behavioral characterization with biochemical, molecular, and electrophysiological techniques and treatments (behavioral, pharmacological and viral) to obtain a vertically integrated perspective on neural aging, from the molecular to the cognitive level. I have been continuously funded through NIH as a principle investigator since 1992 and my work includes over 100 publications on memory mechanisms and the aging brain.

- a) **Foster, T.C.** (2012) Dissecting the age-related decline on learning and memory tasks in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca²⁺ channels in senescent synaptic plasticity. *Prog Neurobiol* 96:283-302. **PMID: 22307057.**
- b) Kumar A and **Foster TC.** Linking redox regulation of NMDAR synaptic function to cognitive decline during aging. *J Neurosci*, 2013; 33: 15710-15715. **PMID: 24089479**
- c) Guidi, M., Kumar, A., and **Foster T.C.**, Impaired attention and synaptic senescence of the prefrontal cortex involves redox regulation of NMDA receptors. *J Neurosci*, 2015, 35(9) 3966-3977. **PMID: 25740525**

B. Positions and Honors

Positions and Employment

Assistant Professor, 1991-1992, Dept. Psych. University of Connecticut
Assistant Professor, 1992-1998, Dept. Psych. University of Virginia
Associate Professor, 1998-2003, Dept. Pharmacology, University of Kentucky Medical School
Associate Professor, 2003-2006, Dept Neurosci, University of Florida
Professor 2006-present, Dept Neurosci, University of Florida

Academic Honors and Awards

National Advisory Council on Aging NIH Method to Extend Research in Time (MERIT) Award (2011-present)
McKnight Chair for Research on Aging and Memory, University of Florida 2003-present
Member of the planning Committee for the Cognitive Aging Summits I (2006) & II (2010)
Associate Editor *Frontiers in Aging Neuroscience* 2009-present
Member for > 10 NIH Special Emphasis Review Panels (2001-2015)
Member NIH IFCN-7 Study Section 1999-2004
Member NIH Learning and Memory study section (7/2014-6/2018)
Shannon Investigators Award, 1992

C. Contribution to Science

1. In general, my research is focused on understanding mechanisms for modifying synaptic transmission and their relationship to memory, particularly in the context of cognitive decline during aging. My early work employed *in vivo* recording and showed that neuronal discharge activity in the hippocampus, a brain structure involved in memory, could represent the history of experience and the association of sensory-motor information.
 - a) **Foster, T.C.**, Christian, E.P., Hampson, R.E., Campbell, K.A. and Deadwyler, S.A. (1986) Sequential dependencies regulate sensory evoked responses of single units in the rat hippocampus. *Brain Research* 408:86-96. **PMID: 3594233**
 - b) **Foster, T.C.**, West, M.O., Hampson, R.E. and Deadwyler, S.A. (1988) Control of sensory activation of granule cells in the fascia dentata by extrinsic afferents: Septal and entorhinal inputs. *Journal of Neuroscience* 8:3869-3878. **PMID: 3193182**
 - c) **Foster, T.C.**, Castro, C.A. and McNaughton, B.L. (1989) Spatial selectivity of rat hippocampal neurons: Dependence on preparedness for movement. *Science* 244: 1580-1582. **PMID: 2740902**
2. Synaptic plasticity is thought to mediate the associative and information storage properties of neurons; however, the mechanisms that regulate the induction and expression of synaptic plasticity remained to be elucidated. Therefore, I developed *in vitro* quantal analysis techniques to determine presynaptic and post synaptic mechanisms for expression changes in synaptic strength due to long-term potentiation (LTP), aging, or due to differential experience. The results provided a frame work for the cellular basis of memory.
 - a) **Foster, T.C.** and McNaughton, B.L. (1991) Long-term enhancement of synaptic transmission is due to increased quantal size, not quantal content. *Hippocampus* 1:79-91. **PMID: 1669344**
 - b) **Foster, T.C.**, Barnes, C.A., Rao, G. and McNaughton, B.L. (1991) Increase in perforant path quantal size in aged F-344 rats. *Neurobiology of Aging* 12:441-448. **PMID: 1776764**
 - c) **Foster, T.C.** and Dumas, T.C. (2001) Mechanisms for increased hippocampal synaptic strength following differential experience. *Journal of Neurophysiology* 85: 1377-1383. **PMID: 11287462**
 - d) McNaughton, B.L. and **Foster, T.C.** (1990) The cellular basis of memory. *Science* 249:1487. **PMID: 2271062**
3. Intracellular calcium (Ca²⁺) levels occupy a pivotal position in regulating the induction of synaptic plasticity and Ca²⁺ regulation is disrupted during aging, providing a possible link between age-related cognitive decline and senescent synaptic function. My work established this linked, showing that altered synaptic plasticity in region CA1 of the hippocampus is related to a specific age-related decline in episodic spatial memory and revealed the mechanisms for dysregulation of Ca²⁺ sources due to an oxidized redox state.
 - a) Norris, C.M., Korol, D.L. and **Foster, T.C.** (1996) Increased susceptibility to induction of long-term depression and long-term potentiation reversal during aging. *Journal of Neuroscience* 16: 5382-5392. **PMID: 8757251**
 - b) Norris, C.M., Halpain, S. and **Foster, T.C.** (1998) Reversal of age-related alterations in synaptic plasticity by blockade of L-type Ca²⁺ channels. *Journal of Neurosciences*, 18: 3171-3179. **PMID: 9547225**
 - c) Bodhinathan, K., Kumar A., **Foster, T.C.** Intracellular redox state alters NMDA receptor response during aging through Ca²⁺/calmodulin-dependent protein kinase II. *Journal of Neurosciences* 2010; 30(5):1914-1924. **PM:20130200**
 - d) Kumar, A. and **Foster, T.C.** Linking redox regulation of NMDAR synaptic function to cognitive decline during aging. *Journal of Neuroscience* 2013; 33: 15710-15715. **PMID: 24089479**
4. Questions remain as to the factors that contribute to the aging mechanisms we have described and could determine the well-characterized variability in cognitive decline. For example, gender differences have been described for the rate of cognitive decline during aging suggesting a possible role of sex steroids (e.g. estrogen) in preserving cognitive function. My work demonstrates a critical window for effective estrogen treatment in aging female rats, and reveals differential role for various estrogen receptors in regulating estrogen effects on cognition and the maintenance of neuronal health.
 - a) **Foster, T.C.**, Sharrow, K.M., Kumar, A. and Masse, J. (2003) Interaction of age and chronic estradiol replacement on memory and markers of brain aging. *Neurobiology of Aging*, 24: 839-852, **PMID 12927766**.
 - b) **Foster, T.C.**, Rani, A., Kumar, A., Cui, L. and Semple-Rowland, S.L. (2008) Viral vector mediated delivery of estrogen receptor-alpha to the hippocampus improves spatial learning in adult estrogen receptor-alpha knockout mice. *Molecular Therapy*, 16: 1587-1593, **PMID: 18594506**.

- c) Han, X., Aenlle, K.K., Bean, L.A., Rani, A., Semple-Rowland, S.L., Kumar, A., and Foster, T.C. Role of estrogen receptor alpha and beta in preserving hippocampal function during aging. *Journal of Neuroscience* 2013, 33: 2671-2683. PMID: 23392694
- d) Bean, L.A., Kumar, A., Rani, A., Guidi, M., Rosario, A., Cruz, P., Golde, T., Foster, T.C. Re-opening the critical window for estrogen therapy. *Journal of Neuroscience*, 2015, 35 16077-1693. PMCID: 4682778.

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/thomas.foster.1/bibliography/40906731/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

NIA R01 AG037984-11 (PI: Foster) Estrogen and cognition over the lifespan	9/15/2010 to 7/31/2016	\$193,725/year
NIA R37 AG036800-05 (PI: Foster) Signaling cascades and memory deficits during aging	10/05/2010 to 08/31/2019	\$186,208/year
NIA R01 AG049711-01(PI: Foster) Systemic inflammation in regulating the onset and progression of brain aging-1	09/01/2015 to 4/30/2020	\$205,000/year
NIA R01 AG052258-01(PI: Foster) Systemic inflammation in regulating the onset and progression of brain aging-2	05/05/2016 to 4/30/2021	\$250,000/year
NIA P30AG028740 (PI Pahor) Claude D. Pepper Older Americans Independence Center	7/1/2006-3/31/2017	\$728,001
NINDS R37 NS040389 (PI Ranum) Molecular Genetic Characterization of SCA8	8/1/2015 – 7/31/2019	\$639,179/year
R21NS091435 (PI Notterpek) Targeting the Chaperone Pathway for Myelin Repair in Hereditary Neuropathies	9/1/2017 – 8/31/2017	\$250,000/year
Preceptor		
T32 AG046371 (PI: Fillingim) Integrative and Multidisciplinary Pain and Aging Research Training (IMPART)	9/1/2015-3/31/2019	\$244,256

Damon Geoffrey Lamb, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Lamb, Damon

eRA COMMONS USER NAME (credential, e.g., agency login): dglamb

POSITION TITLE: Research Health Science Specialist (Malcom Randall VAMC), Assistant Professor (UF)

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Maryland, College Park, MD	BS	05/2003	Mathematics
University of Maryland, College Park, MD	BS	12/2003	Computer Engineering
University of Chicago, Chicago, IL	MS	12/2005	Computer Science
MBL, Woods Hole, MA	~	07/2009	Neural Systems & Behavior
Emory University, Atlanta, GA	PHD	08/2013	Neuroscience

A. Personal Statement

My long term goal is to bridge cutting edge basic science and clinical/treatment focused research. The goal of this research proposal is to improve our understanding of autonomic function and modulations of learning and memory. In particular, I am investigating transcutaneous vagal nerve stimulation (tVNS) as a novel treatment for amnesic mild cognitive impairment (aMCI) to enhance cognition both in healthy individuals as well as amnesic mild cognitive impairment. tVNS is an exciting approach based on our understanding of the neurophysiological basis of memory and cognitive function, as well as pilot data. I look forward to extending our knowledge of this mechanistic impact of this innovative tool, laying a foundation for future clinical applications. I also have DARPA funding to further elucidate the neural circuit impacted by vagal nerve stimulation, providing complementary animal model data for the development of this approach. Apropos the mission of the Cognitive Aging and Memory Clinical Translational Research Program, I have been instrumental in the development of a multi-modal neuroimaging pipeline and I will be using these tools to assist members of the CAM in processing their data efficiently and accurately using high performance parallel computing.

B. Positions and Honors

Positions and Employment

2001 - 2001	Product Engineer, Hughes Network Systems, Germantown, MD
2001 - 2004	Research Software Developer, University of Iowa
2002 - 2004	Research Assistant, Institute for Research in Electronics and Applied Physics, University of Maryland, College Park, MD
2003 - 2005	Acoustic Modeling Software Developer, Acoustic Design Ahnert
2004 - 2007	Data Analyst, Brain-Body Center, University of Illinois, Chicago, IL
2007 - 2013	Graduate Student, Emory University, Atlanta, GA
2013 -	Research Health Science Specialist, Malcom Randal VAMC, Gainesville, FL
2013 -	Assistant Professor, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

Member, Society for Neuroscience
Member, American Association for the Advancement of Science
Member, Organization for Computational Neurosciences

Honors

2005	Computer Science Faculty Commendation, University of Chicago
2007	IGERT: Hybrid Neural Microsystems Fellow, NSF (Georgia Tech & Emory University)
2009	MBL Neural Systems and Behavior Fellow, Frank R. Lillie Fellowship and Scholarship
2009	Scholar, Burroughs Wellcome Fund
2011	Research Partners Fellow, Howard Hughes Medical Institute

C. Contribution to Science

1. Correlated ionic conductances and interactions underlie coordinated neuronal activity

Neurons can have widely differing intrinsic membrane properties, in particular the density of specific conductances (or resistance to ionic flow through ion channels), but how these contribute to characteristic neuronal activity or pattern formation is not well understood. My biophysical modeling work on small neuronal networks investigated how these ionic conductances contribute to coordinated motor output. Previous work had elucidated relationships between pairs of conductances, but they were generally required to be similar in their time courses, although of opposing polarity. My work showed that much more complex correlational relationships contribute to the output of neuronal networks, as well as providing an explanation of the basis for these relationships. Outside of the novel modeling approaches and the combination of algorithmic optimization approaches, computational tools, and biological data I used, this work has implications for the variability of individual response to psychoactive medication. The primary publication from this large, multi-year modeling work has already been cited 11 times over the past two years since its publication and extensions of the work are ongoing.

- a. **Lamb DG, Calabrese RL.** Correlated conductance parameters in leech heart motor neurons contribute to motor pattern formation. *PLoS One*. 2013;8(11):e79267. **PubMed PMID: 24260181; PubMed Central PMCID: PMC3832487.**
- b. **Lamb DG, Calabrese RL.** Small is beautiful: models of small neuronal networks. *Curr Opin Neurobiol*. 2012 Aug;22(4):670-5. **PubMed PMID: 22364687; PubMed Central PMCID: PMC3817830.**
- c. **Lamb DG, Calabrese RL.** Neural circuits controlling behavior and autonomic functions in medicinal leeches. *Neural Syst Circuits*. 2011 Sep 28;1(1):13. **PubMed PMID: 22329853; PubMed Central PMCID: PMC3278399.**

2. Experimental and data analysis software & hardware

Throughout my scientific career I have applied my technical skills to the design, development, and deployment of computer software and hardware to improve and enable research. An example of the data processing tools I have developed is CardioEdit/CardioBatch, which allows efficient raw data processing and analysis of electrocardiogram signals for the extraction of heart rate variability measures, which are an index of autonomic nervous system function. I used these tools to conduct collaborative research with both animal and human biological psychology researchers, but they were also made freely available to the research community. As a testament to the utility of this software, over 65 papers cite using my software to process and analyze their data. In 2000, I developed a multi-center data collection and aggregation tool that enabled distributed, offline collection of child abuse and maltreatment information collected by social workers, police, and researchers. This tool has been a critical tool for at least 18 papers, and the ideas about aggregating multi-site data have led to subsequent tools developed by other scientific programmers. More applicable to the proposed investigation, I also programmed the Dynamic Affect Recognition Experiment software and built the initial hardware, a test which presents subjects with a morphing emotional face whose emotion they identify.

- a. Bal E, Harden E, **Lamb D**, Van Hecke AV, Denver JW, Porges SW. Emotion recognition in children with autism spectrum disorders: relations to eye gaze and autonomic state. *J Autism Dev Disord*. 2010 Mar;40(3):358-70. **PubMed PMID: 19885725.**
- b. Vaughan Van Hecke A, Lebow J, Bal E, **Lamb D**, Harden E, Kramer A, Denver J, Bazhenova O, Porges SW. Electroencephalogram and heart rate regulation to familiar and unfamiliar people in children with autism spectrum disorders. *Child Dev*. 2009 Jul-Aug;80(4):1118-33. **PubMed PMID: 19630897.**
- c. Grippo AJ, **Lamb DG**, Carter CS, Porges SW. Social isolation disrupts autonomic regulation of the heart and influences negative affective behaviors. *Biol Psychiatry*. 2007 Nov 15;62(10):1162-70. **PubMed PMID: 17658486; PubMed Central PMCID: PMC2144909.**
- d. Grippo AJ, **Lamb DG**, Carter CS, Porges SW. Cardiac regulation in the socially monogamous prairie vole. *Physiol Behav*. 2007 Feb 28;90(2-3):386-93. **PubMed PMID: 17107695; PubMed Central PMCID: PMC1839927.**

3. Time-resolved particle-beam emittance

Early in my research career, I gathered the first time-resolved particle-beam emittance data. This experiment looked into how a 100ns charged particle beam varied along its length. Such a measurement was technically challenging at many levels, and the success of this experiment relied on two key control systems I programmed: one controlling the electro-magnetic focusing and bending optics, and the other an adaptive control system for the beam-measurement apparatus. The data and the functional measurement system that resulted from this work directly contributed to journal papers and referred conference papers, and enabled other researchers to investigate otherwise inaccessible research questions.

- a. Walter M, Quinn B, **Lamb D**, Bernal S, Godlove T, Haber I, Holloway M, Kishkek RA, Li H, O'Shea PG, Reiser M. Experimental tests of the injection Y on the University of Maryland Electron Ring. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*. 2005 May 21; 544(1-2):374-377.
- b. Bernal S, Beaudoin B, Cui Y, Glanzer M, Godlove TF, Harris J, Holloway M, Haber I, Kishkek RA, Lee W, **Lamb D**, Quinn B, Quirus M, Reiser M, Valfells A, Walter M, Wilson M, Yun R, Zou Y, O'Shea PG. Intense beam transport experiments in a multi-bend system at the University of Maryland Electron Ring. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*. 2004 February 21; 519(1-2):380-387.
- c. Walter M, **Lamb D**, Bernal S, Haber I, Kishkek R, Li H, Quinn B, Snowel M, Valfells A, Reiser M, O'Shea P. Time resolved emittance measurement in the University of Maryland Electron Ring. Proceedings of the 2003 Particle Accelerator Conference. *Particle Accelerator Conference*; 2003; c2003.
- d. Walter M, Quinn B, **Lamb D**, Bernal S, Godlove T, Haber I, Holloway M, Kishkek R, Li H, O'Shea P, Reiser M. Experimental tests of the injection Y on the University of Maryland Electron Ring. Proceedings of the 2003 Particle Accelerator Conference. *Particle Accelerator Conference*; 2003; c2003.

My NCBI bibliography is available at: <http://www.ncbi.nlm.nih.gov/sites/myncbi/1j9Hg8t4ygtkW/bibliography/47495904/public/?sort=date&direction=descending>

A citation report is available on google scholar:

<http://scholar.google.com/citations?user=X49GAQkAAAAJ&hl>

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

12179085 (BAA-16-24 – Targeted Neuroplasticity Training), DARPA

Otto, Kevin (PI)

01/01/2017-12/31/2020

Cognitive Augmentation through Neuroplasticity (TNT-CAN)

Role: Performer

1IK2RX000707, Veterans Health Administration

Williamson, John (PI)

08/25/13-04/30/17

White Matter Changes Emotional and Autonomic Consequences

Role: Co-Investigator

5I01CX000744, VA Clinical Science Research & Development

Heilman, Kenneth (PI)

08/25/13-09/30/17

Vertical Neglect

Role: Co-Investigator

1R56HL127175, NIH-NHLBI

Williamson, John (PI)

09/08/15-08/31/17

Brain and cognition effects of cardio-resynchronization therapy in heart failure

Role: Co-Investigator

Completed Research Support

0214BRRC-17, VA Rehabilitation Research & Development

Lamb, Damon (PI)

02/21/14-12/31/14

External autonomic nervous system modulation for the treatment of PTSD

Role: Co-PI

Debra E. Lyon, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Debra E. Lyon

eRA COMMONS USER NAME: delyon

POSITION TITLE: Kirbo Endowed Chair, Interim Associate Dean for Research and Scholarship, University of Florida, College of Nursing

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Virginia Commonwealth University, Richmond, VA	B.S.	1984	Nursing
Virginia Commonwealth University	M.S.	1993	Child-Family MH Nursing
Virginia Commonwealth University	Certificate	1996	Family Nurse Practitioner
Virginia Commonwealth University	Ph.D.	1997	Nursing
Postdoctoral Fellow (T32AT000052, Ann G. Taylor, PD), Training Program In Complementary and Alternative Medicine, National Center for Complementary and Alternative Therapy	Postdoctoral fellow	2001-2003	Complementary and Alternative Therapy Research Methods

A. Personal Statement

I have a broad background in oncology and symptom science, with expertise in complementary and alternative modalities, clinical trials and health disparities. Over the past 15 years, I have been PI or Co-I on multiple grants that have examined biobehavioral symptoms science, including the interplay of biological factors, symptoms, and symptom clusters, and pain in individuals with serious illnesses (e.g. human immunodeficiency virus (HIV), breast cancer, and fibromyalgia). Currently, I am MPI, with Dr. Colleen Jackson-Cook, on an NIH-R01 study, "Epigenetics and Psychoneurologic Symptoms in Women with Breast Cancer," which examines the role of epigenetic and genomic factors associated with symptoms experienced by women treated with chemotherapy. I am formerly the Co-Director of the Science Core at VCU School of Nursing's P30 Center of Excellence in Biobehavioral Approaches to Symptom Management, where I led the team on disseminating symptom research across multiple diverse populations, such as patients with sickle cell disease and fibromyalgia. Since my appointment as Executive Associate Dean at the UF College of Nursing, I have developed collaborations with multiple investigators at the University of Florida, including Dr. Odenina, and I am committed to the further education of a cadre of racially and ethnically diverse, well-trained scientists focused on cancer research. Due to my success as PI on multiple clinical research projects and my history of training students at all levels. I have the expertise to contribute to the research training component of the UF-FAMU Florida Minority Cancer Research & Training to apprise and culturally sensitize URM undergraduate, post-baccalaureate, and post-masters students of the need to reduce the disproportionate prostate cancer (CAP) burden in minority populations through basic, clinical, and behavioral research. I will also assist with the dissemination of research findings to national and international venues.

1. **Lyon, D. E.,** Taylor, A. G. & Schubert, C. (2010). Pilot study of cranial stimulation for symptom management in breast cancer. *Oncology Nursing Forum*, 37(4), 476-83. **PMCID: PMC3061342**
2. Starkweather, A. R., **Lyon, D. E.,** & Schubert, C. M. (2011). Pain and inflammation in women with early-stage breast cancer prior to induction of chemotherapy. *Biological Research for Nursing*, 15(2), 234-241.
3. **Lyon, D.,** McCain, N., Elswick, R. K., Sturgill, J., Ameringer, S., Jallo, N., . . . Grap, M. J. (2014). Biobehavioral examination of fatigue across populations: Report from a P30 Center of Excellence. *Nursing Outlook*, 62(5), 322-331.

4. Starkweather, A. R., Coyne, P., Lyon, D. E., Elswick, R. K., An, K., & Sturgill, J. (2015). Decreased low back pain intensity and differential gene expression following Calmare®: Results from a double-blinded randomized sham-controlled study. *Research in Nursing & Health*, 38(1), 29-38.

B. Positions and Honors

Positions

7/84 - 4/85	Army Nurse Corps Officer, Emergency Room Nurse, Eisenhower Med. Ctr., Fort Gordon, GA
4/85 - 12/92	Clinical Nursing Positions
1/93 - 5/95	Graduate Teaching assistant, RN to BS program, VCU
8/95 - 8/98	Clinical Instructors, VCU School of Nursing, Richmond, VA
7/01 - 7/03	Post-Doctoral Fellow, University of Virginia School of Nursing
8/98 - 8/04	Assistant Professor, University of Virginia School of Nursing
7/04- 6/10	Associate Professor, Virginia Commonwealth University School of Nursing
12/04- 12/13	Member Scientist, Massey Cancer Center, Virginia Commonwealth University
12/04-07/09	Center Affiliate, Center for Biobehavioral Research, School of Nursing, VCU
08/09-12/13	Center Scientist, Center for Excellence in Biobehavioral Symptom Management, SON
01/09-04/10	Interim Chair, Department of Family and Community Health Nursing
04/10-3/13	Chair, Department of Family and Community Health Nursing
04/13-12/13	Associate Dean for Research, VCU
4/15-Pres	Emeritus Professor, VCU
01/14-Pres	Kirbo Endowed Chair and Executive Associate Dean, University of Florida, College of Nursing

Special Awards, Fellowships and Other Honors

1980-84	VCU Honors Program Student
1992-94	National Institute of Mental Health (NIMH) Trainee Recipient
1994-96	A.D. Williams Doctoral Scholarship; Whitehead Scholarship, Master's Program
1997	Summer Institute Fellow, University of Pennsylvania School of Nursing
2000	Seven Society Faculty Award
2000, 2003	Faculty Marshall, UVA School of Nursing
2001, 2002	University of Virginia Teaching Fellow
2005	Junior Researcher Award, VCU School of Nursing
2008	Fellow, Grace Harris Leadership Institute
2009	National Academies of Practice, Fellow
2010	Jean D. Wood Nursing Scholarship Award, Southern Nursing Research Society
2010	Exceptional Researcher Award, AD Williams Foundation
2011	Fellow, American Academy of Nursing
2011	Alumni Star, VCU Alumni Association

Membership – Scientific, Honorary and Professional Societies

1998-pres	American Nurses' Association/Virginia Nurses' Association
1994-pres	Phi Kappa Phi, Virginia Commonwealth University
1992-pres	Sigma Theta Tau, Gamma Omega Chapter, Counselor (1997-98)
1996-pres	Southern Nursing Research Society, Member Biobehavioral RIG (2004-pres)
1999-pres	Association of Nurses in AIDS Care
2000-pres	Oncology Nursing Society, Associate Editor, Clinical Journal of Oncology Nursing
2002-pres	Council for the Advancement of Nursing Science
2011-pres	Co-Chair, Biobehavioral Research Group, Southern Nursing Research Society
2012-pres	Co-Chair, Genetics Expert Panel, American Academy of Nursing.

Licensure and Certification

1984-pres	Registered Nurse, Commonwealth of Virginia – 0001093520
1996-pres	Nurse Practitioner, Commonwealth of Virginia
1995-pres	A.N.A. Clinical Specialist in Adult Psychiatric & Mental Health Nursing
1996-pres	A.N.A. Family Nurse Practitioner Certification

Memberships on Review Panels

April 2003	Health Services Research Fund. Government Secretariat, Hong Kong, The People's Republic of China. SARS and Psychological Distress
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Aug 2003	Health Research Board, Dublin, Ireland. HRB Research Fellowships in Primary Care
2005-pres	Oncology Nursing Society: Symptom Management Grants. Breast Cancer Grants. Lung Cancer Grants. Core Biology Reviewer (2009-2012)
2007, 2008	Department of Defense, Breast Cancer Program, Pre-doctoral Proposal Review
May 2009	Center for Scientific Review, National Institutes of Health, Mail Reviewer. Challenge Grants
Oct 2009	National Institutes of Health, Member, Special Emphasis Panel/Scientific Review Group ZRGI HDM-F. Cost – Effectiveness and CAM Research. P50 Applications
Nov 2009	National Institutes of Health, Member, Special Emphasis Panel/Scientific Review Group ZAT1 SM (17). PCCTR International Centers for Research in CAM, U 19 Applications
Feb 2010	Center for Scientific Review, National Institutes of Health, Ad Hoc Member, Scientific Review Group BCHI (Biomedical Computing and Health Informatics)
July 2010-14	Center for Scientific Review, National Institutes of Health, Standing Member, Scientific Review Group BCHI (Biomedical Computing and Health Informatics)

C. Contributions to Science

As a nurse scientist, the long-term goal of my program of research is to elucidate the biological mechanisms associated with the development and persistence of distressing symptoms in patients with chronic diseases. My early publications focused on the interplay of biopsychosocial variables, mental health outcomes, and health disparities in individuals living with human immunodeficiency virus.

- a. **Lyon, D. E.** & Munro, C. L. (2001). Disease severity and symptoms of depression in black Americans infected with HIV. *Applied Nursing Research*, 14(1), 3-9.
 - b. **Lyon, D. E.** & Younger, J. B. (2001). Purpose in life and depressive symptoms in persons living with HIV disease. *Journal of Nursing Scholarship (formerly Image)*, 33(2), 137-141.*
 - c. McCain, N. L., **Lyon, D. E.**, Higginson, R., Settle, J., Robins, J. L. W. & Fisher, E. J. (1998). Revision of the HIV Center Medical Staging Scale. *Journal of the Association of Nurses in AIDS Care*, 9(5), 19-23.
2. After a post-doctoral fellowship, my work transitioned to women with breast cancer, and I have been PI/Co-I on multiple projects that examined biological markers and quantitative methods with sophisticated technology. This research transitioned from single symptoms to symptom clusters. This translational work focuses on developing the interdisciplinary teams that will support the integration of basic science, symptom science, and quantitative models for better elucidating the relationship among molecular markers and symptoms.
- a. **Lyon, D. E.**, Walter, J, McCain, N. L., Schubert, C. (2008). Cytokine comparison of women with negative breast biopsy with women with breast cancer. *Nursing Research*, 57(1), 51-58. PMID: 2234268.
 - b. **Lyon, D.**, Elmore, L., Aboalela, N., Merrill-Schools, J., McCain, N., Starkweather, A., . . . Jackson-Cook, C. (2013). Potential epigenetic mechanism(s) associated with the persistence of psychoneurological symptoms in women receiving chemotherapy for breast cancer: A hypothesis. *Biological Research for Nursing*, 16(2), 160-174. PMID: 3872254
 - c. Menzies, V., **Lyon, D.E.**, Archer, K., Zhou, Q., Brumelle, J., Jones, K.H., Gao, G., York, T. & Jackson-Cook, C. (2013). Epigenetic alterations and an increased frequency of micronuclei in women with fibromyalgia. *Nursing Research and Practice*, 2013, 795784.
 - d. Starkweather, A. R., **Lyon, D. E.**, Elswick Jr, R. K., Montpetit, A., Conley, Y., & McCain, N. L. (2013). Symptom cluster research in women with breast cancer: A comparison of three subgrouping techniques. *Advances in Breast Cancer Research*, 2(4), 107.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/debra.lyon.1/bibliographahy/40754910/public/?sort=date&direction=ascending>

D. Research Support

R01 NR012667 [Lyon/Jackson-Cook (MPI)] 10/01/2010-08/30/2016

NIH/NINR Epigenetics and Psychoneurologic Symptoms in Women with Breast Cancer

The proposed study will prospectively characterize PN symptoms (cognitive dysfunction; depressive symptoms and anxiety; fatigue; sleep disturbances; pain), inflammatory activation [(levels of C-reactive protein (CRP), tumor necrosis factor alpha (TNF- α), interleukin 1 beta (IL-1 β), and interleukin 6 (IL-6)], and epigenetic alterations in 75 women diagnosed with early-stage breast cancer across 5 timepoints: prior to their receipt of chemotherapy, at the time of their fourth chemotherapy treatment, and at 6, 12, and 24 months following the initiation of chemotherapy. Epigenetic alterations will be detected by quantifying the: (1)frequency and

genome-wide location of methylation; (2) expression of a histone methyltransferase, EZH2, which is a Polycomb group protein that is thought to be important for the early steps in the epigenetic “decision” process; and (3) telomere attrition, which can lead to alterations in chromatin compaction/gene expression. The study results may potentially deepen understanding regarding the biological processes underlying PN symptoms and lead to improved strategies for symptom management in women with breast cancer.

Role: Multiple PI

R01 NR013932 (Starkweather) 08/01/2013-06/30/2016

NIH/NINR *Pain Sensitivity in Low Back Pain*

Although a precise structural etiology of persistent low back pain is rarely present, recent studies report functional alteration in the peripheral and central nervous system of patients with persistent low back pain that are associated with enhanced pain sensitivity. It has recently been shown that genetic polymorphisms and modifications in gene expression patterns can influence pain sensitivity; however, this has never been studied in patients with low back pain. Therefore, the proposed study will investigate the role of enhanced pain sensitivity on the risk of persistent low back pain through characterization of pain sensitivity and pain-sensitivity candidate gene profiling.

Role: Co-investigator

UF CTSI Pilot Study Horgas (PI) 10/01/2014-09/30/2015

NIH/NCATS Clinical and Translational Science Award to the University of Florida UL1 TR00006

Biobehavioral Predictors of Persistent Post-surgical Pain in Women Undergoing Breast Cancer Treatment

The primary goal of the proposed project is to investigate biological and psychological factors that are associated with the development of persistent pain in women with early stage breast cancer.

Role: Co-investigator

Completed Support

P30 NR011403-01 (Grap) 08/10/2009-05/31/2014

NIH/NINR

Center of Excellence in Biobehavioral Approaches to Symptom Management

The major goals of this project are to (1) expand biobehavioral research capacity for scientists conducting nursing research by centralizing research resources and infrastructure for biobehavioral clinical research focused on symptom management; (2) advance biobehavioral approaches for symptom management, including fatigue and its associated symptoms, in diverse populations; (3) facilitate the development and expansion of biobehavioral programs of independent, investigator-initiated biobehavioral research that have a common theme of fatigue; and (4) establish a mature environment of sustainable biobehavioral research in symptom management that is clinically focused and interdisciplinary in nature.

Role: Science Core, Co-Director (15% effort)

R01CA127446-01A (Lyon) 04/01/2009-01/31/2015

NIH/NCI Cranial Stimulation for Chemotherapy Symptoms in Breast Cancer

Cranial Stimulation for Symptoms in Early Stage Breast Cancer

The aims of this project are: (1) to examine the relationships among selected markers of inflammation, symptoms and quality of life (QOL); (2) to examine whether the symptoms of depression, anxiety, fatigue, sleep disturbances and pain form a cluster, and (3) to examine the effects of cranial stimulation on QOL.

Role: Principal Investigator

Massey Cancer Center 08/10/2013-05/31/2014

Cognitive Impairment and Work Outcomes in Women with Breast Cancer

The specific aims of proposed project are: (1) To determine the extent to which CD and symptom burden (symptoms of depression and anxiety, fatigue and pain), affect initially employed women’s labor supply (measured as employment and weekly hours worked), productivity (using the Work Limitations Questionnaire), and ability to perform specific job tasks (including physically demanding and cognitive tasks); and (2) To explore the relationships among CD theoretically relevant biomarkers and women’s labor supply and work productivity.

Role: Multiple Principal Investigator (MPI)

2016 Publications

Lyon, D. E., Cohen, R., Chen, H., Kelly, D. L., McCain, N. L., Starkweather, A., ... & Jackson-Cook, C. K. (2016). Relationship of systemic cytokine concentrations to cognitive function over two years in women with early stage breast cancer. *Journal of Neuroimmunology*.

- Lyon, D. E.**, Cohen, R., Chen, H., Kelly, D. L., McCain, N. L., Starkweather, A., ... & Jackson-Cook, C. K. (2016). Relationship of systemic cytokine concentrations to cognitive function over two years in women with early stage breast cancer. *Journal of Neuroimmunology*.
- Kelly, D. L., **Lyon, D. E.**, Garvan, C., & Wingard, J. R. (2016). Associations Among Inflammation, Perceived Stress, and Lifestyle Behaviors of Individuals with Chronic Graft-Versus-Host Disease (cGVHD) Following Allogeneic Blood and Marrow Transplantation. *Biology of Blood and Marrow Transplant*, 22(3), S187.
- Starkweather, A., Kelly, D. L., Thacker, L., Wright, M. L., Jackson-Cook, C. K., & **Lyon, D. E.** (2017). Relationships among psychoneurological symptoms and levels of C-reactive protein over 2 years in women with early-stage breast cancer. *Supportive Care in Cancer*, 25(1), 167-176.
- Starkweather, A. R., Ramesh, D., **Lyon, D. E.**, Siangphorn, U., Deng, X., Sturgill, J., ... & Greenspan, J. (2016). Acute Low Back Pain: Differential Somatosensory Function and Gene Expression Compared to Healthy No-pain Controls. *The Clinical Journal of Pain*.
- Starkweather, A. R., Heineman, A., Storey, S., Rubia, G., **Lyon, D. E.**, Greenspan, J., & Dorsey, S. G. (2016). Methods to measure peripheral and central sensitization using quantitative sensory testing: A focus on individuals with low back pain. *Applied Nursing Research*, 29, 237-241.
- Starkweather, A. R., **Lyon, D. E.**, Kinser, P., Heineman, A., Sturgill, J. L., Deng, X., ... & Dorsey, S. G. (2016). Comparison of Low Back Pain Recovery and Persistence A Descriptive Study of Characteristics at Pain Onset. *Biological research for nursing*, 18(4), 401-410.
- Ahn, H., Weaver, M., **Lyon, D.**, Kim, J., Choi, E., Staud, R., & Fillingim, R. B. (2016). Differences in Clinical Pain and Experimental Pain Sensitivity between Asian Americans and Whites with Knee Osteoarthritis. *The Clinical Journal of Pain*.
- Kim, J., Ahn, H., **Lyon, D. E.**, & Stechmiller, J. (2016, January). Building a Biopsychosocial Conceptual Framework to Explore Pressure Ulcer Pain for Hospitalized Patients. In *Healthcare* (Vol. 4, No. 1, p. 7). *Multidisciplinary Digital Publishing Institute*.
- Wright, M. L., Dozmorov, M. G., Wolen, A. R., Jackson-Cook, C., Starkweather, A. R., **Lyon, D. E.**, & York, T. P. (2016). Establishing an analytic pipeline for genome-wide DNA methylation. *Clinical epigenetics*, 8(1), 1.
- Kelly, D. L., **Lyon, D. E.**, Yoon, S. L., & Horgas, A. L. (2016). The Microbiome and Cancer: Implications for Oncology Nursing Science. *Cancer nursing*, 39(3), E56-E62.
- Williams, J.K., Katapodi, M.C., Starkweather, A., Badzek, L., Cashion, A.K., Coleman, B., Fu, M.R., **Lyon, D.**, Weaver, M.T. and Hickey, K.T., 2016. Advanced nursing practice and research contributions to precision medicine. *Nursing outlook*, 64(2), pp.117-123.
- Redwine, C. L., Odedina, F., **Lyon, D.**, & Nguyen, J. (2016). Abstract A18: Exploration of Florida blacks' understanding of precision medicine for cancer care and treatment. *Cancer Epidemiology Biomarkers & Prevention*, 25(3 Supplement), A18-A18.
- Ahn, H., Cowan, L., Garvan, C., **Lyon, D.**, & Stechmiller, J. (2016). Risk Factors for Pressure Ulcers Including Suspected Deep Tissue Injury in Nursing Home Facility Residents: Analysis of National Minimum Data Set 3.0. *Advances in skin & wound care*, 29(4), 178-190.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: MAURER, ANDREW

eRA COMMONS USER NAME (agency login): DREWMAURER

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)

Table with 4 columns: INSTITUTION AND LOCATION, DEGREE (if applicable), Completion Date MM/YYYY, FIELD OF STUDY. Rows include University of Pittsburgh, University of Arizona, and University of Arizona with degrees BS, PHD, and Postdoctoral Fellow.

A. Personal Statement

Throughout my scientific career, I have been focused on trying to understand the mechanisms that govern information propagation in the brain. As a graduate student, I worked with Dr. Bruce McNaughton, acquiring skills in the acquisition and analysis of high-density single-unit electrophysiological recordings from awake-behaving rats.

After relocating to the University of Florida, where there is a strong research focus on the neurobiology of cognitive decline, I have become interested in extending my research approach to incorporate technological advancements. The fifty-five million Americans that are projected to be over the age of sixty five by 2020 presents a significant financial and public health crisis.

B. Positions and Honors

Positions and Employment

- 2002 - 2004 Undergraduate Research Assistant, Dr. Bill Yates' Vestibular Research laboratory (U of Pitt), Pittsburgh, PA
2004 - 2008 Graduate Research Associate, Dr. Bruce McNaughton's Neural Systems, Memory and Aging Laboratory (U of Arizona), Tucson, AZ
2005 - 2006 Graduate Teaching Assistant, Course- "Memory mechanisms & Neural Computation", Tucson, AZ
2009 - 2014 Postdoctoral Research Fellow, Evelyn F. McKnight Brain Institute with Dr. Carol Barnes, Tucson, AZ
2014 - Affiliate faculty member, Department of Biomedical Engineering, University of Florida, Gainesville, FL
2014 - Assistant Professor, Department of Neuroscience, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

- 2002 - Member, Society for Neuroscience
2014 - North Central Florida Chapter of the Society for Neuroscience

Honors

- 2003 Cum Laude, University of Pittsburgh
2007 Recipient of Conference Travel Award, Society for Neuroscience
2008 Recipient of the D.B. Marquis Behavioral Neuroscience Award, Behavioral Neuroscience Journal
2011 Recipient of the Ruth L. Kirschstein National Research Service Award, National Institute of Health

C. Contribution to Science

- 1. Prior to my thesis research, only two studies investigated hippocampal dynamics in the posterior/ventral region of the hippocampus. Therefore, I sought out to determine the firing rate characteristics of neurons in the intermediate portion of

the hippocampus compared to the dorsal. We found that place field size was larger in more posterior regions, associated with a decreased rate of phase precession and a decreased sensitivity to velocity. The examination of hippocampal activity patterns across the long axis of the hippocampus is a central component of the current proposal.

- a. **Maurer AP**, Vanrhoads SR, Sutherland GR, Lipa P, McNaughton BL. Self-motion and the origin of differential spatial scaling along the septo-temporal axis of the hippocampus. *Hippocampus*. 2005;15(7):841-52. **PubMed PMID: 16145692**.
 - b. **Maurer AP**, Cowen SL, Burke SN, Barnes CA, McNaughton BL. Organization of hippocampal cell assemblies based on theta phase precession. *Hippocampus*. 2006;16(9):785-94. **PubMed PMID: 16921501**.
 - c. **Maurer AP**, Cowen SL, Burke SN, Barnes CA, McNaughton BL. Phase precession in hippocampal interneurons showing strong functional coupling to individual pyramidal cells. *J Neurosci*. 2006 Dec 27;26(52):13485-92. **PubMed PMID: 17192431**.
2. Theta phase precession has long been thought to be a mechanism by which the brain temporally organizes events in order to facilitate learning and memory. The basic neuronal mechanisms, from ion channels to network dynamics governing this phenomenon, however, are not well understood. In order to elaborate and test the models of theta phase precession, I designed an experiment in which we trained rats to ambulate backwards, thereby, dissociating self-motion from head direction. These data support a view that head-direction input is not critical for theta phase precession.
- a. **Maurer AP**, McNaughton BL. Network and intrinsic cellular mechanisms underlying theta phase precession of hippocampal neurons. *Trends Neurosci*. 2007 Jul;30(7):325-33. **PubMed PMID: 17532482**.
 - b. **Maurer AP**, Lester AW, Burke SN, Ferng JJ, Barnes CA. Back to the future: preserved hippocampal network activity during reverse ambulation. *J Neurosci*. 2014 Nov 5;34(45):15022-31. **PubMed PMID: 25378167; PubMed Central PMCID: PMC4220031**.
3. One of the prominent characteristics of hippocampal pyramidal cell activity is their firing correlates with short-term predictions of future locations. Of course ambulatory characteristics will modulate both the future location and the distance covered. We have determined how ambulation alters firing patterns as well as tested models of hippocampal updating by training rodents to walk backwards on a linear track and found that when rodents walk backwards, hippocampal activity patterns continue to predict future locations regardless of head direction.
- a. **Maurer AP**, Burke SN, Lipa P, Skaggs WE, Barnes CA. Greater running speeds result in altered hippocampal phase sequence dynamics. *Hippocampus*. 2012 Apr;22(4):737-47. **PubMed PMID: 21538659; PubMed Central PMCID: PMC3367321**.
 - b. **Maurer AP**, Lester AW, Burke SN, Ferng JJ, Barnes CA. Back to the future: preserved hippocampal network activity during reverse ambulation. *J Neurosci*. 2014 Nov 5;34(45):15022-31. **PubMed PMID: 25378167; PubMed Central PMCID: PMC4220031**.
4. While the size of hippocampal spatial receptive fields increases along the dorsal to ventral longitudinal axis, we asked the additional question on whether non-spatial factors could influence the firing rate characteristics. By placing objects on the track, we showed that the spatial metric of hippocampal receptive fields can be reduced. This work produced new insights regarding the impact of sensory information along the hippocampal longitudinal axis and highlights the productive collaborative efforts of Dr. Burke and myself.
- a. Burke SN, **Maurer AP**, Nematollahi S, Uprety AR, Wallace JL, et al. The influence of objects on place field expression and size in distal hippocampal CA1. *Hippocampus*. 2011 Jul;21(7):783-801. **PubMed PMID: 21365714; PubMed Central PMCID: PMC3314262**.
 - b. Burke SN, **Maurer AP**, Hartzell AL, Nematollahi S, Uprety A, et al. Representation of three-dimensional objects by the rat perirhinal cortex. *Hippocampus*. 2012 Oct;22(10):2032-44. **PubMed PMID: 22987680; PubMed Central PMCID: PMC3447635**.
 - c. Burke SN, **Maurer AP**, Nematollahi S, Uprety A, Wallace JL, et al. Advanced age dissociates dual functions of the perirhinal cortex. *J Neurosci*. 2014 Jan 8;34(2):467-80. **PubMed PMID: 24403147; PubMed Central PMCID: PMC3870932**.
5. Interneurons have been hypothesized to provide the “scaffold” by which neuronal activity is structured within neural networks. In this sense, they can both govern the rate that information propagates through neural circuits as well as perform computational operations on the information. In light of these theories, we were enthusiastic to discover that putative basket cells exhibited theta phase precession, plausibly inherited from afferent pyramidal cell activity.

- a. **Maurer AP**, Cowen SL, Burke SN, Barnes CA, McNaughton BL. Phase precession in hippocampal interneurons showing strong functional coupling to individual pyramidal cells. *J Neurosci*. 2006 Dec 27;26(52):13485-92. **PubMed PMID: 17192431**.

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/andrew.maurer.1/bibliography/43942059/public/?sort=date&direction=ascending>

D. RESEARCH SUPPORT

2016/01/01-2020/11/31

1R01AG049722-01A1, NIH – National Institute on Aging

Burke, Sara (PI), **MAURER, ANDREW (Co-I)**

Contribution of Declines in Functional Connectivity to Cognitive Aging.

The major goal of this proposal is to interrogate prefrontal-medial temporal lobe interactions in order to determine how alterations in systems-level neural coordination in old animals produce cognitive impairments.

15% effort

1R21DA039701, NIH – National Institute on Drug Abuse

MAURER, ANDREW (M-PI), Setlow, Barry (M-PI)

Development of a rat model of cannabis smoke self-administration

In conjunction with Dr. Barry Setlow (a leading expert in drug addiction), we designed an apparatus that will allow precisely-calibrated, response-contingent delivery of cannabis smoke using experimental designs similar to those employed with other drugs of abuse. We will use this apparatus to determine whether rats will reliably show operant responding for cannabis smoke delivery. Successful development of a rodent cannabis smoke self-administration model will lay the groundwork for a larger research program on neurobehavioral mechanisms of cannabis smoking as well as allow us to bridge animal and human research.

15% effort

2015/08/15-2017/05/31

1R03AG049411, NIH – National Institute on Aging

MAURER, ANDREW (M-PI), Burke, Sara (M-PI), Ormerod, Brandi (M-PI)

Neurogenesis and Memory Network Dynamics during Normal Aging.

This collaborative R03 is designed to develop preliminary data aimed at understanding of the role of neurogenesis in memory and learning. Simply, there has yet to be a high-density electrophysiological investigation of dentate gyrus neural dynamics in aged, freely-behaving animals. As this region appears to be highly vulnerable to the aging process, we are in the process of relating functional change to alteration in neurogenesis.

5% effort

BIOGRAPHICAL SKETCH

NAME: Okun, Michael S.

eRA COMMONS USER NAME (credential, e.g., agency login): MSOKUN

POSITION TITLE: Professor

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Florida State University, Tallahassee, FL	BS	1993	History
University of Florida, Gainesville, FL	MD	1993-1996	Medicine
University of Florida, Gainesville, FL		1996-	Internship
University of Florida, Gainesville, FL		1997-2000	Neurology Residency
Emory University, Atlanta, GA		2000-2002	Movement Disorders Fellowship

A. Personal Statement

Michael S. Okun, M.D. is currently the Acting Chair, Administrative Director, and the Co-Director of the Center for Movement Disorders and Neurorestoration, and the Adelaide Lackner Professor of Neurology. Dr. Okun is considered an international expert in Parkinson's Disease and is the current National Medical Director of the National Parkinson Foundation (NPF), and serves as Co-Chair of the Medical Advisory Board for the Tourette Syndrome Association (TSA). He runs the Ask the Doctor International web-forum, and has been on the steering committee for the worldwide NPF quality improvement clinical-research database (every Parkinson's patient at each center has one page of data collected every year). In his role with TSA, Dr. Okun assists with the medical direction of the foundation, and also administers the TSA International Deep Brain Stimulation Registry for Tourette Syndrome. His expertise has included many physiology and brain imaging projects as well as many device related projects. Dr. Okun is an established Movement Disorders researcher with over 300 peer-reviewed publications and he has been PI, co-PI, or co-I on multiple NIH Parkinson's Disease intervention studies including the PI of a recently completed and published randomized controlled study of cognition and mood with deep brain stimulation (The NIH COMPARE study). Dr. Okun's research efforts have focused on the motor and non-motor aspects of deep brain stimulation as applied to basal ganglia disorders. He is currently Multi-PI of a R01 on mobile computing platforms. Dr. Okun has been involved in many projects examining cognitive-limbic-motor basal ganglia function. Finally, Dr. Okun has trained more than 30 M.D. fellows in movement disorders and many with an emphasis on imaging. There are four papers that are particularly relevant to my experience in DBS and in TS DBS for this current application.

1. **Okun MS.** Deep-brain stimulation – entering the era of human neural-network modulation. *N Engl J Med.* 2014 Oct 9;371(15):1369-73. doi: 10.1056/NEJMp1408779. Epub 2014 Sep 8. **PubMed PMID: 25197963.**
2. Schrock LE, Mink JW, Woods DW, Porta M, Servello D, Visser-Vandewalle V, Silburn PA, Foltynie T, Walker HC, Shahed-Jimenez J, Savica R, Klassen BT, Machado AG, Foote KD, Zhang JG, Hu W, Ackermans L, Temel Y, Mari Z, Changizi BK, Lozano A, Auyeung M, Kaido T, Agid Y, Welter ML, Khandhar SM, Mogilner AY, Pourfar MH, Walter BL, Juncos JL, Gross RE, Kuhn J, Leckman JF, Neimat JA, **Okun MS**; Tourette Syndrome Association International Deep Brain Stimulation (DBS) Database and Registry Study Group. Tourette syndrome deep brain stimulation: A review and updated recommendations. *Mov Disord.* 2015 Apr;30(4):448-71. doi: 10.1002/mds.26094. Epub 2014 Dec 5. **PubMed PMID: 25476818.**
3. **Okun MS,** Foote KD, Wu SS, Ward HE, Bowers D, Rodriguez RL, Malaty IA, Goodman WK, Gilbert DM, Walker HC, Mink JW, Merritt S, Morishita T, Sanchez JC. A trial of scheduled deep brain stimulation for Tourette syndrome: moving away from continuous deep brain stimulation paradigms. *JAMA Neurol.* 2013 Jan;70(1):85-94. doi: 10.1001/jamaneurol.2013.580. **PubMed PMID: 23044532.**

- Maling N, Hashemiyoon R, Foote KD, **Okun MS**, Sanchez JC. Increased thalamic gamma band activity correlates with symptom relief following deep brain stimulation in humans with Tourette's syndrome. *PLoS One*. 2012;7(9):e44215. doi: 10.1371/journal.pone.0044215. Epub 2012 Sep 6. Erratum in: *PLoS One*. 2012;7(11):doi/10.1371/annotation/446ec4cb-63da-42d2-afc6-7e8459b2abbe. **PubMed PMID: 22970181; PubMed Central PMCID: PMC3435399.**

B. Positions and Honors

Positions and Employment

2000-2001	Fellowship, Emory University Movement Disorders
2001-2002	Fellowship, Emory University Stereotactic Neurosurgery for Movement Disorders
2002-2006	Assistant Professor, Co-Director Movement Disorders Center University of Florida Department of Neurology
2003-present	Director, National Parkinson Foundation Center of Excellence
2006-present	National Medical Director, National Parkinson Foundation
2006-present	Adelaide Lackner Endowed Professor
2007-2012	Associate Professor of Neurology
2012-present	Professor of Neurology (with tenure)
2013- present	Associate Chair of Neurology

Professional Positions

1998-present	Executive Co-Director of the University of Florida Society for the History of Medicine
1999-2000	Chief Resident, University of Florida Department of Neurology
2001-present	Diplomate, American Board of Psychiatry and Neurology
Member:	American Academy of Neurology (AAN)
	American Neurological Association (ANA)
	Movement Disorders Society
	Alpha Omega Alpha Honor Society
	Phi Beta Kappa

C. Contribution to Science

Google Scholar Publicly Available Database of Research Papers: <https://scholar.google.com/citations?user=YXrkisUAAAAJ&hl=en>

- Historical Background:** Deep Brain Stimulation for Parkinson's disease has evolved as an important treatment for patients, however there existed an important knowledge gap in choosing the appropriate brain target for symptom specific and personalized therapy. Scientific Problem: There was a major controversy about using the subthalamic nucleus (STN) versus the globus pallidus internus (GPI) for treatment of medication refractory Parkinson's disease. Central Finding: We conducted a randomized NIH study comparing the STN target to the GPI DBS target in Parkinson's disease. This was the first adequately powered randomized target comparison, and it was also the first randomized comparison to show that there was no difference in motor outcomes between targets. A year after publication of our study (in the *Annals of Neurology*), the large bilateral VA study was published and the results were consistent with our findings. We were able to elucidate target specific differences between STN and GPI and advantages and disadvantages of a unilateral approach. Our team has been instrumental over many years in the development of DBS screening tools and the development and implementation of multidisciplinary DBS screening teams. Influence of the Finding: Today, targets and approaches (unilateral versus bilateral) to DBS are tailored to the individual needs of patients through the use of multidisciplinary screening teams and this approach was influenced by our many publications in this area. Today groups are more likely to use unilateral implants for select patients, and to carefully choose targets based on symptom profiles. Specific Role: PI on the NIH grant (The COMPARE Trial).

Relevant Papers:

- Taba HA, Wu SS, Foote KD, Hass CJ, Fernandez HH, Malaty IA, Rodriguez RL, Dai Y, Zeilman PR, Jacobson CE, **Okun MS**. A closer look at unilateral versus bilateral deep brain stimulation: results of the National Institutes of Health COMPARE cohort. *J Neurosurg*. 2010 Dec;113(6):1224-9. doi: 10.3171/2010.8.JNS10312. Epub 2010 Sep 17. Erratum in: *J Neurosurg*. 2013 Oct;119(4):1086. **PubMed PMID: 20849215.**
- Zahodne LB, **Okun MS**, Foote KD, Fernandez HH, Rodriguez RL, Wu SS, Kirsch-Darrow L, Jacobson CE 4th, Rosado C, Bowers D. Greater improvement in quality of life following unilateral deep brain stimulation surgery in the globus pallidus as compared to the subthalamic nucleus. *J Neurol*. 2009 Aug;256(8):1321-9. doi: 10.1007/s00415-009-5121-7. Epub 2009 Apr 12. **PubMed PMID: 19363633; PubMed Central PMCID: PMC3045861.**
- Okun MS**, Fernandez HH, Wu SS, Kirsch-Darrow L, Bowers D, Bova F, Suelter M, Jacobson CE 4th, Wang X, Gordon CW Jr, Zeilman P, Romrell J, Martin P, Ward H, Rodriguez RL, Foote KD. Cognition and mood in Parkinson's disease in subthalamic

nucleus versus globus pallidus interna deep brain stimulation: the COMPARE trial. *Ann Neurol*. 2009 May;65(5):586-95. doi: 10.1002/ana.21596. **PubMed PMID: 19288469; PubMed Central PMCID: PMC2692580.**

4. **Okun MS**, Wu SS, Fayad S, Ward H, Bowers D, Rosado C, Bowen L, Jacobson C, Butson C, Foote KD. Acute and Chronic Mood and Apathy Outcomes from a randomized study of unilateral STN and GPi DBS. *PLoS One*. 2014 Dec 3;9(12):e114140. doi: 10.1371/journal.pone.0114140. eCollection 2014. **PubMed PMID: 25469706; PubMed Central PMCID: PMC4254912.**
2. **Historical Background:** Cognitive and mood issues following DBS surgery can limit the overall success of this procedure, and there was a need for a better analysis of this issue. Scientific Problem: Issues such as verbal fluency declines were emerging as potentially limiting factors in deep brain stimulation surgery. Central Finding: Our studies revealed that verbal fluency was the most common cognitive side effect of DBS and it is now recognized as the most common cognitive side effect of DBS for Parkinson's disease. Our NIH COMPARE trial revealed that there was no difference in verbal fluency overall outcome whether blindly on or off stimulation, though outcome could be changed by target and by location of the stimulation field. We were the also the lead authors on the St. Jude randomized constant current DBS trial that led to FDA approval of a new DBS device. This trial had a randomized group with implants but no activation of the DBS, and it corroborated the findings of the initial NIH COMPARE trial. It also added to our previous decade of publications on the microlesional effects of DBS therapy. Influence of Finding: Our group contributed much of the basic understanding of the most common cognitive effect of deep brain stimulation and we demonstrated that the effect was more surgical (traumatic) than stimulation induced. The effect could be modulated somewhat within the target by altering the stimulation field. Patients and clinicians now use this information in pre-operative discussions and post-operative management. Additionally, much of our early work has helped to define lesion and placebo effects of DBS. Specific Role: PI of the NIH grant, and lead author on the randomized St. Jude trial.

Relevant Papers:

1. **Okun MS**, Foote KD, Wu SS, Ward HE, Bowers D, Rodriguez RL, Malaty IA, Goodman WK, Gilbert DM, Walker HC, Mink JW, Merritt S, Morishita T, Sanchez JC. A trial of scheduled deep brain stimulation for Tourette syndrome: moving away from continuous deep brain stimulation paradigms. *JAMA Neurol*. 2013 Jan;70(1):85-94. doi: 10.1001/jamaneurol.2013.580. **PubMed PMID: 23044532.**
2. Dietz J, Noecker AM, McIntyre CC, Mikos A, Bowers D, Foote KD, **Okun MS**. Stimulation region within the globus pallidus does not affect verbal fluency performance. *Brain Stimul*. 2013 May;6(3):248-53. doi: 10.1016/j.brs.2012.05.011. Epub 2012 Jun 16. **PubMed PMID: 22766102; PubMed Central PMCID: PMC3491090.**
3. Mikos A, Bowers D, Noecker AM, McIntyre CC, Won M, Chaturvedi A, Foote KD, **Okun MS**. Patient-specific analysis of the relationship between the volume of tissue activated during DBS and verbal fluency. *Neuroimage*. 2011 Jan;54 Suppl 1:S238-46. doi: 10.1016/j.neuroimage.2010.03.068. Epub 2010 Mar 31. **PubMed PMID: 20362061; PubMed Central PMCID: PMC2908727.**
4. Zahodne LB, **Okun MS**, Foote KD, Fernandez HH, Rodriguez RL, Kirsch-Darrow L, Bowers D. Cognitive declines one year after unilateral deep brain stimulation surgery in Parkinson's disease: a controlled study using reliable change. *Clin Neuropsychol*. 2009 Apr;23(3):385-405. doi: 10.1080/13854040802360582. Epub 2008 Sep 23. **PubMed PMID: 18821180; PubMed Central PMCID: PMC3045862.**
3. **Historical Background:** There are many neuropsychiatric diseases and symptoms that may possibly be addressed by DBS. Scientific Problem: Understanding targets and symptoms as well as developing a safety profile and outcome predictors for DBS in Obsessive Compulsive Disease and Tourette syndrome has been a challenge. Additionally, the development of non-continuous stimulation strategies (scheduled and responsive) will be important to the future of the field. Central Finding: We performed the first NIH funded trial of OCD DBS and we have performed two other studies in Tourette DBS showing the potential for scheduled rather than continuous stimulation. We are also developing outcome predictors for DBS in neuropsychiatric disorders inclusive of clinical (e.g. the smile response) and physiological measures (e.g. oscillation changes). We have also performed the first human closed loop DBS experiments on Tourette patients. Our laboratory runs the Tourette Syndrome Association International DBS registry, Influence of Finding: Our data was pooled and used for the FDA HDE approval for OCD and we plan to submit our data to the FDA for Tourette syndrome. We are helping to pioneer the necessary database monitoring for DBS in neuropsychiatric diseases. We also hold the DBS think tank each year at UF and there is an associated published proceedings. The think tank is designed to create cutting edge DBS collaboration for neurological and neuropsychiatric diseases. We have recently led the publication for the updated recommendations for Tourette DBS in Movement Disorders. Closed loop smart DBS may provide an important alternative to standard DBS and may be used to modulate individual symptoms in real-time. Our lab has ongoing closed loop experiments. Specific Role: Co-I on NIH OCD grant, PI on two NIH Tourette DBS grants.

R01NS075012-01A1 Vaillancourt (PI) 07/16/12-07/15/17
NIH
Non-Invasive Markers of Neurodegeneration in Movement Disorders
The Major Goal of this project is to examine the imaging aspects of neurodegenerative disorders such as Parkinson's disease and no examine imaging changes that may serve as markers of disease.
Role: Co-Investigator

TSA International Data Base **Okun (PI)** 11/01/12-06/3019
TSA International Database of deep Brain Stimulation Studies in Tourette Syndrome
The Major Goal of this project is to create an international registry of Tourette DBS procedures. The database contains DBS procedure parameters, scale measurements, and more information on an individual's deep brain stimulation surgery for the treatment of Tourette Syndrome.
Role: PI

NEUROMODULATION 2014 **Okun (PI)** 01/16/15-01/15/17
Michael J Fox Foundation (MJFF)
A Responsive Closed-Loop Approach to Treat Freezing of Gait in Parkinson's Disease
The major goal of this study is to provide a rapid, automated closed-loop algorithm prototyping. Our approach will identify the local field potentials (LFP) occurring in GPI and PPN during normal walking and during maneuvers known to instigate freezing episodes. We will use an algorithm to facilitate a responsive train of stimulation to break freezing episodes.
Role: PI

NIH CTSI KL2 Gunduz (PI) 01/15/15-01/14/17
The Human Tic Detector: A Responsive Deep Brain Stimulator for the Treatment of Tourette Syndrome
The goal of this study is to detect the neural signatures of Tourette Syndrome to initiate and terminate deep brain stimulation.
Role: Mentor

UF Research Foundation Gunduz (PI) 06/01/15-05/31/17
Uncovering an Electrical Biomarker for Freezing of Gait in Parkinson's Disease
The goal of this study is to investigate biomarkers of freezing of gait in Parkinson's Disease during ambulation using wireless EEG systems.
Role: Co-PI

Publications 2016

Randomized, Blinded Pilot Testing of Nonconventional Stimulation Patterns and Shapes in Parkinson's Disease and Essential Tremor: Evidence for Further Evaluating Narrow and Biphasic Pulses (2016) U Akbar, RS Raike, N Hack, CW Hess, J Skinner, D Martinez-Ramirez, S DeJesus, **MS Okun** *Neuromodulation: Technology at the Neural Interface*

TA Mestre, AE Lang, **MS Okun**. Factors influencing the outcome of deep brain stimulation: Placebo, nocebo, lessebo, and lesion effects (2016) *Movement Disorders* 31 (3), 290-298

Is blast injury a modern phenomenon?: early historical descriptions of mining and volcanic traumatic brain injury with relevance to modern terrorist attacks and military warfare. (2016) LN Bowen, DF Moore, **MS Okun**; *The neurologist* 21 (2), 19-22

Comparison of Two Methods for Inducing Reflex Cough in Patients With Parkinson's Disease, With and Without Dysphagia (2016) KW Hegland, MS Troche, A Brandimore, **MS Okun**, PW Davenport; *Dysphagia* 31 (1), 66-73

Motivational engagement in Parkinson's disease: Preparation for motivated action (2016) JB Renfroe, MM Bradley, **MS Okun**, D Bowers; *International Journal of Psychophysiology* 99, 24-32

Association of Parkinson disease age of onset with DRD2, DRD3 and GRIN2B polymorphisms (2016) A Hassan, MG Heckman, JE Ahlskog, ZK Wszolek, DJ Serie, RJ Uittik, JA van Gerpen, **MS Okun**, S Rayaprolu, OA Ross; *Parkinsonism & related disorders* 22, 102-105

Mood Differences Among Parkinson's Disease Patients With Mild Cognitive Impairment (2016) JD Jones, P Mangal, J Lafo, **MS Okun**, D Bowers; *The Journal of neuropsychiatry and clinical neurosciences*, appi. *neuropsych* 15090221

Gray and White Matter Contributions to Cognitive Frontostriatal Deficits in Non-Demented Parkinson's Disease (2016) CC Price, J Tanner, PT Nguyen, NA Schwab, S Mitchell, E Slonena, B Brumback, **MS Okun**, TH Mareci, D Bowers; *PloS one* 11 (1), e0147332

Proceedings of the Fourth Annual Deep Brain Stimulation Think Tank: A Review of Emerging Issues and Technologies (2016) W Deeb, JJ Giordano, PJ Rossi, AY Mogilner, A Gunduz, JW Judy, BT Klassen, CR Buston C Van Horne, D Deny, DD Dougherty, D Rowell, GA Gerhardt, GS Smith, FA Ponce, HC Walker, HM Bronte-Stewart, HS Mayberg, HJ Chizeck, JP Langevin, J Volkmann, JL Ostrem, JB Shute, J Jimenez-Shahed, KD Foote, AW Shukla, MA Rossi, M Oh, M Pourfar, PB Rosenberg, PA Silburn, C de Hemptine, PA Starr, T Denison, U Akbar, WM Grill, **MS Okun**; *Frontiers in Integrative Neuroscience* 10

Occurrence of Dysphagia Following Botulinum Toxin Injection in Parkinsonism-related Cervical Dystonia: A Retrospective Study (2016) A Patterson, L Almeida, CW Hess, D Martinez-Ramirez, **MS Okun**, RL Rodriguez, V Rundle-Gonzalez, AW Shukla, IA Malaty; *Tremor and Other Hyperkinetic Movements* 6

New Strategies to Optimize Care for Patients With Parkinson's Disease Psychosis (2016) JG Goldman, **MS Okun**, D Weintraub; *Supplement to Neurology Reviews*; *mededibus.com*

Parkinson Disease: Treatment (2016) D Martinez-Ramirez, **MS Okun** *Scientific American* 5, 16

State of the Art for Deep Brain Stimulation Therapy in Movement Disorders: A Clinical and Technological Perspective (2016) AW Shukla, **MS Okun**; *IEEE Reviews in Biomedical Engineering* 9, 219-233

A phase II study of fornix deep brain stimulation in mild Alzheimer's disease (2016) AM Lozano, L Fosdick, MM Chakravarty, JM Leoutsakos, C Munro, E Oh, KE Drake, CH Lyman, PB Rosenberg, WS Anderson, DF Tang-Wai, JC Pendergrass, S Salloway, WF Asaad, FA Ponce, A Burke, M Sabbagh, DA Wolk, G Baltuch, **MS Okun**, KD Foote, MP McAndrews, P Giacobbe, SD Targum, CG Lyketsos, GS Smith; *Journal of Alzheimer's Disease* 54 (2), 777-787

Rare copy number variants in NRXN1 and CNTN6 increase risk for Tourette syndrome(2016) AY Huang, D Yu, LK Davis, JH Sul, F Tsetsos, V Ramensky, I Zelaya, EM Ramos, L Osiecki, JA Chen, LM McGrath, C Illmann, P Sandor, CL Barr, M Grados, HS Singer, MM Noethen, J Hebebrand, RA King, Y Dion, G Rouleau, CL Budman, C Depienne, Y Worbe, A Hartmann, KR Muller-Vahl, M Stuhmann, H Aschauer, M Stamenkovic, M Schloegelhofer, A Konstantinidis, GL Lyon, WM McMahon, C Barta, Z Tarnok, P Nagy, JR Batterson, R Rizzo, DC Cath, T Wolanczyk, C Berlin, IA Malaty, **MS Okun**, DW Woods, ERees, CN Pato, MT Pato, JA Knowles, D Posthuma, DL Pauls, NJ Cox, BM Neale, NB Freimer, P Paschou, CA Mathews, J M Scharf, G Coppola; *bioRxiv*, 062471

apathy, novelty Processing, and the P3 Potential in Parkinson's Disease (2016) DAS Kaufman, D Bowers, **MS Okun**, R Van Patten, WM Perlstein; *Frontiers in neurology* 7

A nonlinear regression technique for manifold valued data with applications to Medical Image Analysis (2016) M Banerjee, R Chakraborty, E Ofori, **MS Okun**, DE Viallancourt, BC Vemuri *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition* 4424-4432

Post-mortem Findings in Huntington's Deep Brain Stimulation: A Moving Target Due to Atrophy (2016) V Vedam-Mai, D Martinez-Ramirez, JD Hilliard, S Carbutaru, AT Yachnis, J Bloom, P Keeling, L Awe, KD Foote, **MS Okun**; *Tremor and Other Hyperkinetic Movements* 6

The International Deep Brain Stimulation Registry and Database for Gilles de la Tourette Syndrome: How Does It Work? (2016) W Deeb, PJ Rossi, M Porta, V Visser-Vandewalle, D Servello, P Silburn, T Coyne, JF Leckman, T Foltynie, MHariz, EM Joyce, L Zrinzo, Z Kefalopoulou, ML Welter, C Karachi, L Mallet, JL Houeto, J Shahed-Jimenez, FG Meng, BT Klassen, AY Mogilner, MH Pourfar, J Kuhn, L Ackermans, T Kaido, Y Temel, RE Gross, HC Walker, AM Lozano, SM Khandhar, BL Walter, E Walter, Z Mari, BK Changizi, E Moro, JC Baldermann, D Huys, SE Zauber, LE Schrock, JG Zhang, WHu, KD Foote, K Rizer, JW Mink, DW Woods, AGunduz, **MS Okun**; *Frontiers in neuroscience* 10

Proceedings of the Third Annual Deep Brain Stimulation Think Tank: A Review of Emerging Issues and Technologies (2016) PJ Rossi, A Gunduz, J Judy, L Wilson, A Machado, JJ Giordano, WJ Elias, MA Rossi, CL Butson, M D Fox, CC McIntyre, N Pouratian, NC Swann, C de Hemptinne, RE Gross, HJ Chizeck, M Tagliati, AM Lozano, W Goodman, JP Langevin, RL Alterman, UAkbar, GA Gerhardt, WM Grill, M Hallett, T Herrington, J Herron, C van Horne, BH Kopell, AE Lang, C Lungu, D Martinez-Ramirez, AY Mogilner, RMolina, E Opri, KJ Otto, KG Oweiss, Y Pathak, AW Shukla, J Shute, SA Sheth, LC Shih, GK Steinke, AI Tröster, N Vanegas, KA Zaghloul, L Cendejas-Zaragoza, L Verhagen, KD Foote, **MS Okun**; *Frontiers in neuroscience* 10

Deep Brain Stimulation Battery Longevity: Comparison of Monopolar Versus Bipolar Stimulation Modes (2016) L Almeida, PV Rawal, B Ditty, BL Smelser, H Huang, **MS Okun**, BL Guthrie, HC Harrison; *Movement Disorders Clinical Practice*

Association between antidepressants and falls in Parkinson's disease (2016) D Martinez-Ramirez, JC Giugni, L Almeida, R Walz, B Ahmed, FA Chai, V Rundle-Gonzalez, AR Bona, E Monari, AW Shukla, CW Hess, CJ Hass, **MS Okun**; *Journal of neurology* 263 (1), 76-82

Cystic Lesions as a Rare Complication of Deep Brain Stimulation (2016) VD Sharma, AR Bona, A Mantovani, S Miocinovic, P Khemani, MP Goldberg, KD Foote, LA Whitworth, S Chitnis, **MS Okun**; *Movement Disorders Clinical Practice* 3 (1), 87-90

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Porges, Eric

eRA COMMONS USER NAME (credential, e.g., agency login): eporges

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)

Table with 4 columns: INSTITUTION AND LOCATION, DEGREE (if applicable), Completion Date MM/YYYY, FIELD OF STUDY. Rows include Hampshire College, University of Chicago, and University of Florida.

A. Personal Statement

Dr. Porges is currently an Assistant Professor at the University of Florida, where he is a CTSA KL2 Scholar. He received his PhD from the University of Chicago in the field of Integrative Neuroscience under the mentorship of Dr. Jean Decety. His dissertation focused on individual differences in central and peripheral neurophysiological responses to social stressors, with an emphasis on the autonomic nervous system as a modulator of these responses.

The proposed training and research plan will provide the necessary training, mentorship, research experience, and pilot data to ensure that Dr. Porges will develop into an independent researcher with the skills necessary to secure R01 funding.

The proposed study will examine the impact of heavy alcohol consumption by HIV+ adults on cognitive flexibility (the ability to infer and switch between concepts or rules) and GABA concentrations measured using MRS.

Since joining the University of Florida, Dr. Porges has been very productive. He currently has had 19 peer-reviewed manuscripts accepted. Ten of these manuscripts, as well as a book chapter, were accepted in the past 12 months.

- 1. Seider TR, Gongvatana A, Woods AJ, Chen H, Porges EC, Cummings T, Correia S, Tashima K, Cohen RA. Age exacerbates HIV-associated white matter abnormalities. J Neurovirol. 2016 Apr;22(2):201-12. PubMed PMID: 26446690; PubMed Central PMCID: PMC4783252.
2. Porges Eric C, Woods AdamJ, Edden RA, Harris AD, Huaihou H, Garcia AM, Lamb DG, Williamson JohnB, Cohen RA. Frontal GABA concentrations are associated with cognitive performance in older adults. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2016; in press.

3. Woods AdamJ, **Porges Eric C**, Bryant V, Seider TR, Gongvatana A, Kahler CW, de la Monte S, Monti PM, Cohen RA. Heavy alcohol consumption is associated with greater cognitive impairment in older adults. *Alcoholism: Clinical and Experimental Research*. 2016; in press.
4. **Porges EC**, Decety J. Violence as a source of pleasure or displeasure is associated with specific functional connectivity with the nucleus accumbens. *Front Hum Neurosci*. 2013 Aug 13;7:447. **PubMed PMID: 23964226; PubMed Central PMCID: PMC3741555.**

B. Positions and Honors

Positions and Employment

- 1999 - 2002 Emergency Medical Technician, Hampshire College Emergency Medical Services, Amherst, MA
 2001 - 2002 Director of Hampshire College Emergency Medical Services, Hampshire College Emergency Medical Services, Amherst, MA
 2002 - 2002 Project Manager, Greenleaf Medical, Palo Alto, CA
 2003 - 2003 Intern, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL
 2004 - 2005 Research Coordinator, University of Illinois at Chicago, Chicago, IL
 2006 - 2008 Lab Manager, Social Cognitive Neuroscience Lab, University of Chicago, Chicago, IL
 2008 - 2013 Graduate Student, Integrative Neuroscience program, Department of Psychology, University of Chicago, Chicago, IL
 2013 - 2015 Postdoctoral Associate, Department of Aging and Geriatric Research, Institute on Aging, Center for Cognitive Aging and Memory, University of Florida, Gainesville, FL
 2016 - Assistant Professor, Center for Cognitive Aging and Memory, Institute on Aging, Department of Aging and Geriatric Research, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

- 2010 - Member, Society for Neuroscience
 2011 - Member, Society for Social Neuroscience
 2011 - Ad Hoc Reviewer, *International Journal Psychophysiology*
 2012 - Member, Cognitive Neuroscience Society
 2012 - Member, Society for Psychophysiological Research
 2012 - Social Neuroscience, Ad Hoc Reviewer
 2013 - Ad Hoc Reviewer, *Developmental Review*
 2014 - Review Editorial Board, *Frontiers in Psychology*; Emotion Science
 2015 - Review Editorial Board, *Frontiers in Psychology*, section Psychology for Clinical Settings
 2015 - Ad Hoc Reviewer, *Experimental Gerontology*

Honors

- 2010 Norman Henry Anderson Award, Department of Psychology at the University of Chicago
 2011 Research Award, University of Chicago Psychology graduate student organization
 2011 Norman Henry Anderson Award, Department of Psychology at the University of Chicago
 2012 Student Poster Award, Society for Psychophysiological Research
 2012 Travel Award, University of Chicago Psychology graduate student organization
 2012 Norman Henry Anderson Award, Department of Psychology at the University of Chicago
 2016 CTSA Institutional K Scholar, University of Florida

C. Contribution to Science

1. **Neurocognitive aging:** Neurochemical and anatomical changes that are protected by social behaviors are associated with changes in GABA concentrations and accelerated by physiological challenges such as HIV. My research has developed a theoretical framework to explain and predict these associated changes. Below are examples of recent work that investigates cognitive aging in a healthy aging cohort and an HIV+ population.
 - a. Seider TR, Gongvatana A, Woods AJ, Chen H, **Porges EC**, Cummings T, Correia S, Tashima K, Cohen RA. Age exacerbates HIV-associated white matter abnormalities. *J Neurovirol*. 2016 Apr;22(2):201-12. **PubMed PMID: 26446690; PubMed Central PMCID: PMC4783252.**
 - b. Seider TR, Fieo RA, O'Shea A, **Porges EC**, Woods AJ, Cohen RA. Cognitively Engaging Activity Is Associated with Greater Cortical and Subcortical Volumes. *Front Aging Neurosci*. 2016 May 2;8:94. **PubMed PMID: 27199740; PubMed Central PMCID: PMC4852201.**

- c. **Porges Eric C**, Woods AdamJ, Edden RA, Harris AD, Huaihou H, Garcia AM, Lamb DG, Williamson JohnB, Cohen RA. Frontal GABA concentrations are associated with cognitive performance in older adults. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. 2016; in press.
 - d. Clark DJ, Rose DK, Ring SA, **Porges EC**. Utilization of central nervous system resources for preparation and performance of complex walking tasks in older adults. *Front Aging Neurosci*. 2014 Aug 25; 6:217. **PubMed PMID: 25202270; PubMed Central PMCID: PMC4142860**.
2. **Individual differences in central and peripheral nervous system response to violent stimuli:** Violence is a salient stimulus and an important environmental signal with survival consequences, conveying information about potential threats to personal health and safety. This research employed a multilevel approach to identify features that contribute to individual differences in peripheral and central physiological responses to observed violence. Studies (a) and (b) employed behavioral manipulations that altered the within-subject relationship to violent stimuli. Study (c) employed functional connectivity analyses of brain imaging data and demonstrated that the modulation of neurophysiological recruitment of specific brain areas was related to individual differences in subjective appraisals. Study (d) employed measures of peripheral physiology to demonstrate an inverse relationship between parasympathetic tone and testosterone release to observed violence, demonstrating the influence of autonomic regulation on physiological response to external stimuli. The work presented here demonstrates across different methods that individual responses to observed violence can be predicted by preexisting traits and manipulated by altering a participant's relationship to the stimuli.
- a. **Porges EC**, Smith KE, Decety J. Individual differences in vagal regulation are related to testosterone responses to observed violence. *Front Psychol*. 2015 Feb 24;6:19. **PubMed PMID: 25759673; PubMed Central PMCID: PMC4338751**.
 - b. **Porges EC**, Decety J. Violence as a source of pleasure or displeasure is associated with specific functional connectivity with the nucleus accumbens. *Front Hum Neurosci*. 2013 Aug 13;7:447. **PubMed PMID: 23964226; PubMed Central PMCID: PMC3741555**.
 - c. Decety J, **Porges EC**. Imagining being the agent of actions that carry different moral consequences: an fMRI study. *Neuropsychologia*. 2011 Sep;49(11):2994-3001. **PubMed PMID: 21762712**.
 - d. Lamm C, **Porges EC**, Cacioppo JT, Decety J. Perspective taking is associated with specific facial responses during empathy for pain. *Brain Res*. 2008 Aug 28;1227:153-61. **PubMed PMID: 18619426**.
3. **Traumatic brain injury (TBI):** Patients with TBI often develop Post-Traumatic Stress Disorder (PTSD). This syndrome, defined and diagnosed by psychological and behavioral features, is associated with symptoms such as anxiety, anger, increased arousal, and vigilance, as well as flashbacks and nightmares. Several of the symptoms observed in PTSD may be in part the result of altered autonomic nervous system (ANS) activity in response to psychological and physical challenges. Brain imaging has documented that TBI often induces white matter damage to pathways associated with the anterior limb of the internal capsule and uncinate fasciculus. Since these white matter structures link neocortical networks with subcortical and limbic structures that regulate autonomic control centers, injury to these pathways may induce a loss of inhibitory control of the ANS. Our work suggests that TBI-induced damage to networks that regulate the ANS increase vulnerability to PTSD. This provides the possibility that vulnerability to PTSD can be measured in patients with TBI.
- a. Falchook AD, **Porges EC**, Nadeau SE, Leon SA, Williamson JB, Heilman KM. Cognitive-motor dysfunction after severe traumatic brain injury: A cerebral interhemispheric disconnection syndrome. *J Clin Exp Neuropsychol*. 2015;37(10):1062-73. **PubMed PMID: 26340588**.
 - b. Williamson JB, **Porges EC**, Lamb DG, Porges SW. Maladaptive autonomic regulation in PTSD accelerates physiological aging. *Front Psychol*. 2015 Jan 21;5:1571. **PubMed PMID: 25653631; PubMed Central PMCID: PMC4300857**.
 - c. Williamson JB, Heilman KM, **Porges EC**, Lamb DG, Porges SW. A possible mechanism for PTSD symptoms in patients with traumatic brain injury: central autonomic network disruption. *Front Neuroeng*. 2013 Dec 19;6:13. **PubMed PMID: 24391583; PubMed Central PMCID: PMC3867662**.
4. **Neuroendocrine function:** Neuroendocrine functions related to individual variability in response to high intensity social stimuli can impact the quality of interpersonal relationships and health outcomes. At the extremes, these differences can lead to interpersonal conflict or a strengthening of social bonds. I have had a long-term interest in exploring central and peripheral physiological predictors (e.g., parasympathetic activity) of individual differences in response to high-intensity social stimuli (e.g., violence and parental interaction). Note: Smith and Porges are co-first authors on "Oxytocin receptor gene variation predicts empathic concern and autonomic arousal while perceiving harm to others."

- a. Ebner NC, Chen H, **Porges E**, Lin T, Fischer H, Feifel D, Cohen RA. Oxytocin's effect on resting-state functional connectivity varies by age and sex. *Psychoneuroendocrinology*. 2016 Jul;69:50-9. **PubMed PMID: 27032063; PubMed Central PMCID: PMC4942126.**
 - b. Zamzow RM, Ferguson BJ, Stichter JP, **Porges EC**, Ragsdale AS, Lewis ML, Beversdorf DQ. Effects of propranolol on conversational reciprocity in autism spectrum disorder: a pilot, double-blind, single-dose psychopharmacological challenge study. *Psychopharmacology (Berl)*. 2016 Apr;233(7):1171-8. **PubMed PMID: 26762378.**
 - c. Smith KE, **Porges EC**, Norman GJ, Connelly JJ, Decety J. Oxytocin receptor gene variation predicts empathic concern and autonomic arousal while perceiving harm to others. *Soc Neurosci*. 2014 Feb;9(1):1-9. **PubMed PMID: 24295535; PubMed Central PMCID: PMC3923324.**
 - d. Carter CS, **Porges EC**. Parenthood, stress, and the brain. *Biol Psychiatry*. 2011 Nov 1;70(9):804-5. **PubMed PMID: 21986092.**
5. Advanced neuroimaging: Dr. Porges has played an integral role in the development and application of advanced neuroimaging methods to target populations. These inquiries have generated novel findings, including specific and unique functional connectivity from amygdala sub-nuclei to cortical targets that are predicted by psychopathic traits and the first exploration of the relationship between GABA MRS and higher-order cognitive function in older adults. Several of these advanced methods, including those for functional connectivity, have been disseminated in methods papers.
- a. Chen H, Zhao B, **Porges EC**, Cohen RA, Ebner NC. Edgewise and subgraph-level tests for brain networks. *Stat Med*. 2016 Nov 30;35(27):4994-5008. **PubMed PMID: 27397632; PubMed Central PMCID: PMC5096985.**
 - b. **Porges Eric C**, Woods AdamJ, Edden RA, Harris AD, Huaihou H, Garcia AM, Lamb DG, Williamson JohnB, Cohen RA. Frontal GABA concentrations are associated with cognitive performance in older adults. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. 2016; in press.
 - c. Chen H, Zhao B, Guanqun C, **Porges EC**, O'Shea A, Woods AdamJ, Cohen RA. Statistical approaches for the study of cognitive and brain aging. *Frontiers in Aging Neuroscience*. 2016; in press.
 - d. Yoder KJ, **Porges EC**, Decety J. Amygdala subnuclei connectivity in response to violence reveals unique influences of individual differences in psychopathic traits in a nonforensic sample. *Hum Brain Mapp*. 2015 Apr;36(4):1417-28. **PubMed PMID: 25557777; PubMed Central PMCID: PMC4837469.**

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1KL2TR001429-01, CTSI University of Florida

Porges, Eric (PI)

07/01/16-06/30/18

Cognitive and functional deficits associated with reduced cortical GABA in HIV-infected heavy drinkers: Potential target for clinical intervention

This project will investigate important hypotheses regarding the relationship between regional cerebral γ -aminobutyric acid (GABA) concentrations and cognitive flexibility in HIV+ heavy drinkers. To ensure an independent career post award, two critical areas of training will be addressed: 1) Behavioral and biological consequences of alcohol use in the context of HIV and 2) the development of expertise in the measurement of γ -Aminobutyric acid (GABA), the principal inhibitory neurotransmitter, using Magnetic Resonance Spectroscopy (MRS). The PI is a cognitive neuroscientist with a strong research background in aging, cognition, experimental design, autonomic measurement, and magnetic resonance imaging (fMRI & MRI).

Role: PI

F31AA024060, NIAAA

Vaughn Bryant (PI)

05/15/15-04/30/18

Working memory: a critical factor underlying alcohol reduction intervention response

Project to evaluate the role of working memory function in response to an effective alcohol reduction intervention in HIV+/- adults. The student will receive training in functional and structural MRI.

Role: CSU

IK2 RX000707, VA Rehabilitation Research & Development

John B. Williamson (PI)

01/08/13-03/31/17

White matter changes and mild TBI: Emotional and autonomic consequences

Department of Veterans Affairs funding to investigate the interaction of mTBI white matter damage and PTSD, resulting in dysregulation of autonomic nervous system.

Role: Co-Investigator

Neuroimaging Consortium Grant , McKnight Brain Research Foundation

Clinton Wright (PI)

05/01/15-05/01/17

UF Neuroimaging Consortium Cohort

The goal of this project is to develop a cohort of 200 adults 85 years and older across four sites using multimodal neuroimaging and cognitive assessment.

Role: PDA

BIOGRAPHICAL SKETCH

NAME: Williamson, John B

eRA COMMONS USER NAME: wjohnb

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
The Florida State University, Tallahassee Florida	BA	04/1996	Psychology
Virginia Polytechnic Institute and State University, Blacksburg, VA	PHD	05/2004	Clinical Psychology, Neuropsychology
University of Chicago, Chicago IL	Resident	07/2004	Clinical Psychology Internship
University of Illinois, Chicago	Postdoctoral Fellow	07/2006	Neuropsychology
University of Illinois, Chicago	Postdoctoral Fellow	07/2008	Neuroscience

A. Personal Statement

I am a clinical scientist (neuropsychologist). I have conducted clinical neuroscience research that has incorporated neuroimaging, cognitive and autonomic data in the study of neurodegenerative disease, emotion, memory and cognitive function in the context of TBI and cerebrovascular disease. In my current role, I am the PI on a study examining factors influencing emotional, cognitive and physiological differences in patients with TBI in the context of white matter changes (CDA-2 supported). I have over 40 peer-reviewed research articles related in topics and I have been the PI or Co-I on multiple grants funded by the NIH and the VA that employ neuroimaging, neuropsychological, and psychophysiological methods. I completed postdoctoral work (NIH F32 supported study) with Dr. Stephen Porges, focusing on psychophysiological methods and applications to disease models. I am currently the PI on an NIH supported R56 to understand the relationship between changes in cardiac function, cerebral hemodynamics and executive function.

I recently completed a pilot project funded by our Center of Excellence (BRRRC, VA) to examine the impact of an intervention on emotional cognition and autonomic behavior in patients with TBI and PTSD. I am the sub-PI on a testbed for controlling hypothalamic nuclei for an NSF proposed pending engineering research center to understand how the autonomic nervous system can be manipulated to produced better behavioral outcomes.

B. Positions and Honors

Positions and Employment

- 2012- Research Psychologist, Dept of Veteran Affairs, Gainesville FL
- 2008-2012 Research Health Scientist. Dept of Veteran Affairs, Gainesville FL
- 2009-2012 Research Assistant Professor, Department of Neurology University of Florida
- 2012- Assistant Professor (tenure track), Department of Neurology, University of Florida
- 2013- Assistant Professor Departments of Aging and Geriatric Research, and Clinical and Health Psychology, University of Florida
- 2016- Assistant Professor, Department of Neuroscience, University of Florida

Other Experience and Professional Memberships

- 2002- Member, International Neuropsychological Society
- 2008- Member, Florida Society of Neurology
- 2013- Member, American Academy of Clinical Neuropsychology

C. Contributions to Science

1. *Advanced understanding of neurophysiological and cognitive consequences of mood and personality trait differences.* Dr. Williamson's early research focused on the role of differences in fronto-subcortical brain systems and laterality as a function of subclinical individual differences in mood and personality states and traits in the manifestation of autonomic mobilization to regional brain tasks. We demonstrated that, in a college aged population, that high trait hostility resulted in elevated autonomic responses to tasks that recruited right hemisphere resources and that performance on these right hemisphere tasks was also degraded compared to their low trait hostility peers. Further we showed motor asymmetries in children and men with symptoms of depression and hostility. This research has been replicated multiple times by other groups and lead to a capacity model for understanding the interaction of personality traits on psychophysiological profiles that have been

correlated to cardiovascular and cerebrovascular diseases later in life.

- a) **Williamson JB**, Harrison DW. Functional cerebral asymmetry in hostility: A dual task approach with fluency and cardiovascular regulation. *Brain and Cognition* 2003; 52:167-174.
 - b) Demaree HA, Higgins D, **Williamson JB**, Harrison DW. Asymmetry in handgrip strength and fatigue in low- and high-hostile men. *International Journal of Neuroscience* 2002; 112:415-428.
 - c) Everhart DE, Harrison DW, Shenal BV, **Williamson JB**, Wuensch KL. Grip-strength, fatigue and motor perseveration in anxious men without depression. *Neuropsychiatry, neuropsychology and behavioral neurology* 2002; 15:122-142.
 - d) Emerson CS, Harrison DW, Everhart D, **Williamson JB**. Hand fatigue asymmetry in motor performances of depressed boys. *Neuropsychiatry, neuropsychology, and behavioral neurology* 2001; 14:130-134.
2. *Furthered the knowledge base of factors relating to cognition, emotion and autonomic disturbance in cerebrovascular disease and neurological injury.* Because of relationships between autonomic disruptions in trait hostility and other mood related features to later development of cardiovascular and cerebrovascular disease, Dr. Williamson became interested in research aimed at achieving a greater understanding of the bases of vascular dementia, and the contributions of vascular factors to the development of cognitive and emotional dysfunction in the elderly. This led to numerous studies of vascular cognitive impairment (dementia precursor) resulting in evidence showing the contribution of WMHs in VCI to cognition and also Dr. Williamson's early work on the use of DTI as a sensitive tool for assessing the relationship of regional white matter disruption on cognitive and mood indicators. Further, Dr. Williamson was funded by an F32 mechanism to study the relationship of regional white matter disease in stroke patients on mobilization of autonomic resources to perform cognitive and motor tasks.
- a) **Williamson JB**, Nyenhuis DL, Pedelty L, Byrd S, Jhaveri M, Wang C, deTeledo-Morrell L, Sripathirathan K, Gorelick P. Baseline differences between Vascular Cognitive Impairment No Dementia reverts and nonreverts. *Journal of Neurology, Neurosurgery, and Psychiatry* 2008;79:1208-1214.
 - b) **Williamson JB**, Nyenhuis DL, Stebbins GT, Gorelick PB. Regional differences in apparent white matter integrity, cognition and mood in patients with ischemic stroke. *Journal of Clinical and Experimental Neuropsychology* 2010, 32, 673-681.
 - c) **Williamson JB**, Lewis GF, Grippo A, Lamb D, Harden E, Handleman M, Lebow J, Carter CS, Porges SW. Autonomic predictors of recovery following surgery: A comparative study. *Autonomic Neuroscience* 2010:156, 60-66.
 - d) **Williamson JB**, Lewis GF, Nyenhuis DL, Stebbins GT, Murphy C, Handelman M, Harden E, Heilman KM, Gorelick PB, Porges SW. The effects of cerebral white matter changes on cardiovascular responses to cognitive and physical activity in a stroke population. *Psychophysiology* 2012; 49:1618-1628.
3. *Elucidated impact of chronic lateralized stroke on spatial cognition as well as normal perturbations of sensory performance on laterality of spatial cognition and autonomic support.* These efforts led to several related lines of investigation to examine risk factors contributing to the development of spatial performance deficits in patients with cerebrovascular disease.
- a) **Williamson JB**, Haque S, Harciarek M, Burtis DB, Lamb D, Zilli E, Heilman KM. The influence of stimulus proximity on judgments of spatial location in patients with chronic unilateral right and left hemisphere stroke. *Journal of Clinical and Experimental Neuropsychology* 2014; 36:787-793.
 - b) Finney G, **Williamson JB**, Burtis DB, Drago V, Mizuno T, Jeong Y, Crucian G, Haque S, Heilman KM. Effects of chronic right hemisphere damage on the allocation of spatial attention: Alterations of accuracy and reliability. *Journal of the International Neuropsychological Society* 2015; 21:1-5.
 - c) Burtis DB, **Williamson JB**, Mishra M, Heilman KM. The blindside: Impact of monocular occlusion on spatial attention. *Journal of Clinical and Experimental Neuropsychology* 2013; 35: 291-297.
 - d) Burtis DB, Heilman KM, Mo J, Wang C, Lewis GF, Davilla MI, Ding M, Porges SW, **Williamson JB**. The effects of constrained left and right monocular viewing on the autonomic nervous system. *Biological Psychology* 2014; 100:79-85.
4. Provided theoretical model to advance the understanding of traumatic brain injury on manifestation of emotional dysregulation and also the impact of chronic emotional dysregulation on accelerated aging. TBI and PTSD are both critical issues that affect today's veteran population. Understanding neurological mechanisms of emotional disruption in this population is critical to developing appropriate treatments. The presented models provide clear testable hypotheses that may lead to effective diagnosis and treatments for this population. This work is ongoing (Williamson's CDA-2) and we are developing several lines of inquiry from the project including a CDA-2 submission this cycle (Damon Lamb) on tVNS and its impact in the context of our model on GABA and fMRI shifts in the limbic system in patients with mTBI/PTSD and the proposed merit submission integrating my mechanistic work (CDA-2) and the impact of tVNS on emotional cognition/autonomic behavior.
- a) **Williamson JB**, Heilman KM, Porges EC, Lamb DG, Porges SW. A possible mechanism for PTSD symptoms in patients with traumatic brain injury: central autonomic disruption. *Frontiers in neuroengineering* 2013.
 - b) **Williamson JB**, Porges EC, Lamb DG, Porges SW. Maladaptive autonomic regulation in PTSD accelerates physiological aging. *Frontiers in psychology* 2015.

My bibliography is available at: <http://www.ncbi.nlm.nih.gov/sites/myncbi/john.williamson.2/bibliography/48036192/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support

NIH 1R56HL127175-01

09/01/2015-08/31/2017

Brain and cognition effects of cardio resynchronization therapy in heart failure.

The goal of this funding is to characterize brain changes as a result of improved cardiac output in heart failure including hemodynamic, task dependent regional brain activation and brain metabolic changes associated with improved cognitive performance and brain health.

Role: PI

VAMC 1 LK2RX000707-09 CDA-2

4/01/2012 – 03/31/2017

White matter changes and mild TBI: Emotional and autonomic consequences.

The goal of this funding is to extend knowledge of white matter damage contributions after TBI to the development of emotional dysregulation in veterans with PTSD. Preliminary analyses demonstrate independent (of PTSD symptom severity) contributions of TBI to emotional cognition. White matter and fMRI post-processing is ongoing.

Role: PI

VAMC Merit Review

10/2012-10/2016

Vertical Neglect

The goal of this funding is to examine the impact of unilateral stroke and aging on dorsal and ventral streams in vertical attention.

Role: Co-I (PI = Kenneth M. Heilman, MD)

McKnight Brain Research Foundation

Cohen (PI)

10/15/13-10/15/16

The ACTIVE Brain Study

The goal of this funding is to provide neuroimaging biomarkers of successful aging. Methods include MRI based measures including DTI, MRS (GABA, NAA, Cho, etc. . .), resting state fMRI, task dependent fMRI (CVMT, N-Back), NIH Tool Box, indices of personality trait and mood state, etc. . .

Role: Co-I

Completed Research Support

VAMC Merit Review 2008-2012

Approach-Avoidance Spatial Neglect

The goal of this funding was to examine the contribution of unilateral stroke to neglect.

Role: Co-I (PI = Kenneth Heilman)

1 F32 AG027648-01A1 2006-2008

NIA funded individual training grant

White matter integrity and autonomic stress response

The goal of this study was to provide data on the effect of white matter disease on mobilization of autonomic resources to perform cognitive tasks.

Role: PI

VAMC Career Development Award 1

2013-2015

Traumatic Brain Injury and Motor Disorders

This career developmental award was designed to assess lateralized motor disorder presentations in patients with TBI. The central hypothesis was that corpus callosal injury would result in different forms of apraxia at the left and right hand driven by the laterality of motor control and communication deficits induced by the injury preventing normal performance of motor activities. The primary findings of this study did demonstrate laterality differences and this work is currently under review at a high impact factor journal.

Role: Co-I (PI = Adam Falchook, MD).

VAMC BRRC Pilot Award 2014

External Non-invasive vagal nerve stimulation for the treatment of post-traumatic stress disorder.

The goal of this funding was to provide pilot data for the effect of transcutaneous vagal nerve stimulation on emotional cognition and physiology in patients with TBI and PTSD. Preliminary data analyses demonstrate alleviation of anxiety (state) in patients with TBI/PTSD.

Role: PI

Adam Joshua Woods, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Woods, Adam Joshua

eRA COMMONS USER NAME (credential, e.g., agency login): AJWOODS

POSITION TITLE: Assistant Professor of Aging and Geriatric Research

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Alabama at Birmingham	B.S.	05/03	Psychology
George Washington University	Ph.D.	05/10	Cognitive Neuroscience
University of Pennsylvania	Post-Doctoral	06/13	Cognitive Neuroscience

A. Personal Statement

Dr. Woods is Assistant Director of the Center for Cognitive Aging and Memory (CAM) in the McKnight Brain Institute at UF. He is the Director of the Neurophysiology and Neuromodulation Research Core in the CAM. Dr. Woods is also an Assistant Professor in the Department of Clinical and Health Psychology at UF, with a joint appointment to Neuroscience. He is a cognitive neuroscientist with expertise in non-invasive brain stimulation and neuroimaging. He is a national leader in the field of transcranial direct current stimulation (tDCS), and runs an international training workshop for this technology, held in the past in NYC, Singapore, Gainesville, and other locations each year. He has trained over 500 researchers and clinicians around the world to safely and appropriately use tDCS. He also works with numerous groups around the country for ongoing tDCS collaborations at the University of Pennsylvania, University of Arkansas for Medical Sciences, University of California San Diego, University of Arizona, University of New Mexico and University of Miami. Dr. Woods' research focuses on discovery and application of novel non-invasive brain stimulation interventions for enhancing cognitive function in adults with and without neurodegenerative disease. This includes work in a variety of comorbid conditions that may accelerate the brain aging process, including HIV, stroke, obesity, and surgery. Dr. Woods has expertise in multi-disciplinary cognitive neuroscience methodologies (MRI/fMRI, electrophysiology, non-invasive brain stimulation), extensive experience with aging-related disorders, and past research with neurological diseases. His background, experience, and training in analysis and interpretation of human neuroimaging and tDCS outcomes will be important for his role as a scientific contributor on the current project. Dr. Woods has established a multimodal semi-automated neuroimaging pipeline using 1000 cores of the HiperGator super-computer at the University of Florida, specifically dedicated to the Center for Cognitive Aging and Memory. This infrastructure will serve as an important part of the overall success of the project. He is also the PI of the largest phase 3 RCT for non-invasive electrical brain stimulation using transcranial direct current stimulation (tDCS), the ACT study. Dr. Woods will not only use his expertise in non-invasive brain stimulation, but also neuroimaging to facilitate the interpretation and understanding of tDCS application in the current proposal. He will setup and analyze functional neuroimaging data from the N-Back working memory BOLD fMRI task in Specific Aim 3 of the proposal, a task that he has used successfully in his lab over the past five years in over 200 participants. This will build off of his existing work using this N-back task in scanner with tDCS. In addition, the N-back and working memory are a central component of his ongoing Phase III randomized clinical trial using tDCS paired with cognitive training: the ACT study (Woods, PI). ACT is the largest tDCS clinical trial to date. The information gained in the current proposal has great potential for enhancing clinical trials like ACT in the future. In summary, his primary role as a scientific contributor on this project will involve overseeing in scanner application of tDCS with an in scanner N-back working memory task, analyses of fMRI data, and assistance in interpretation and write-up of data related to this study.

- a. Bikson, M., Grossman, P., Thomas, C., Jiang, J., Adnan, T., Mourdoukoutas, P., Kronberg, G., Troung, D., Boggio, P., Brunoni, A., Charvet, L., Fregni, F., Fritsch, B., Gillick, B., Hamilton, R., Hampstead, B., Jankford, R., Kirton, A., Knotkova, H., Liebetanz, D., Liu, A., Loo, C., Nitsche, M., Richardson, J., Rotenberg, A., Turkeltaub, P., & **Woods, A.J.** Safety of transcranial Direct Current Stimulation (tDCS): evidence based update 2016. *Brain Stimulation*. Accepted June 2016, available online June 15, 2016. **PMCID: in process.**

- b. **Woods, A.J.**, Antal, A., Bikson, M., Boggio, P.S., Brunoni, A.R., Celnik, P., Cohen, L.G., Fregni, F., Herrmann, C.S., Kappenman, E., Knotkova, H., Liebetanz, D., Miniussi, C., Miranda, P.C., Paulus, W., Priori, A., Reato, D., Stagg, C., Wenderoth, N., Nitsche, M.A. (2016). A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clinical Neurophysiology*. 127(2): 1031-1048. **PMCID: PMC4747791**
- c. Porges, E.C., **Woods, A.J.**, Edden, R., Harris, A., Chen, H., Garcia, A., Lamb, D., Williamson, J.W., Cohen, R.A. Frontal GABA concentrations are associated with cognitive performance in older adults. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. Accepted June 2016. **PMCID: in process.**

B. Positions and Honors

Positions and Employment

- 2010-2013 Post-Doctoral Fellow, Department of Neurology, University of Pennsylvania, Philadelphia, PA
- 2013- Assistant Professor, Department of Aging and Geriatric Research, University of Florida, Gainesville, FL
- 2013-2014 Cognitive Aging and Memory Clinical Translational Research Program Scholar, University of Florida, Gainesville, FL
- 2013-2014 Pepper Scholar, Institute on Aging, University of Florida, Gainesville, FL
- 2014- Assistant Director, Center for Cognitive Aging and Memory, Institute on Aging, University of Florida, Gainesville, FL

Academic and Professional Honors

- 2006-2009 National Science Foundation (NSF) Graduate Research Fellowship
- 2008 Research Enhancement Fund grant award for advanced dissertation research, GWU
- 2009-2010 Graduate Research Fellowship, GWU
- 2009-2010 Thelma Hunt Research Fellowship in Psychology, GWU
- 2010-2013 Post-Doctoral Fellowship, Intellectual and Developmental Disabilities Research Center, Children's Hospital of Philadelphia
- 2013-2015 Pepper Center/CAM-CTRP Scholar, Cognitive Aging and Memory Clinical Translational Research Program, University of Florida, Gainesville, FL
- 2014 Appointed Assistant Director of the Center for Cognitive Aging and Memory
- 2014 KL2 Scholar, Clinical Translational Science Institute
- 2014 Junior Fellow of the World Academy of Arts and Sciences
- 2015 Young Investigator Award in Neuromodulation, NYC Neuromodulation 2015, New York, NY, USA

C. Contributions to Science

Over the past six years, I have focused my research on the technical and basic science application of non-invasive brain stimulation techniques as novel interventions for enhancement of cognitive function. This work includes both transcranial direct current stimulation and transcranial magnetic stimulation. To further the field, I co-founded a CME certified practical training course in tDCS that has trained over 500 researchers and students to safely and consistently apply this method of non-invasive brain stimulation. I have published numerous papers aimed at enhancing replicability and safety for the method, in addition to exploring its impact on a variety of cognitive functions in the brain. In addition, I was awarded the 2015 NYC Neuromodulation Young Investigator Award for my technical and educational contributions to the field. Furthermore, I just completed leading a 20-author field consensus paper on technical and methodological standards in the field of tDCS and related non-invasive brain stimulation tools, in addition to senior authorship on a 27 author field standards safety paper currently. Collectively, this work provides me with a strong foundation in the technical elements and application standards of tDCS.

- a. Minhas, P., Bikson, M., **Woods, A.J.**, Rosen, A., Kessler, S. (2012). *Transcranial direct current stimulation in the pediatric versus adult brain: A computational modeling study*. *IEEE Xplore: EMBC*, 63: 859-862. **PMCID: PMC3641645**
- b. Kessler, S., Minhas, P., **Woods, A.J.**, Rosen, A., Bikson, M. (2013). Dose considerations for transcranial direct current stimulation in children: a computational modeling study. *PLoS ONE*, 8(9): e76112. **PMCID: PMC3785412**
- c. **Woods, A.J.**, Bryant, V., Sacchetti, D., Gervits, F., Hamilton, R. (2015). Effects of electrode drift on transcranial direct current stimulation. *Brain Stimulation*. 8(3): 515-519. **PMCID: PMC4461479**
- d. Knotkova, H., **Woods, A.J.**, Bikson, M., Nitsche, M. (2015). Transcranial direct current stimulation (tDCS): What pain practitioners need to know. *Practical Pain Management*. 15:58-66.

My work in neuroimaging has focused on understanding what brain networks underlie cognitive processes and how these processes are altered by age and medical disorders exacerbating aging of the human brain. This work has primarily used structural and functional magnetic resonance imaging and diffusion weighted imaging, but now includes magnetic resonance spectroscopy. Through multimodal neuroimaging, this work aims to identify markers predictive of cognitive decline in older adults, as well as markers of intervention effectiveness.

- a. **Woods, A.J.**, Hamilton, R.H., Kranjec, A., Bikson, M., Minhaus, P., Yu, J., Chatterjee, A. (2014). Space, time, and causal inference in the human brain. *NeuroImage*, 92, 285-297. **PMCID: PMC4008651**
- b. Dotson, V.M., Szymkowicz, S.M., Sozda, C.N., Kirton, J.W., Green, M.L., O'Shea, A., McLaren, M.E., Anton, S.D., Manini, T.M. & **Woods, A.J.** (2015). Age differences in prefrontal thickness and volumes in middle aged to older adults. *Frontiers in Aging Neuroscience*. 7: 250. **PMCID: PMC4717301**
- c. Seider, T., Gongvatana, A., **Woods, A.J.**, Porges, E., Chen, H., Cummings, T., Kahler, C.W., Monti, P.M., Cohen, R.A. (2016). Age exacerbates HIV associated white matter abnormalities. *Journal of Neurovirology*. 22(2): 201-212. **PMCID: PMC4783252**
- d. Szymkowicz, S.M., McLaren, M.E., Kirton, J.W., O'Shea, A., **Woods, A.J.**, et al. (2016). Depressive Symptom Severity Is Associated with Increased Cortical Thickness in Older Adults. *International Journal of Geriatric Psychiatry*. 31(4): 325-333. **PMCID: PMC4724336**

Over the past ten years, I have studied attentional processes in the brain using a variety of attention research methods in spatial neglect following stroke and healthy cognitive populations to understand the relative contributions of frontal and parietal systems in attention. My prior work in the relative contribution of frontal vs. parietal brain systems to attentional processing will be an important factor facilitating my contribution to the current grant.

- a. Mennemeier, M., Pierce, C., Dowler, R., Chatterjee, A., Anderson, B., Jewell, G., **Woods, A.J.**, Mark, V.W. (2005). Biases in attentional orientation and magnitude estimation explain crossover: neglect is a disorder of both. *Journal of Cognitive Neuroscience*, 17, 1194-1211.
- b. **Woods, A.J.**, Mennemeier, M., Garcia-Rill, E., Meythaler, J., Mark, V.W., Jewell, G.R., Murphy, H. (2006). Bias in magnitude estimation following left hemisphere injury. *Neuropsychologia*, 44, 1406-12.
- c. **Woods, A.J.**, Lehet, M., Chatterjee, A. (2012). Context modulates the contribution of time and space in causal inference. *Frontiers in Psychology*, 3, 371. doi: 10.3389/fpsyg.2012.00371 **PMCID: PMC3498891**
- d. **Woods, A. J.**, Mennemeier, M., Garcia-Rill, E., Huitt, T., Chelette, K. C., McCullough, G., Munn, T., Brown, G., Kiser, T. S. (2012). Improvement in arousal, visual neglect, and perception of stimulus intensity following cold pressor stimulation. *Neurocase*, 18, 115-122. **PMCID: PMC3266979**

One area of in my prior work investigated the impact of stroke and aging on visual search and executive function. This work has spanned investigation of early development of visual search processes (age 2-18 years) to effects in later life (ages 60+) and following focal lesions to frontal and parietal brain systems. My background in age-related executive decline will be germane to the current project and interpretation of improvement following anti-inflammatory intervention.

- a. Mark, V.W., **Woods, A.J.**, Ball, K.K., Roth, D.L., Mennemeier, M. (2004). Disorganized search is not a consequence of neglect. *Neurology*, 63(1), 78-84.
- b. **Woods, A.J.**, Mark, V.W. (2007). Convergent validity of executive organization measures on cancellation. *Journal of Clinical and Experimental Neuropsychology*, 29(7), 719-723. **PMCID: PMC3275913**
- c. **Woods, A.J.**, Goksun, T., Chatterjee, A., Zeloni, S., Mehet, A., Smith, S. (2013). The development of organized visual search. *Acta Psychologica*. 143(2), 191-199. doi: 10.1016/j.actpsy.2013.03.008 **PMCID: PMC3651801**
- d. Piedimonte, A., **Woods, A.J.**, Chatterjee, A. (2015). Disambiguating ambiguous motion perception: what are the cues? *Frontiers in Psychology*. 6: 902. **PMCID: PMC4496557**

Much of my current and past work focuses on successful cognitive aging interventions, in a variety of populations. This work has evaluated not only the cognitive and functional consequences of aging and various disorders, but also improvement in these processes following intervention. This line of my research attempts to identify novel markers (e.g., neuroimaging, etc.) and methods for prevention (e.g., anti-inflammatory intervention) of age and disease related cognitive. This line of research is directly relevant to the current project.

- a. Mark, V.W., **Woods, A.J.**, Mennemeier, M., Abbas, S., Taub, E. (2006). Cognitive assessment for CI therapy in the outpatient clinic. *Neurorehabilitation*, 21, 139-46.
- b. **Woods, A.J.**, Mark, V.W., Pitts, A., & Mennemeier, M. (2011). Pervasive cognitive impairment in acute rehabilitation patients "without" brain injury. *PM&R*, 3(5), 426-432. **PMCID: PMC3275913**
- c. **Woods, A.J.**, Cohen, R.A., Pahor, M. (2013). Cognitive frailty: frontiers and challenges. *Journal of Nutrition, Health, and Aging*. 17, 741-743. **PMCID: PMC4471842**
- d. Anton, S., **Woods, A.J.**, Ashizawa, T., Barb, D., Buford, T., et al., Successful aging: Advancing the science of physical independence in older adults. *Aging Research Reviews*. 24, 304-27. **PMCID: PMC4661112**

Complete List of Published Work in MyBibliography: <http://www.ncbi.nlm.nih.gov/sites/myncbi/adam.woods.1/bibliography/45511051/public/?sort=date&direction=descending>

D. Research Support

Ongoing Research Support

NIA R01AG054077 (Woods/Cohen/Marsiske; MPis) 09/01/16-08/31/21

National Institutes of Health

Augmenting Cognitive Training in Older Adults (ACT)

This study is a Phase III definitive multi-site randomized clinical trial with an adaptive design that will establish the benefit of delivering adjunctive transcranial direct current stimulation (tDCS) with cognitive training in older adults to combat cognitive aging. This trial measures both trial success and intervention mechanisms using multimodal neuroimaging and magnetic resonance spectroscopy, as well as comprehensive neurocognitive and functional assessment.

Role: PI

NIA K01AG050707-A1 (Woods; PI) 09/30/16-05/31/21

NIH

Neuromodulation of Cognition in Older Adults

The goal of this study will be to investigate the ability of transcranial direct current stimulation to enhance the effectiveness of cognitive training targeting attention, speed of processing, and working memory function in older adults. Training will focus on cognitive aging interventions and advanced magnetic resonance imaging and spectroscopy methods.

Role: PD/PI

Industry Sponsored Trial (Woods; PI) 07/01/16-06/31/18

Osato Research Institute

Impact of Fermented Papaya Product on brain energetics, neuroinflammation, and cognition: The Efficient Brain Study

The goal of this study is to perform a pilot clinical trial investigating the influence of Fermented Papaya Product on brain energetics, neuroinflammation, and cognition in older adults with elevated systemic inflammation using multimodal neuroimaging (fMRI, DWI) and spectroscopy (31P, 1H-MRS), as well as assessment of systemic inflammation and cognition.

Role: PI

Samuel S. Wu, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Wu, Samuel S

eRA COMMONS USER NAME: gatorwu

POSITION TITLE: Professor and Associate Chair, Department of Biostatistics

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	MM/YY	FIELD OF STUDY
Peking University, Beijing, PR China	B.S.	08/1989	Probability & Statistics
Nankai University, Tianjin, PR China	M.A.	08/1992	Probability & Statistics
Cornell University, Ithaca, New York	M.S.	08/1997	Statistics
Cornell University, Ithaca, New York	Ph.D.	08/1998	Statistics

A. Personal Statement

I have published numerous journal articles at the cutting edge of trial design, particularly in the area of adaptive experiment. Also, I have led the experimental design, data management and statistical analysis on many clinical trials: deep brain stimulation effects on mood & cognition in Parkinson's disease; scheduled and responsive brain stimulation for the treatment of Tourette syndrome; Prevention Of Low back pain in the Military (POLM); a six-year study of locomotor training programs for stroke patients; a study of Biopsychosocial Influence on Shoulder Pain (BISP); Functional Ambulation: Standard Treatment vs. Electrical Stimulation Therapy (FASTEST) trial in chronic post-stroke subjects with foot drop; and a study to assess the safety and efficacy of the Bioness® StimRouter™ neuromodulation system in the treatment for patients with chronic pain of peripheral nerve origin. Currently, I am co-investigator on the following trials: Augmenting Cognitive Training in Older Adults, Scaling and Sequencing Motor Output in Humans, and Biopsychosocial Influence on Shoulder Pain (phase 2). Also, I have served as Director of Biostatistics Core at the VA Rehabilitation Outcome Research Center (2002-2012), the VA Brain Rehabilitation Research Center (2002-2014), and the UF Claude D. Pepper Older American Independence Center (2013-present). Thus, my training and experience have given me extensive practical knowledge of the issues faced by clinical researchers.

In addition, collaborating with Dr. Chen from computer science, we invented some privacy-preserving data collection methods, which enable that nobody sees the actual data, but standard statistical analysis can still be performed with the same results for masked data as for the original data. We have jointly published 5 papers, mainly in the area of data confidentiality and privacy protection.

Furthermore, I have strong experience to identify good treatment practices in tertiary clinical care centers through NPF's Quality Improvement Initiative. Also, I have a strong team who assists me in data management and analysis. Dr. Pei, Mr. Dai and Mr. Chen have worked with me as programmer and statistical research coordinator for several years. All of them have solid training in statistics and proficient skills in REDCap, SAS and R software.

In summary, my role in the project builds logically on my experience, my position, my interests and my proven abilities.

B. Positions and Honors

1998-2001	Research Assistant Professor, Department of Statistics, University of Florida
2001-2005	Assistant Professor, Department of Statistics, University of Florida
2005-2008	Assistant Professor, Dept. of Epidemiology & Health Policy Research, University of Florida
2008-2010	Tenured Associate Professor, Dept. of Epidemiology & Health Policy Research, Univ. of Florida
2010-2012	Associate Professor and Interim Chair, Department of Biostatistics, University of Florida
2012-2014	Associate Professor and Associate Chair, Department of Biostatistics, University of Florida
2002-2014	Director of Biostatistics Core, the VA RORC and the VA BRRC
2013-present	Director of Biostatistics Core, the UF Claude D. Pepper Older American Independence Center
2014-present	Professor and Associate Chair, Department of Biostatistics, University of Florida

Other Experience and Professional Memberships

Member, American Statistical Association, 1998 - present

Member, the Institute of Mathematical Statistics, 1998 - present

C. Contribution to Science

1. My primary research focus is on adaptive design of clinical trials and simultaneous statistical inference. Adaptive designs use continuously updated data to modify certain aspects of a trial without undermining its validity. Simultaneous inference techniques enable experimenters to answer many related questions efficiently while maintaining valid type I error control. Both topics are particularly relevant to medical research, where study information arrives incrementally and multiple cohorts often are compared on multiple outcomes across multiple time periods. I have published numerous journal articles at the cutting edge of clinical trial design. My accomplishments include developing methods for incorporating pilot study information into the testing procedure of a subsequent trial; establishing confidence limits for hypothesis tests commonly performed in two-stage, drop-the-losers clinical trials; deriving improved methods of estimating treatment effects in adaptive trial designs.
 - a. **Wu SS**, Tu Y, He Y. (2014). Testing for efficacy in adaptive clinical trials with enrichment. *Statistics in Medicine*. 33(16):2736-45. (PMID: 24577792)
 - b. Lu X, Sun A, **Wu SS**. (2013). On estimating the mean of the selected normal population under the LINEX loss function in two-stage adaptive designs, *Journal of Statistical Planning and Inference*, 143, 1215 - 1220.
 - c. Neal D, Casella G, Yang MCK, **Wu SS**. (2011). Interval estimation in two-stage, drop-the-losers clinical trials with flexible treatment selection, *Statistics in Medicine*, 30(23), 2804-2814. (PMID:21823142)
 - d. **Wu SS**, Wang W, Yang MCK. (2010). Interval estimation for drop-the-loser designs, *Biometrika*, 97, 405-418.
2. Collaborating with Dr. Chen from computer science, we invented technologies for privacy-preserving collection and analysis of confidential data (US Provisional Patent Application #1123). The method enables that nobody sees the actual data, but standard statistical analysis can still be performed with the same results for masked data as for the original data. Moreover, in contrast to previous work that performs masking at a central place after all data are collected, our masking procedure is performed in a distributed way at each participant's data-generating device: one subject (or a group of subjects) at a time, giving great flexibility for incremental data collection and processing. We believe these technologies will greatly increase people's willingness to reveal sensitive information in medical and social studies.
 - a. **Wu SS**, Chen SG, Burr D, and Zhang L. A New Data Collection Technique for Preserving Privacy, to appear in *Journal of Privacy and Confidentiality*.
 - b. **Wu SS**, Chen SG, Burr D, and Zhang L. *New Technologies for Full Privacy Protection in Data Collection and Analysis*, in *Proceedings of 2014 Joint Statistical Meetings*, Boston, Massachusetts, USA, August 2014.
 - c. Pei QL, Chen SG, Xiao Y, and **Wu SS**. *Privacy-preserving Data Collection and Its Application in HIV Studies*, to appear in *Current HIV Research*.
 - d. Tao L, **Wu SS**, Chen SG, Yang MCK. *Energy efficient algorithms for the RFID estimation problem*. Proc. IEEE INFOCOM10, pp. 1019-1027, San Diego, CA, US, March 2010.
3. In addition to methodological research, I have established as a productive collaborator for researchers on neurological disorders and stroke. I have acted as lead statistician and Co-director of the Data Management and Analysis Center for several randomized controlled clinical trials. Among them, Locomotor Experience Applied Post-Stroke (LEAPS) was the largest of its kind funded by the NIH's National Institute of Neurological Disorders and Stroke and National Center for Medical Rehabilitation Research.
 - a. George SZ, Parr J, Wallace MR, **Wu SS**, Borsa PA, Dai Y, Fillingim RB. (2014). Biopsychosocial influence on exercise-induced injury: genetic and psychological combinations are predictive of shoulder pain phenotypes. *J Pain*. 15(1):68 - 80. (PMID: 24373571)
 - b. Duncan PW, Sullivan KJ, Behrman AL, Azen SP, **Wu SS**, Nadeau SE, Dobkin BH, Rose DK, Tilson JK, Cen S, Hayden SK for The LEAPS Investigative Team. (2011). Body-weight-supported treadmill rehabilitation after stroke. *New England Journal of Medicine*. 364(21):2026-2036.
 - c. Okun, M. S., Fernandez, H. H., **Wu, S. S.**, Kirsh-Darrow, L., Bowers, D., Suelter, M., Jacobson, C. C., Wang, X., Gordon, C. W., Zeilman, P., Romrell, J., Martin, P., Rodriguez, R. L., Foote, k. D. (2009). Cognition and Mood in Parkinson disease STN versus GPi DBS. The COMPARE Trial. *Annals of Neurology*, 65(5): 586-595.
 - d. Kluding P, Dunning K, O'Dell M, **Wu SS**, Ginosian J, Feld J, McBride K. (2013). Foot drop stimulation versus ankle foot orthosis following stroke: 30 week outcomes, *Stroke*, 44, 1660-1669.
4. I have applied adaptive theory to military and sports topics as well. Mo et al. (2001) considered a decision-making problem in defense in which a number of missiles will be fired sequentially to destroy as many targets as possible. We provided a

backward induction proof showing that the myopic strategy, defined as choosing the next target as the one with the highest intact posterior probability, is the optimal strategy. And in a series of papers on sports statistics, we provided more efficient designs for baseball, football and World Cup tournaments by scheduling matches using accumulated competition results. These papers showed that adaptive designs are often far superior to current methods, and these results have potential applications to clinical trials with pair-wise comparisons.

- a. **Wu SS**, Yang MCK. (2008). An improved double-elimination tournament with application to the FIFA world cup. *The Mathematical Scientist*. 33: 79-92.
 - b. Annis D, **Wu SS**. (2007). Improved college football scheduling using a modified Swiss system. *Chance*. 20(1): 6-10.
 - c. Annis D, **Wu SS**. (2006). A comparison of potential playoff systems for NCAA I-A football. *The American Statistician*. 60(2):151-157.
 - d. Mo S, **Wu SS**, Chen R, Yang MCK. (2001). Optimal sequential allocation with imperfect feedback information. *J. Applied Probability*. Vol. 38, No.1: 248-254.
5. I have also contributed in genetic linkage analysis and statistical inference for orthogonal saturated designs, both involving simultaneous hypothesis tests. We developed a statistical method for linkage analysis of polymorphic markers; a statistical model for detecting major genes responsible for growth trajectories; and a hierarchical model for detecting major genes based on progeny tests of outcrossing species. Wu et al. (2006) introduced the so-called shrinkage tests that have the best average power for testing multivariate linear hypotheses. For the first time in the area, Wu and Wang (2007) derived step-up simultaneous testing procedures that control the experiment-wise error rate at a given level in the strong sense for orthogonal saturated designs.
- a. **Wu SS**, Wang WZ. (2007). Step-up simultaneous tests for identifying active effects in orthogonal saturated designs, *Annals of Statistics*, 35(1), 449-463.
 - b. **Wu SS**, Li HY, Casella G. (2006). Tests with optimal average power in multivariate analysis, *Statistical Sinica*, 16(1), 255-266.
 - c. **Wu SS**, Wu RL, Ma CX, Zeng ZB, Yang MCK, Casella G. (2001). A multivalent pairing model of linkage analysis in autotetraploids, *Genetics*, 159(3), 2001, 1339-50.
 - d. Wu RL, Li BL, **Wu SS**, Casella G. (2001). A maximum likelihood-based method for mining major genes affecting a quantitative character, *Biometrics*, 57(3), 2001, 764-768.

Complete List of Published Work in MyBibliography: <http://www.ncbi.nlm.nih.gov/sites/myncbi/1lu1dogjq8GAv/bibliography/47276308/public/?sort=date&direction=ascending>.

D. Research Support

Ongoing Research Support

Project Number: 1R01AG054077-01

Dates of Approved/Proposed Project

Source: NIH/NIA

09/01/2016 to 08/31/2021

Title of Project: Augmenting Cognitive Training in Older Adults – The ACT Grant

This randomized clinical trial examines the effect of augmenting cognitive training with transcranial direct current stimulation to maximize cognitive and functional outcomes in older adults experiencing age-related cognitive decline.

Role: Co-I

Project Number: 2R01NS052318-13

Dates of Approved/Proposed Project

Source: NIH/ NINDS

08/1/2016 to 05/31/2021

Title of Project: Scaling and Sequencing Motor Output in Humans: fMRI Study

This project will study the effect of an MAO-inhibitor on the measures of progression (free-water in the posterior substantia nigra; blood oxygen level dependent fMRI in the posterior putamen, primary motor cortex, and supplementary motor area) in Parkinson's disease.

Role: Co-I

Project Number: 1R01NS096008

Dates of Approved/Proposed Project

Source: NIH/NINDS

07/01/2016 - 06/30/2021

Title of Project: The Human Thalamocortical Network in Tourette Syndrome

This project will determine the physiological underpinnings of Tourette Syndrome and how tics can be modulated by electrical stimulation.

Role: Co-I

Project Number: U01AG050499
Source: NIH
Title of Project: The ENRGISE Study
This project will assess whether lowering low-grade chronic inflammation improves mobility in older persons.
Role: Co-I

Dates of Approved/Proposed Project
09/01/2015 to 07/31/2018

Project Number: 1R01DK099334
Source: NIH
Title of Project: Biopsychosocial Influence on Shoulder Pain (phase 2)
This project will determine mechanisms and efficacy of personalized pharmaceutical and personalized psychological pain interventions designed to target the genetic and psychological factors that comprise the high risk subgroup.
Role: Co-I

Dates of Approved/Proposed Project
05/01/2015 to 03/31/2020

Project Number: 1R01DK099334
Source: NIH
Title of Project: Obesity and type-2 diabetes: Bariatric surgery effects on brain function
This project is to determine the effect of bariatric surgery on brain function in adults with obesity and type-2 diabetes.
Role: Co-I

Dates of Approved/Proposed Project
06/25/2014 to 05/31/2019

Project Number: P30AG028740
Source: NIH
Title of Project: The UF Claude D. Pepper Older Americans Independence Center (OAIC)
The mission of OAIC is to assess the risk factors of physical disability in older adults, develop and test effective prevention therapies, and train new investigators in research on aging and disability, while developing their leadership qualities.
Role: Director of Biostatistics Core

Dates of Approved/Proposed Project
4/1/2013 to 3/31/2022

Project Number: NA
Source: NIDRR
Title of Project: Restoring Lost Functions after Spinal Cord Injury: Combination Therapy with Dalfampridine and Locomotor Training in Persons with Chronic, Motor Incomplete Spinal Cord Injury
This project is to determine the efficacy, safety, and tolerability of combination therapy with dalfampridine and locomotor training in persons with chronic, motor incomplete spinal cord injury.
Role: Co-I

Dates of Approved/Proposed
10/1/2011 to 9/30/2017

Project Number: NA
Source: Dept of Veterans Affairs
Title of Project: Biostatistical Research Support Services for VAMC Rehabilitation Studies
The object of the contracts is to provide statistical support in design, analysis, and interpretation of a series of rehabilitation studies at the Center of Innovation on Disability & Rehabilitation Research and at the Brain Rehabilitation Research Center.
Role: PI

Dates of Approved/Proposed
10/1/2011 to 9/30/2017

Completed Research Support (in last 5 years)

Project Number: KK-G000790
Source: APTA
Title of Project: Creation of the Orthopaedic Physical Therapy Investigative Network (OPT-IN) for the Optimal Screening for Prediction of Referral and Outcome (OSPRO) Cohort Study.
The purpose of this research study is to develop a new questionnaire to direct physical therapy management of individuals seeking care for pain complaints at the back, neck, knee, or shoulder.
Role: Lead statistician and Co-I

Dates of Approved/Proposed
1/29/2013 to 1/29/2016

Project Number: NA
Source: NPF
Title of Project: Biostatistical Research Support Services for National Parkinson Foundation
The purpose of the contract is to provide statistical support in design, analysis, and interpretation of studies titled "NPF's Quality Improvement Initiative," to identify good treatment practices in tertiary clinical care centers that specialize in Parkinson's disease care.
Role: PI

Dates of Approved/Proposed
4/1/2012 to 6/30/2016

TSU Contracts
Texas State University
Contract between Texas State University – San Marcos and UF
Provide statistical support in development of TSU's Air Force Medical Support Agency SG9S grant proposal titled "Implications of Timing and Quality of Physical Therapy and Chiropractic Care on Low Back Pain Utilization and Costs in the Military Health System."
Role: PI

Dates of Approved/Proposed
10/01/2011 – 09/30/2013

1R01AR055899
NIH / NINDS

Dates of Approved/Proposed
07/01/2008 – 04/30/2013

Biopsychosocial influence on shoulder pain

This study will develop a novel biopsychosocial model that considers the potentially interactive roles played by psychological and genetic risk factors in the development of chronic shoulder pain. Completion of the proposed studies will provide important information on how chronic musculoskeletal pain syndromes develop and could alter standard of care by allowing for early and accurate identification of individuals likely to develop chronic shoulder pain.

Role: Co-I

UF Award
University of Florida

Dates of Approved/Proposed
10/01/2011 – 07/30/2012

Faculty Enhancement Opportunity Award

The aim of the project is to develop new systematic and stochastic approaches that can efficiently extract radial velocity signals in the Multi-object APO Radial Velocity Exoplanet Large-area Survey (MARVELS) data and detect new planets.

Role: PI

R01 NS050506
NIH / NINDS&NCMRR

Dates of Approved/Proposed
07/01/2005 - 06/30/2012

Locomotor Experience Applied Post-Stroke (LEAPS)

The goals of the LEAPS trial are to determine if a specialized locomotor training program that includes use of a body weight support system and a treadmill as a treatment modality can result in a functionally significant improvement in walking of individuals post-stroke compared to a control group and whether timing of therapy, severity of impairments, and the number of treatments make a difference.

Role: Lead statistician and Co-I

Publications (2016)

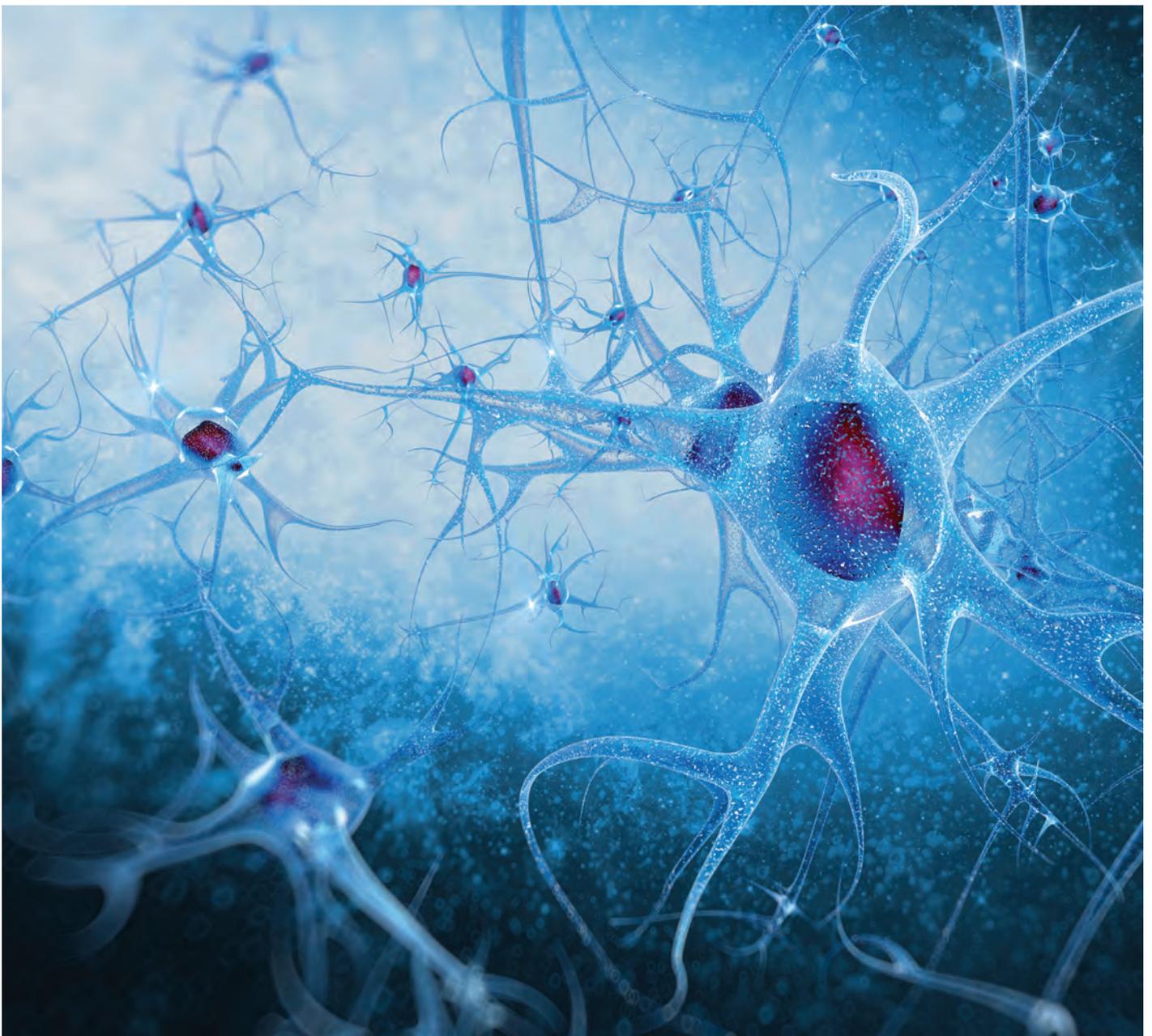
1. Pei QL, Chen SG, Xiao Y, and **Wu SS**. (2016). Applying triple-matrix masking for privacy preserving data collection and sharing in HIV studies. *Current HIV Research*. 14(2): 121 - 9. (PMID: 26511340).
2. Liphart J, Gallichio J, Tilson JK, Pei Q, **Wu SS**, Duncan PW. (2016). Concordance and discordance between measured and perceived balance and the effect on gait speed and falls following stroke. *Clin Rehabil*. 30(3):294-302 (PMID: 25810385)
3. Jia H, Pei Q, Sullivan CT, Cowper Ripley DC, **Wu SS**, Bates BE, Vogel WB, Bidelspach DE, Wang X, Hoffman N. (2016). Poststroke rehabilitation and restorative care utilization: a comparison between VA community living centers and VA-contracted community nursing homes. *Med Care* 54(3):235-42. (PMID: 26807537)
4. Lentz TA, Beneciuk JM, Bialosky JE, Zeppieri G Jr, Dai Y, **Wu SS**, George SZ. (2016). Development of a yellow flag assessment tool for orthopaedic physical therapists: Results from the optimal screening for prediction of referral and outcome (OSPRO) cohort. *Journal of Orthopaedic & Sports Physical Therapy* 46(5):327-43. (PMID: 26999408)
5. Valiani V, Gao S, Chen Z, Swami S, Harle CA, Lipori G, Sourdet S, **Wu S**, Nayfield SG, Sabba C, Pahor M, Manini TM. (2016). In-hospital mobility variations across primary diagnoses among older adults. *J Am Med Dir Assoc*. 17(5):465.e1-8. (PMID: 26971132)
6. George SZ, Wu SS, Wallace MR, Moser MW, Wright TW, Farmer KW, Greenfield WH 3rd, Dai Y, Li H, Fillingim RB. (2016). Biopsychosocial influence on shoulder pain: Genetic and psychological combinations are predictive of 12 month post-operative pain and disability outcomes. *Arthritis Care Res*. 68(11):1671-1680. (PMID: 26945673)
7. Nadeau SE, Dobkin B, **Wu SS**, Pei Q, Duncan PW; LEAPS Investigative Team. (2016). The effects of stroke type, locus, and extent on long-term outcome of gait rehabilitation: the LEAPS experience. *Neurorehabil Neural Repair*. 30(7):615-25. (PMID: 26498434)

Presentations (2016)

1. **Wu SS.** *“Statistical inference for 2 by 2 factorial designs.”* The Institute of Statistics and Big Data, Renmin University, Beijing, China. January 4, 2016. (Invited)
2. **Wu SS, Bhattacharjee A.** *“Identifying Main Effects in Multi Factor Clinical Trials.”* ICSA Applied Statistics Symposium, Atlanta, GA, June 12 - 15, 2016. (Invited)
3. **Wu SS, Lu X, He Y.** *“Interval Estimation in Multi-stage Drop-the-losers Designs.”* ICSA Applied Statistics Symposium, Atlanta, GA, June 12 - 15, 2016. (Invited)
4. **Wu SS, Lu X, Kairalla J, Zeng H.** *“Graphical Approach to Multiple Test Procedures in 2 by 2 Factorial Designs.”* ICSA Applied Statistics Symposium, Atlanta, GA, June 12 - 15, 2016. (Invited)
5. **Wu SS.** *“Introduction to biostatistics and clinical trials.”* The 7th Symposium of Science, Engineering, and Biomedicine, Jacksonville, FL, September 2 - 5, 2016. (Invited)
6. **Wu SS.** *“New data collection methods with privacy protection.”* Department of Mathematics, Clarkson University, Potsdam, NY. October 20, 2016. (Invited)

Affiliate Faculty Biographical Sketches

In addition to primary faculty, the CAM-CTRP maintains a broad network of active cross-college affiliated faculty. These collaborations are central to the mission of the CAM-CTRP in understanding cognitive aging and identifying new approaches to combating the cognitive aging process. The affiliate faculty's research areas represent important lines of investigation for shedding light on underlying brain-based changes that occur with aging. These faculty collaborate closely with the primary CAM-CTRP faculty on funded and pending research projects focused on cognitive aging. In combination, the primary and affiliate faculty of the CAM-CTRP provide a broad base of knowledge and expertise for studying cognitive aging and memory.



Dawn Bowers, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Dawn Bowers, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): dbowers

POSITION TITLE: Professor of Clinical & Health Psychology and Neurology

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Emory University	--	1968-1970	Chemistry
University of Florida	B.S.	1972	Psychology
University of Florida	M.S.	1974	Clinical Psychology
Boston University	internship	1977	Neuropsychology
University of Florida	Ph.D.	1978	Clinical & Health Psychology
University of Florida	Post-Doc	1979	Behavioral Neurology

A. Personal Statement

I am a university professor, a board certified neuropsychologist, and a clinical researcher. I have longstanding research and clinical expertise in cognitive and emotional changes that are associated with Parkinson disease. As lead neuropsychologist for the UF Movement Disorders Center, I oversee the neurocognitive module of the INFORM database, which currently has neurocognitive data on over 1800 individuals with movement disorders. I have a keen understanding of the cognitive and emotional sequelae of Parkinson disease and other movement disorders, along with statistical methods inherent in conducting reliable change analyses over time. My current research focuses on electro-psychophysiological signatures of apathy and depression, emotion regulation and executive function, and the interactive effects of mindfulness, cognitive training, and novel therapies on mood and cognition. I have been a funded researcher for over 30 years, including several randomized clinical trials, one for treatment of apathy using rTMS, another for treatment of masked faces in Parkinson disease, and others combining cognitive training with exercise in older adults (McKnight Research Foundation). I currently direct an NINDS funded T32 predoctoral grant focusing on interdisciplinary training in movement disorders. My Cognitive Neuroscience Laboratory includes five doctoral students, who are using various tools (ERP, startle, pupillometry, computational modeling, NIRS, advanced statistical approaches) to better understand mechanisms that underlie emotional regulation and cognitive changes in individuals with movement disorders.

B. Positions and Honors

Positions and Employment

1976 - 1977 Teaching Fellow in Neurology, Boston University College of Medicine
 1976 - 1977 Internship in Clinical Psychology/Neuropsychology, Boston VAMC
 1976 - 1977 Externship in Geriatric Neuropsychology, Framingham Heart Study, MA
 1979 Post-doctoral Fellowship, Behavioral Neurology, UF College of Medicine
 1980 - 1998 Associate Professor in Neurology [Assistant 1980-85], UF College of Medicine
 1984 - 1998 Neuropsychologist, State of Florida Memory Disorders Clinic
 1998- Professor of Clinical & Health Psychology [Associate 1998-2002]
 2006- Area head, Neuropsychology Area, Dept. Clinical & Health Psychology
 2006- Director, Neuropsychology Post-doctoral Program

Other Positions and Professional Memberships

- 2016 - Chair, Fellows Committee Division 40, American Psychological Association
- 2013 - 17 Merit Review Panel for Mental Health and Behavioral Sciences-B, BLRD and SCR&D;
Department of Veterans Affairs
- 2013 - Fellows Committee, Division 40, APA (Member 2013-16; Chair 2016-)
- 2012 - 14 Chair & Vice Chair, Faculty Council, College of PHHP
- 2012 - 15 Board of Governors, International Neuropsychological Society
- 2012 Panel Member, NIH Review of LRP proposals
- 2011 - 12 Ad hoc Member, Special Emphasis Panel, Clinical and Imaging Translations Study Section (ZRG1 DTCS Y(81)).
- 2004 - 05 Ad hoc Member, NIH Biobehavioral Mechanisms of Emotion, Stress, and Health Study Section
- 2000 - Editorial Boards, The Clinical Neuropsychologist, JINS
- 1999 - 2003 Special Review Panel, Minority Research Infrastructure Support Program (MRISP), NIMH
- 1995 - 1998 Member, Merit Review Committee, Mental Health & Behavioral Science, Dept. Veterans Affairs

Membership: American Psychological Association (Divisions 12, 20, & 40), International Neuropsychology Society, American Academy of Clinical Neuropsychology, Society for Neuroscience, Cognitive Neuroscience Society

Honors

- 2006 - UF Foundation Research Professor
- 2012 Fellow, American Psychological Association, Division 40
- 2013 Board Certification in Clinical Neuropsychology (ABBP/cn)
- 2014 Paul Satz Career Mentoring Award, International Neuropsychology Society
- 2014 Edith Kaplan Neuropsychology Award, Massachusetts Psychological Society
- 2015 Doctoral Mentoring Award, College of Public Health and Health Professions, UF
- 2015 Audrey Shumacher Teaching Award, Department of Clinical & Health Psychology
- 2015 Research Award, Department of Clinical & Health Psychology
- 2016 University of Florida Doctoral Mentoring Award

C. Contribution to Science

- Pseudoneglect.** Early in my career, I described a mild asymmetry in spatial attention whereby normal individuals tend to favor the left side of space. This bias was initially observed during a tactile line bisection, when participants made systematic errors to the right. I referred to this phenomenon as 'pseudoneglect', since the spatial error was opposite that observed in patients with right parietal strokes who exhibit left sided spatial neglect. Since the initial discovery, the pseudoneglect (or spatial asymmetry) has been observed in 100's of studies ranging from judgements of brightness, numerosity, size, and representation to neuroimaging studies. The original study initially describing this phenomenon (Bowers & Heilman, 1980) has been cited over 631 times, with a meta-analysis completed several years ago. It continues to be studied today
 - Bowers D, Heilman KM (1980)** Pseudoneglect: effects of hemispace on a tactile line bisection task. *Neuropsychologia* 18: 491-498
 - Heilman KM, **Bowers D**, Watson RT. (1984). Pseudoneglect in a patient with partial callosal disconnection. *Brain*. 107 (Pt 2):519-32.
 - Watson RT, Heilman KM, **Bowers D**. (1985). Magnetic resonance imaging (MRI, NMR) scan in a case of callosal apraxia and pseudoneglect. *Brain*, 108 (Pt 2):535-6
- Apathy/depression in Parkinson Disease.** Over the past decade, much of my research has focused on cognitive and emotional sequelae of Parkinson disease, an alpha-synuclein disorder that affects the dopaminergic system but others as well. One line of research has involved apathy, which we consider the primary neuropsychiatric signature in Parkinson disease. Indeed, we and others have shown that apathy is distinct from depression, affects between 30-70% of PD patients, and progressively worsens with disease severity. We view depression as a mood disorder and apathy a motivational disorder that is directly linked to dopamine deregulation. In my lab, we have examined psychometric properties of different apathy scales and shown how depression and apathy differentially predict cognitive, physiologic (startle, ERP), and trajectory of motor decline in Parkinson disease. Why is this important? The importance relates to treatment, in that pharmacologic therapy for depression with SSRI's actually worsens apathy due to serotonergic modulation of midbrain dopamine systems. In one small RCT, we found that apathy significantly abated following treatment with rTMS and this effect maintained for 3 months; however, patients assigned to a sham rTMS condition showed similar improvement, perhaps due to placebo and/or behavioral activation. Pragmatically, either mechanism is informative and helpful. Currently, we are completing an NINDS funded ERP study examining the ability of Parkinson patients to 'intentionally' upregulate their emotional reactivity and whether success

in doing so is related to executive dysfunction. Finally, a current post-doc in my lab recently completed a telehealth feasibility study showing that apathy in PD was improved via a goal-setting approach.

- a. **Bowers, D.**, Miller, K., Mikos, A., Kirsch-Darrow, L., Springer, S., Fernandez, H., Foote, K., Okun, M.S. (2006). Startling facts about emotion in Parkinson disease: Blunted reactivity to aversive stimuli. *Brain*, 129, 3345-3365.
 - b. Kirsch-Darrow, L., Fernandez, H., Okun, M., **Bowers, D.** (2006). Dissociating apathy and depression in Parkinson's disease. *Neurology*. 67(1), 20-27.
 - c. Zahodne, L., Marsiske, M., Okun, M.S., Rodriguez, R., Malaty, I, **Bowers, D.** (2012) Mood and motor symptoms in Parkinson's Disease: a Multivariate latent growth curve modeling. *Neuropsychology*, 26, 71-80. **PMID: 22142359**
 - d. Jordan, L., Zahodne, L., Okun, M.S., **Bowers, D.** (2013). Hedonic and behavioral deficits associated with apathy in Parkinson's disease. *Movement Disorders*, 28(9):1301-4 [epub 2013 May]. **PubMed PMID: 23712560; PubMed Central PMCID: PMC3760996.**
 - e. Jones, J., Mangal, P., Lafo, P., Okun, M.S., **Bowers, D.** (2016, in press). Mood differences among Parkinson disease patients with mild cognitive impairment. *J. Neuropsychiatry and Clinical Neuroscience*. **PMID: 26792098.**
 - f. Butterfield, L., Cimino, C., Salazar, R., Lee, C., Haley, W., Sanchez-Ramos, J., Okun, M.S., **Bowers, D.** (2016, in press). The Parkinson's Active Living (PAL) program: A behavioral intervention targeting apathy in Parkinson's disease. *J. Geriatric Psychiatry and Neurology*.
3. **Vascular Comorbidities and Cognition in Parkinson Disease.** Despite clinical lore that patients with Parkinson disease are impervious to effects of hypertension due to the blood pressure lowering effects of various dopamine medications, we have shown that those with hypertension and other vascular comorbidities 'take a hit' in terms of executive function, similar to that of non-PD older adults.
- a. Jones, J., Malaty, I., Price CC, Okun, MS., **Bowers, D.** (2012) Health comorbidities and cognition in 1948 patients with idiopathic Parkinson disease. *Parkinsonism and Related Disorders*. 18 (10), 1073-1078 **PMID:22776043**
 - b. Jones, J., Jacobson, C., Murphy, M.C., Price, C.E., Okun, M.S., **Bowers, D.** (2014). Influence of hypertension on neurocognitive domains in non-demented Parkinson's disease patients. *Parkinson's Disease*, 2014, Article ID 507529, [<http://dx.doi.org/10.1144/2014/507529>]. **PMID: 24587937 PMCID: PMC3920751.**
 - c. Jones, J., Tanner, J., Okun, M.S., Price, C.P., **Bowers, D.**, (2016, submitted). Is cognition more vulnerable to the effects of cardiovascular comorbidities in Parkinson patients vs controls? Neuroimaging and behavioral correlates
4. **Retrosplenial Amnesia.** This body of work described the first human case of a pure amnesic syndrome due to an isolated lesion of the left retrosplenial region, located beneath the posterior cingulum; it receives direct input from the fornix of the hippocampus, projects forward via the cingulum and serves as way station for input from the adjacent parietal region. We conceptualized the amnesia induced by this lesion as a 'disconnection' variant, and a neuroimaging study (PET) of this patient showed hypometabolism of the thalamus and hypermetabolism of the ipsilateral frontal lobe. We and others subsequently described other cases of retrosplenial amnesia. More recent research has implicated this region in Alzheimer's disease.
- a. Valenstein, E., **Bowers, D.**, Verfaellie, M., Heilman, K., Day, A., and Watson, R. (1987). Retrosplenial amnesia. *Brain*, 110, 1631-1646.
 - b. **Bowers, D.**, Verfaellie, M., Valenstein, E., and Heilman, K. (1988). Impaired acquisition of temporal information in retrosplenial amnesia. *Brain and Cognition*, 8, 47-66
 - c. Heilman, K.M., **Bowers, D.**, Watson, R., Day, A., Valenstein, E., Hammond, E., and Duara, R. (1990). Frontal hypermetabolism and thalamic hypometabolism in a patient with abnormal orienting and retrosplenial amnesia. *Neuropsychologia*, 28, 161-170.
 - d. McDonald, C., Crosson, B., Valenstein, E., and **Bowers, D.** (2001). Verbal encoding deficits in a patient with retrosplenial amnesia. *Neurocase*, 7, 407-17.
5. **Cortical Contributions to Emotion.** During an early phase of my academic career, my interests focused on cortical contributions to emotion. This was done primarily using a 'stroke' or 'lesion' model. Much of this work predated fMRI. In brief, we viewed the cortex as modulating underlying limbic systems and the goal was to examine how the two hemispheres might play a differential role in doing so. Some key findings, that have been replicated over the years, were that that posterior

focal hemisphere lesions (temporo-parietal) induced defective perception of emotional faces and prosody (tone of voice) that was not due to basic visuoperceptual disturbances. Instead I proposed a model which involved a nonverbal affective lexicon that was more lateralized to the right than left hemisphere. This was initially inspired by observations of patient with a callosal disconnection and an affect specific anomia (Bowers & Helman, 1984); this observation led to the development of The Florida Affect Battery which enabled us to parcellate out various processing disturbances. What we know now is that more anterior cortical systems play a more direct role in modulating the underlying limbic system, whereas the posterior cortical systems are involved in emotional interpretation that feeds forward to assist in goal selection, with lateralized involvement.

- a. **Bowers, D., Jones, J., Dietz, J. (2014).** Assessment of emotion, mood, and affect associated with neurologic disorders. In Parsons, M. and Hammeck, T. (eds), *Clinical Neuropsychology*. Pocket Handbook of Assessment. American Psychological Press.
- b. **Bowers, D., Bauer, R.M., and Heilman, K. (1993).** The nonverbal affect lexicon: Theoretical perspectives from neuropsychological studies of affect perception. *Neuropsychology*, 7(4), 433-444.
- c. **Bowers, D., Coslett, B., Bauer, R., Speedie, L., and Heilman, K.M. (1987).** Comprehension of emotional prosody following unilateral brain damage: Processing versus distraction defects. *Neuropsychologia*, 25,317-328.
- d. **Bowers, D., Bauer, R., Coslett, B., and Heilman, K. (1985).** Processing of faces by patients with unilateral hemisphere lesions. I. Dissociation between judgments of facial affect and facial identity. *Brain & Cognition*, 4, 258-272.

D. Research Support

Ongoing Research Support

T32- NS082168 MPI: Bowers & Vaillancourt 2015-2020

Interdisciplinary Training in Movement Disorders and Neurorestoration

This grant focuses on interdisciplinary training of predoctoral trainees across cognitive/movement science,

ALZ67-State of Florida PI: Bowers 2016-2018

Pilot Intervention in Mild Cognitive Impairment: A Proof of Concept Study with Transcranial Near Infrared Stimulation

This pilot study will test in a randomized sham controlled trial whether a novel intervention, near infrared brain stimulation, has potential for improving cognitive symptoms in individuals with amnesic mild cognitive impairment.

NIH/ R01-NS096008 MPI: Okun/Gunduz 2016-2021

The Human Thalamocortical Network in Tourette.

The goal of this study is to develop a closed loop neuromodulation solution for Tourette syndrome and to explore the human thalamocortical network in Tourette syndrome

Role: Co-I

UH3-NS095553 MPI: Gunduz/Foote 2016-2021

NIH/NINDS

Closing the Loop on Tremor: A Responsive Deep Brain Stimulator for Treatment of Tremor

This project examines the thalamocortical neurophysiology of tremor,

ALZ-121 State of Florida PI: Wicklund 2016-2018

Consortium for Diagnostic Algorithm with Novel Markers in early Alzheimer's Disease

This Florida multisite study aims to 1) validate novel neuropsychological and imaging measures that are sensitive to the earliest cognitive changes associated with AD, and 2) develop algorithms for fitting patients into various diagnostic categories using multimodal clinical, neuropsychological, and neuroimaging data.

Role: Co-I

R03-MH109333 PI: Dotson 2015-17

Dissociating Components of Anhedonia: Pilot Behavioral and fMRI Data for the Effort Expenditure for Rewards Task

The goal of this study is to examine the neural correlates of anhedonia in older and younger adults.

Role: Co-I

R01 NS082386 PI: Price 2013-2018

White Matter Connectivity and PD Cognitive Phenotype

This grant examines 3 cognitive subtypes of PD in relation to white matter connectivity using cognitive testing and multimodal imaging approaches (DTI, fMRI)

Role: Co-I

Michael J. Fox Foundation PI: Okun 2015-17

A Closed Loop Neuromodulation Solution For Parkinson's Disease Related Freezing

Role: Co-I

Completed Research Support

Village-UF Partnership MPI: Bowers & Marsiske 2014-2016

Vitality Mind-Brain Health: Re-Vitalize, Cedar, & Neuroadvantage

This project tests various hypotheses regarding the basis for cognitive improvement in older adults undergoing various cognitive and behavioral interventions i.e., mindfulness, exergames, LED NIR, etc.)

R21NS079767 PI: Bowers 2012-2015

Emotion Regulation, Executive Function, and Parkinson Disease.

This grant tests whether Parkinson patients can learn to "upregulate" their emotional reactivity, as measured by electrophysiological measures (LPP, ERP), and whether the ability to do so is related to executive functioning.

Role: PI

State of Florida PI: Lowenstein/Wicklund 02/2015-03/30/15

Novel Markers in Alzheimer's Disease,

This multi-site project across 5 institutions in Florida focused on novel experimental measures that might be more sensitive in detecting preclinical changes associated with early Alzheimer's disease.

Role: Co-I

Dawn Bowers, Ph.D., ABPP-CN:

Work Completed from January 2016-December 31, 2016

Peer Reviewed Publications

1. Scott, B.M., Maye, J., Jones, J., Thomas, K., Mangal, P., Trifilio, E., Hass, C., Marsiske, M., **Bowers, D.** (2016) Post-exercise pulse pressure is a better predictor of executive function than pre-exercise pulse pressure in cognitively normal older adults. *Aging, Neuropsychology, and Cognition*. 23(4):464-76. [epub ahead of print Dec 2, 1-13], PMID: 26629911.
2. Renfroe, J.B., Bradley, M.M., Okun, M.S., **Bowers, D.** (2016) Motivational engagement in Parkinson disease: perception and preparation for action. *International Journal of Psychophysiology*, 99, 24-32. PMID:26659013.
3. Higuchi, M., Martinez-Ramirez, D., Mrita, H., Topiol, D., **Bowers, D.**, Ward, H., Warren, L., DeFranco, M., Hicks, J., Hegland, K.W., Troche, M.S., Kulkarni, S., Hastings, E., Foote, K., Okun, M.S. (2016). Interdisciplinary deep brain stimulation screening and the relationship to unintended hospitalizations and quality of life. *PLOS One*, 11(5): e0153785. Doi: 10.1371/ journal.pone. 0153875. Ecollection 2016. PMID:27159519, PMC4861342.
4. Higuchi, M., Martinez-Ramirez, D., Topiol, D., Ahmed, B., Morita, H., Hess, C., **Bowers, D.**, Ward, H., Warren, L., DeFranco, M., Troche, M., Kulkarni, S., Monari, E., Foote, K., Okun, M.S. (2016). Impact of an interdisciplinary deep brain stimulation screening model on post-surgical complications of essential tremor patients. *PLOS One*. Dec 28; 10(12):e0145623. PMID: 26710099
5. Price, C.C., Tanner, J.J., Nguyen, P., Schwab, N., Mitchell, S., Slonena, E., Brumback, B., Okun, M.S., Mareci, T., **Bowers, D.** (2016). Grey and white matter predictors of cognitive frontal-striatal deficits in Parkinson's disease. *PLOS One*, Jan 19, 11(1):20147332. PMID:26784744.
6. Jones, J., Mangal, P., Lafo, P., Okun, M.S., **Bowers, D.** (2016). Mood differences among Parkinson disease patients with mild cognitive impairment. *J. Neuropsychiatry and Clinical Neuroscience*. 28 (3), 211-6. PMID: 26792098
7. Kaufman, D., **Bowers, D.**, Okun, M.S., van Pattern, R., Okun, M.S., Perlstein, W.M. (2016). Apathy, novelty processing, and the P3 potential in Parkinson's disease. *Frontiers Neurology*, 7:95. (June 23), PMID: 27445962. <http://journal.frontiersin.org/article/10.3389/fneur.2016.00095/full>
8. Sisco, S., Slonena, E., Okun, M.S., **Bowers, D.**, Price, C. (2016). Parkinson's disease and the Stroop Color Word Test: Processing speed and interference algorithms. *The Clinical Neuropsychologist*, 6, 1-14. PMID 27264121
9. Loewenstein, D.A., Curiel, R., Greig, M., Rosado, M., Bauer, R., **Bowers, D.**, Wicklund, M., Crocco, e., Abhinay, J., Pontecorvo, M., Rodriguez, R., Barker, W., Hidalgo, J., & Duara, J. (2016). A Novel cognitive stress test for the detection of preclinical Alzheimer's disease. *American Journal of Geriatric Psychiatry*, 24(10), 804-13. doi: 10.1016/j.jagp.2016.02.056. PMID:27160985

10. Rossi, P., Opi, E., Shute, J.B., Molina, R., **Bowers, D.**, Ward, H., Foote, K., Gunduz Aysegul, Okun, M.S. (2016). Scheduled, Intermittent stimulation of the thalamus reduces tics in Tourette syndrome. *Parkinsonism and Related Disorders*, 29, 35-41. [epub Jun 7, doi:10.1016], **PMID 27297737**
11. Renfroe, J., Sege, C., Bradley, M., **Bowers, D.** (2016). Emotion modulation of late positive potential during picture free viewing in older and young adults. *PLOS One*, 11(9),:e0162323.doi: 10.1371/ **PMD: 27589393**
12. Altman, L., Stegemoller, E., **Bowers, D.**, Okun, M.S., Hass, C.L. (2016). Aerobic exercise improved mood, cognition, and language function in Parkinson's disease: Results of a controlled study. *J. International Neuropsychology Society*, 22(9), 878-889. **PMID: 27655232**
13. Butterfield, L., Cimino, C., Salazar, R., Lee, C., Haley, W., Sanchez-Ramos, J., Okun, M.S., **Bowers, D.** (2016, in press). The Parkinson's Active Living (PAL) program: A behavioral intervention targeting apathy in Parkinson's disease. *J. Geriatric Psychiatry and Neurology*.
14. Jones, J., Price, CE, Tanner, J., Okun, M.S., **Bowers, D.** (2016, in press). Is cognition more Vulnerable to the Effects of Cardiovascular Risk in Parkinson Patients versus Controls. *J. International Neuropsychology Society*.
15. Lafo, J., Milkos, A., Mangal, P., Scott, B., Trifilio, E., Okun, M.S., **Bowers, D.** (2016, in press). Emotion modulation of the startle reflex in essential tremor: Blunted physiologic reactivity to unpleasant and unpleasant pictures. *Parkinson Disease and Related Disorders*. Nov 17pii: S1353-8020(16)30434-5. doi: 10.1016
16. Almeida, L., Ahmed,B., Walz, R., DeJesus, S., Patterson, A., Martinez-Ramirez, D., Vaillancourt, D., **Bowers, D.**, Ward, H., Okun, M.S., McFarland, N. (2016, in press). Depressive symptoms are frequent in atypical parkinsonian disorders. *Movement Disorders Clinical Practice*.

Peer Reviewed Presentations

1. Levy, S., Tanner, J., **Bowers, D.**, Price, C.P. (2016, February). One-year reliable change of cognition and mood idiopathic non-dementia Parkinson's disease. Presented at the 44th annual meeting of the International Neuropsychological Society, Boston, MA. (Abstract: *Journal of the International Neuropsychological Society*).
2. Jones, J., Tanner, J., Okun, M.S., Price, C., **Bowers, D.** (2016, February). Cognition and Parkinson's disease: Influence of health comorbidities and leukoaraiosis. Presented at the 44th annual meeting of the International Neuropsychological Society, Boston, MA. (Abstract: *Journal of the International Neuropsychological Society*).
3. Lafo, J., Milkos, A., Scott, B., Okun, M.S., **Bowers, D.** (2016, February). Startling facts about Essential Tremor: Blunted emotional reactivity as indexed by the startle eyeblink response. Presented at the 44th annual meeting of the International Neuropsychological Society, Boston, MA. (Abstract: *Journal of the International Neuropsychological Society*).
4. Mangal, P., Lafo, P., Scott, B., Okun, M.S., **Bowers, D.** (2016, February). Intentional enhancement of electrocortical responses to emotional pictures by Parkinson patients: Relation to executive function. Presented at the 44th annual meeting of the International Neuropsychological Society, Boston, MA. (Abstract: *Journal of the International Neuropsychological Society*).
5. Scott, B., Lafo, J., Almeida, L., Okun, M.S., **Bowers, D.** (2016, February). Differential effects of apathy, depression, and anxiety on cognitive function in Parkinson's disease and Essential Tremor. Presented at the 44th annual meeting of the International Neuropsychological Society, Boston, MA. (Abstract: *Journal of the International Neuropsychological Society*).
6. Trifilio, E., Butterfield, L., Mangal, P., Thomas, K., Jones, J., Marsiske, M., **Bowers, D.** (2016, February). Cognitive correlates of consummatory vs anticipatory anhedonia in older adults. Presented at the 44th annual meeting of the International Neuropsychological Society, Boston, MA. (Abstract: *Journal of the International Neuropsychological Society*).
7. Almeida, L., Ahmed, B., Walz, R., DeJesus, S., Patterson, A., Martinez-Ramierz, D., Vaillancourt, D., **Bowers, D.**, Ward, H., Okun, M.S., Armstrong, M., McFarland, N. (2016, April). Comparison of depressive symptoms among atypical parkinsonisms and Parkinson disease. Paper presented at, Annual meeting of the Academy of Neurology, Vancouver, BC

Invited Professional Talks (outside UF)

1. **Bowers, D.** (5/13/2016). Emotion Regulation and Parkinson Disease. Invited talk, University of Utah, Department of Psychology, Salt Lake City, UT
2. **Bowers, D.** (2016, August) Think Big: Emotion Regulation, Apathy and Parkinson's disease. Invited Address, American Psychological Association, Division 40, Denver, CO (8/5/2016).

3. **Bowers, D.** (2016, September). Age Related Changes in Mood and Emotions. Invited talk, Florida Society of Neurology. Orlando, FL.

Invited Talks (Community)

1. **Bowers, D.** (6/14/2016). Apathy or Depression: Which one is it? PD Expert Briefing Webinar, Parkinson's Disease Foundation. <http://event.netbriefings.com/event/pdeb/Live/apathy/> (listened to by over 1700 individuals)
2. **Bowers, D.,** (2016, November). A tale of two emotions in Parkinson disease: depression vs apathy. Invited talk. Neuro Challenge Foundation Distinguished Speaker Symposium, Naples, FL (11/5/2016),

BIOGRAPHICAL SKETCH

NAME: David J. Clark

eRA COMMONS USER NAME: DJCLARK

POSITION TITLE: Assistant Professor (University of Florida)
Research Health Scientist (US Dept. of Veterans Affairs)

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Massachusetts – Lowell, Lowell MA	BS	05/2001	Exercise Physiology
Boston University, Boston MA	ScD	05/2007	Rehabilitation Sciences
Malcom Randall VA Med Center, Gainesville FL	Post-Doc	08/2009	Motor Control and Neuro-rehabilitation

A. Personal Statement

David Clark is a Research Health Scientist with the Brain Rehabilitation Research Center (BRRC) at the Malcom Randall VA Medical Center, and an Assistant Professor with the Department of Aging and Geriatric Research at the University of Florida. He serves as Co-Leader of the BRRC's Locomotor Research Initiative. His work and training spans the domains of motor control, neuro-rehabilitation, biomechanics and exercise physiology. Dr. Clark's primary area of interest is optimizing the neural control of walking in order to maximize mobility function in compromised populations such as elderly adults and people with neurological injury. He has a well-funded and highly productive research program in this area.

B. Positions and Honors

Positions and Employment

2002 – 2005	Graduate Research Assistant, Neural Control of Movement Lab, Boston University
2003	Teaching Assistant, Exercise Physiology, Boston University
2004 – 2006	Head Teaching Assistant, Exercise Physiology, Boston University
2005 – 2007	Graduate Research Assistant, Nutrition, Exercise Physiology and Sarcopenia Lab Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University
2006	Teaching Assistant, Neuroanatomy and Neurophysiology, Boston University
2007	Head Teaching Assistant, Neuroanatomy and Neurophysiology, Boston University
2007 – 2014	Courtesy Faculty, College of Public Health and Health Professions, University of Florida
2007 – present	Research Health Scientist, Brain Rehabilitation Research Center, Malcom Randall VA
2011 – present	Assistant Professor, Department of Aging and Geriatric Research, College of Medicine, University of Florida
2013 – present	Co-Coordinator, Locomotor Research Initiative, Brain Rehabilitation Research Center, Malcom Randall VA Medical Center
2014 – present	Graduate Faculty, PhD Program in Movement and Rehabilitation Sciences, College of Public Health and Health Professions, University of Florida

Honors

- University of Massachusetts – Lowell Honors Program (1997-2001)
- Alpha Lambda Delta National Honor Society (1998)
- Class President, Exercise Physiology, University of Massachusetts – Lowell (2000-2001)
- Dean's Award, Boston University Science and Engineering Research Symposium (2007)
- Outstanding Poster, University of Florida Neuromuscular Plasticity Symposium (2008)
- Complimentary Registration Award for Workshop on "Aging, the Central Nervous System, and Mobility in Older Adults" at Annual Meeting of the Gerontological Society of America (2012)

- Certificate of Excellence in Reviewing from Experimental Gerontology (2013)
- Pepper Scholar, Pepper Older American's Independence Center, University of Florida (2011 –2014)

C. Contribution to Science

1. fNIRS neuroimaging of cortical/executive control of walking

Functional near infrared spectroscopy (fNIRS) is an optical neuroimaging technology that allows assessment of cortical activity during natural movements, which is a major advantage over other modalities such as fMRI. Although fNIRS is a well-established technology, it has only recently emerged in the literature as a powerful approach for assessing neural control of walking. Dr. Clark's research has provided new insights into the role of executive control resources (quantified by activity in prefrontal cortex) for controlling walking, especially during complex walking tasks and for populations with compromised mobility function. In addition to the publications listed below, Dr. Clark has two additional first/senior authored manuscripts under review. The first is titled "fNIRS neuroimaging of executive control of walking in humans with mobility deficits" and shows higher use of executive resources (prefrontal cortical activity) during walking in adults post-stroke compared to healthy elderly or young adults. The second manuscript is titled "Stroke rehabilitation reduces prefrontal cortical activity during walking: a pilot study". Each of these manuscripts is discussed in the Background and Preliminary Data section of the proposal.

- a. **Clark DJ**, Rose DK, Ring SA and Porges EC. Utilization of central nervous system resources for preparation and performance of complex walking tasks in older adults. *Front Aging Neurosci*, 6:217, 2014. **PMC4142860**.
- b. **Clark DJ**, Christou EA, Ring SA, Williamson JB and Doty L. Enhanced somatosensory feedback reduces prefrontal cortical activity during walking in older adults. *J Gerontol A Biol Sci Med Sci*, 69(11): 1422-8, 2014. **PMC4229993**.
- c. **Clark DJ**. Automaticity of walking: functional significance, mechanisms, measurement and rehabilitation strategies. *Front Hum Neurosci*, 246(9): 1-13, 2015. **PMC4419715**

2. Somatosensation and mobility

My research has revealed strong links between somatosensory function, neural control of walking and mobility function. Somatosensory inputs are a crucial source of afferent input to locomotor circuits in the central nervous system that are important to "automaticity" of locomotor control, and thus play an important role in preserving healthy walking function. My research has shown that elders with impaired somatosensation exhibit marked deficits in balance and walking performance. I have also shown that augmentation of somatosensory input using textured shoe insoles may reduce the demand for compensatory executive control in older adults by enhancing afferent input to locomotor circuits of automaticity. I have recently published a comprehensive article that consolidates knowledge about automaticity, and its functional importance, underlying neurophysiology, measurement, mechanisms of impairment and rehabilitation strategies. This review includes dedicated sections that document the important role of somatosensory input.

- a. Cruz-Almeida Y, Black ML, Christou EA and **Clark DJ**. Site-specific differences in the association between plantar tactile perception and mobility function in older adults. *Front Aging Neurosci*, 6:68, 2014. **PMC3990110**.
- b. **Clark DJ**, Christou EA, Ring SA, Williamson JB and Doty L. Enhanced somatosensory feedback reduces prefrontal cortical activity during walking in older adults. *J Gerontol A Biol Sci Med Sci*, 69(11): 1422-8, 2014. **PMC4229993**.
- c. **Clark DJ**. Automaticity of walking: functional significance, mechanisms, measurement and rehabilitation strategies. *Front Hum Neurosci*, 246(9): 1-13, 2015. **PMC4419715**

3. Intermuscular coordination during walking

Healthy walking requires proper coordination of a larger number of lower extremity muscles. After neurological injury such as stroke, intermuscular coordination can be markedly impaired. The ability to understand and quantify impaired intermuscular coordination is crucial to monitoring the effectiveness of rehabilitation interventions that seek to improve walking function. Prior research revealed that the healthy nervous system is able to coordinate a large set of muscle using a smaller set of control signals. The control signals active groups of muscles that serve particular gait functions (e.g., body weight acceptance, propulsion, swing initiation, etc). These functional grouping are often called "modules" or "synergies". My work was the first to take these ideas about healthy neural control and apply to them to understanding coordination of walking after stroke. Using an elegant approach called non-negative matrix factorization, my colleagues and I determined the number of "modules" needed to coordinate lower extremity muscle activation in stroke participants. We also used biomechanical simulations to determine the functional role of each "module". This research shows: a) Poor inter-muscular coordination following stroke is characterized by a smaller number of modules and abnormal characteristics of the modules; b) poor inter-muscular coordination (fewer modules) leads to poorer walking ability; c) rehabilitation improves inter-muscular coordination (more modules) and walking speed.

- a. **Clark DJ**, Ting LH, Zajac FE, Neptune RR and Kautz SA. Merging of healthy motor modules predicts reduced locomotor performance and muscle coordination complexity post-stroke. *J Neurophys*, 103(2): 844-57, 2010. **PMC2822696**.
 - b. Neptune RR, **Clark DJ** and Kautz, SA. Modular control of human walking: a simulation study. *J Biomech*, 42(9): 1282-7, 2009. **PMC2696580**.
 - c. McGowan CP, Neptune RR, **Clark DJ** and Kautz SA. Modular control of human walking: Adaptations to altered mechanical demand. *J Biomech*, 43(3): 412-419, 2010. **PMC2813323**.
 - d. Routson RL, **Clark DJ**, Bowden MG, Kautz SA and Neptune RR. The influence of locomotor rehabilitation on module quality and post-stroke hemiparetic walking performance. *Gait and Posture*, 38, 511-517, 2013. **PMC3687005**.
4. **Weakness and mobility deficits due to impaired voluntary neuromuscular activation**
- Weakness is widely acknowledged to be a major factor contributing to walking/mobility limitations after stroke and in older adults. Weakness has often attributed to low muscle mass, excessive restraint by antagonist muscles and poor intermuscular coordination (especially after stroke). My work has demonstrated that one of the most significant causes of weakness is poor capability of the nervous system to voluntarily activate agonist muscles. This is particularly true for dynamic (i.e., fast) muscle contractions, which are crucial to daily mobility tasks. These findings are based on rigorous assessments of surface electromyography and force production during maximal voluntary muscle contractions under static and dynamic conditions. Furthermore, these findings come from multiple study designs including longitudinal, interventional and cross-sectional studies. This research has important implications for the design and evaluation of clinical and research interventions that seek to address weakness and walking deficits after stroke and/or in older adults. Some of the major publications from this line of research are listed here:
- a. **Clark DJ**, Condliffe EG, Patten C. Activation impairment alters muscle-torque velocity in the knee extensors of persons with post-stroke hemiparesis. *Clin Neurophysiol*, 117(10): 2328-37, 2006.
 - b. **Clark DJ** and Patten C. Eccentric versus concentric resistance training to enhance neuromuscular activation and walking speed following stroke. *Neurorehabil Neural Repair*, 27(4):335-44, 2013.
 - c. **Clark DJ**, Patten C, Reid KF, Carabello RJ, Phillips EM and Fielding RA. Muscle performance and physical function are associated with voluntary rate of neuromuscular activation in older adults. *J Gerontol A Biol Sci Med Sci*, 66(1): 115-21, 2011. **PMC3011959**.
 - d. **Clark DJ**, Pojednic RM, Reid KF, Patten C, Pasha EP, Phillips EM and Fielding RA. Longitudinal decline of neuromuscular activation and power in healthy older adults. *J Gerontol A Biol Sci Med Sci*, 68(11): 1419-1425, 2013. **PMC3805299**.

A full list of references for Dr. David Clark is available here:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/david.clark.2/bibliography/44237379/public/?sort=date&direction=descending>

D. Research Support

1. VA Merit Review (B1149-R)

6/2014 – 5/2018

Department of Veterans Affairs, Rehabilitation Research and Development Service

Rehabilitation of corticospinal control of walking following stroke

Role: Principal Investigator

The objective of this project is to determine the efficacy of a novel “accurate walking task” rehabilitation intervention for enhancing mobility after stroke by facilitating recovery of damaged cerebral circuits. As Principal Investigator, I am responsible for overseeing all aspects of the study.

2. VA Merit Review

5/2015 – 4/2019

Department of Veterans Affairs, Rehabilitation Research and Development Service

Higher-Than-Replacement Testosterone Plus Finasteride Treatment After SCI

Role: Co-Investigator (PI: JF Yarrow)

The objective of this project is to determine the efficacy of a novel pharmacological intervention of testosterone plus finasteride to enhance skeletal, metabolic and neuromuscular function after spinal cord injury. My role on the project is to oversee aspects of data collection, analysis and dissemination.

3. VA Merit Review (1I01RX002004)
6/2016 – 5/2020
Department of Veterans Affairs, Rehabilitation Research and Development Service
A novel strategy to decrease fall incidence post-stroke
Role: Co-Investigator (PI: DK Rose)

The objective of this project is to assess the use of a novel backwards walking intervention to enhance dynamic balance and mobility function in adults post-stroke.

4. VA Rehabilitation Research and Development Center of Excellence (B9252-C)
7/2014 – 6/2019
Brain Rehabilitation Research Center of Excellence
Role: Co-Investigator and Locomotor Initiative Co-Coordinator (PI: JJ Daly)

This project funds the infrastructure of the VA Brain Rehabilitation Research Center of Excellence, located at the Malcom Randall VA Medical Center. I am responsible for leading the Locomotor Research Initiative, which conducts collaborative multi-disciplinary investigations into the rehabilitation and assessment of impairments affecting mobility in various neurologically compromised populations.

5. NIH/NIA Pilot 2P30-AG028740
7/2015 – 3/2017
via University of Florida Claude D. Pepper Older American's Independence Center
Pain and mobility function in older adults
Role: Co-Investigator (PI: Y Cruz-Almeida)

The objective of this project is to establish cerebral mechanisms of pain that interact and interfere with cerebral mechanisms of mobility in older adults.

6. VA RR&D Small Projects in Rehabilitation Research (SPiRE)
11/2015 – 10/2017
Biofeedback to Increase Propulsion during Walking after Stroke
Role: Co-Investigator (PI: DK Rose)

The objective of this study is to determine if auditory biofeedback of propulsive force generation during walking can improve output from the paretic leg of stroke patients, thereby improving the quality and speed of walking. My role on the project is to oversee aspects of data collection, analysis and dissemination.

Recently Completed Research Support (past 3 years):

1. VA Merit Review (D7675-R)
7/2012 – 6/2016
US Department of Veterans Affairs, Rehabilitation Research and Development Service
Combined cognitive and gait training
Role: Co-Principal Investigator (PI: JJ Daly)

The objective of this project is to determine the efficacy of novel stroke rehabilitation intervention that combines therapy for walking and cognitive function. I am responsible for overseeing aspects of data collection, analysis, and dissemination.

2. NIH/NIA Research Development Project (5P30AG028740-07)
11/2013 – 3/2016
via University of Florida Claude D. Pepper Older American's Independence Center
Development of clinical methods to evaluate neural function in aging
Role: Co-Investigator (PI: TW Buford)

The objective of this project is to develop local expertise in neural assessment methods that can be used to understand age-related neurological impairments underlying mobility deficits in older adults. I am responsible for overseeing aspects of data collection, analysis and dissemination.

3. Brooks Pilot Grant
8/2014 – 7/2015
Brooks Rehabilitation Collaborative Research Fund

Development of a clinical assessment tool for the measurement of walking adaptability post-stroke

Role: Co-Investigator (PI: CK Balasubramanian)

The objective of this project is to develop a battery of walking adaptability tasks that can be considered for inclusion in a future clinical assessment of walking adaptability in people with post-stroke walking deficits. My role on the project is to oversee aspects of data collection, analysis and dissemination.

4. NIH/NIA 2P30-AG028740-06 Pilot Grant
7/2012 – 3/2014
via University of Florida Claude D. Pepper Older American's Independence Center
Cortical control of walking: assessment, mechanisms and functional implications
Role: Principal Investigator

The objective of this project is to examine how cortical demands during walking are affected by task complexity and by sensory inputs. I am responsible for overseeing all aspects of the study.

5. 0214BRRC-13 Brain Rehabilitation Research Center Innovation Award
2/2014 – 9/2014
US Department of Veterans Affairs, Rehabilitation Research and Development Service
Developing the use of skin conductance as an objective physiological indicator of locomotor recovery after stroke
Role: Co-Principal Investigator

The objective of this project is to determine if skin conductance can be used as a robust physiological measurement of the anxiety/arousal occurring during complex walking tasks after stroke. I am responsible for overseeing all aspects of the study.

Publications for time period 1/1/16 – 1/1/17

Clark DJ, Neptune RR, Behrman AL and Kautz SA. A locomotor adaptability task promotes intense and task-appropriate output from the paretic leg during walking. *APMR*, 97(3):493-6, 2016. **PMC4769939**

Conference presentations for time period 1/1/16 – 1/1/17

Clark DJ. Symposium Chair for: Executive Control of Walking in Aging and Neurological Disease: Insights from fNIRS Neuroimaging. Annual Meeting of the Gerontological Society of America. New Orleans LA, November 18 2016.

Hawkins KA, Fox EJ, Daly JJ, Rose DK, Christou EA, Otzel DM, Butera KA, Chatterjee SA, and **Clark DJ**. Executive control of walking in adults with mobility deficits quantified by fNIRS neuroimaging. Society for Neuroscience. San Diego CA, November 16 2016.

Vistamehr A, Balasubramanian CK, **Clark DJ**, Conroy C, Neptune RR, and Fox EJ. Regulation of whole-body angular momentum during a variety of walking adaptability tasks in individuals with post-stroke hemiparesis. 40th Annual Meeting of the American Society of Biomechanics. Raleigh NC, August 2-5 2016.

Burke SN, Rosen-Hernandez A, Campos K, Truckenbrod L, Sakarya Y, **Clark DJ**, Carter CS, and Maurer AP. The Ketogenic Diet as Metabolic Strategy for Improving Motor and Cognitive Functioning in a Rodent Model of Senescence. Pepper Older American Independence Centers Annual Meeting. Bethesda MD, April 15 2016.

Fox EJ, **Clark DJ** and Balasubramanian CK. Symposium: Walking adaptability after neurologic injury: Assessment & intervention. Combined Sections Meeting of the APTA (Neurology Section). Anaheim CA, February 20 2016.

Robert Lewis Cook, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Robert Lewis Cook, MD, MPH

eRA COMMONS USER NAME: cookrl

POSITION TITLE: Professor of Epidemiology and Medicine

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of North Carolina at Chapel Hill, NC	BSPH	05/1986	Biostatistics
University of North Carolina at Chapel Hill, NC	MD, MPH	05/1991	Epidemiology
University of Virginia, Charlottesville, VA	Residency	06/1996	Internal Medicine
University of North Carolina at Chapel Hill, NC	Residency	06/1996	Preventive Medicine
University of North Carolina at Chapel Hill, NC	Fellowship	06/1996	RWJ Clinical Scholars

A. Personal Statement

I am a Professor of Epidemiology, with a joint appointment in Internal Medicine at the University of Florida. I have worked in the field of clinical epidemiology and HIV/STD infections for over 20 years, and most of this work has focused on connections between alcohol consumption and HIV/STD infections. I direct the Southern HIV Alcohol Research Consortium (SHARC), one of five national NIH-funded research groups that support research and training related to alcohol and HIV. The SHARC supports several research projects within the state of Florida, including the Florida Cohort study and a new study to determine the impact of alcohol consumption on brain and cognitive function. This research has provided opportunities to build collaborations across Florida, including university faculty, public health workers, and community partners. Over my academic career, I've had the opportunity to work with a range of trainees, including physicians and medical students, PhD students, and junior faculty. Overall, these mentees have been quite successful, many of them obtaining their first NIH grant and/or completing their first first-authored paper. My mentees also represent a range of diverse backgrounds in terms of gender and race/ethnicity.

In addition to research, I teach graduate courses related to infectious disease epidemiology and AIDS, I continue to practice internal medicine in an outpatient medical clinic, and I participate in several national healthcare organizations and grant review panels.

B. Positions and Honors

1994-1996	Clinical Instructor, University of North Carolina, Chapel Hill, NC
1996-2006	Assistant Professor, Department of Medicine, Division of General Internal Medicine, and Department of Community and Behavioral Health Sciences, University of Pittsburgh, Pittsburgh, PA
2006	Associate Professor, Department of Medicine, University of Pittsburgh, Pittsburgh, PA
2007-2013	Associate Professor with Tenure. Department of Epidemiology, University of Florida, Gainesville FL
2007-2014	Associate Director, Florida Center for Medicaid and the Uninsured, University of Florida
2010-2012	Director, PhD Program in Epidemiology, University of Florida
2012-present	Director, Southern HIV & Alcohol Research Consortium (SHARC), University of Florida, Gainesville, FL
2013- present	Professor (tenured), Departments of Epidemiology and Medicine, University of Florida,

Professional Societies

1993-present	Member, Society of General Internal Medicine
1996-present	Member, American Sexually Transmitted Disease Association
2007-present	Member, American Public Health Association, Florida Public Health Association
2009-present	Member, Research Society on Alcoholism

Awards/Professional Activities

1989-90	Delta Omega Undergraduate Award of Excellence, University of NC
1989-91	Holderness Medical Research Fellowship, University of NC School of Medicine
1990	Cecil G. Sheps Award in Social Medicine, University of NC School of Medicine
1999-2006	Co-Director, Bridging the Gaps Community Health Internship
2010	Delta Omega, Honorary Society of Public Health
2010	University of Florida College of Medicine Excellence in Teaching Award
2013-2016	UF Research Foundation (UFRF) Professor
2013-present	Member, Behavioral and Social Consequences of HIV/AIDS NIH study section

C. Contribution to Science

1. For nearly 20 years, the study of alcohol and HIV infection has been the most consistent theme to my academic work. My research has used both observational and intervention strategies that try to focus on the potential mechanisms by which alcohol may cause harm, and to identify the impact of alcohol within special populations. In 1999, I received a K23 award from NIAAA from which I produced several highly-cited publications, including one of the first major papers demonstrating evidence that linked alcohol consumption to poor HIV medication adherence.
 - a. **Cook RL**, Sereika SM, Hunt SC, Woodward WC, Erlen JA, Conigliaro JC. Problem drinking and medication adherence among persons with HIV infection. *J Gen Intern Med.* 2001; 16:83-88. **PMID: 11251758. PMC1495171.**
 - b. **Cook RL**, Clark DB. Is there an association between alcohol consumption and sexually transmitted diseases? A systematic review. *Sex Transm Dis* 2005; 32:156-64. **PMID: 15729152.**
 - c. **Cook RL**, Comer DM, Wiesenfeld HC, Chang CH, Tarter R, Lave JR, Clark DB. Alcohol and drug use and related disorders: an under recognized health issue among adolescents and young adults attending STD clinics. *Sex Transm Dis.* 2006; 33(9):565-70. **PMID: 16572042.**
2. Over the next several years, I published several findings related to alcohol and HIV from ongoing longitudinal cohort studies, including the VACS, MACS, and WIHS cohorts. I am especially interested in studying how alcohol exposure varies over time, how alcohol is associated with risk behavior (e.g. sexual risk behavior and ART adherence), and how alcohol can impact HIV-related comorbidities.
 - a. **Cook RL**, Zhu F, Belnap BH, Weber K, Cook JA, Vlahov D, Wilson TE, Hessol NA, Plankey M, Howard AA, Cole SR, Sharp GB, Richardson JL, Cohen MH. Longitudinal trends in hazardous alcohol consumption in women with HIV infection: 1995-2006. *American Journal of Epidemiology* 2009;169(8):1025-32. **PMID: 19270052. PMC2727230.**
 - b. **Cook RL**, Zhu F, Belnap BH, Weber, KM, Cole SR, Vlahov D, Cook JA, Hessol NA, Wilson TE, Plankey M, Howard, AA, Sharp GB, Richardson JL, Cohen MH. Long-term trajectories of alcohol consumption in adult women with and without HIV infection. *AIDS and Behavior* 2013, 17(5), 1705-1712. Initially published online July 27, 2012. **PMID: 22836592. PMC3534826. NIHMS396916.**
 - c. **Cook RL**, Kelso NE, Brumback BA, Chen X. Analytic strategies to evaluate the association of time-varying exposures to HIV-related outcomes: Alcohol consumption as an example. *Curr HIV Res.* 2015 Oct 28. [Epub ahead of print] **PubMed PMID: 26511345.**
 - d. Míguez-Burbano MJ, Quiros C, Lewis JE, Espinoza L, **Cook R**, Trainor AB, Richardson E, Asthana D. Gender differences in the association of hazardous alcohol use with hypertension in an urban cohort of people living with HIV in South Florida. *PLoS One.* 2014 Dec 9;9(12):e113122. eCollection 2014. **PubMed PMID: 25490037**
3. My long-term goals include intervention and implementation of preventive interventions into clinical care settings. Intervention research has been a major focus of my work, and I have now led or co-led three, multi-site clinical trials that focused on women with HIV or at high risk for STDs. For our current SHARC U01, we have enrolled over 200 women with HIV into a randomized clinical trial involving women with HIV infection, with study completion in late 2016. Pilot data from the planning study for this RCT were presented in 2015, and the manuscript is currently under review.
 - a. **Cook RL**, Østergaard, L, Hillier SL, Murray PJ, Chang CH, Comer DM, Ness RB for the DAISY study team. Home screening for sexually transmitted diseases in high-risk young women: randomized controlled trial. *Sex Transm Infect* 2007 Jul;83(4):286-91. **PMID: 17301105. PMC2598665.**

- b. Schwebke JR, Lee JY, Lensing S, Philip SS, Wiesenfeld W, Sena AC, Trainor N, Acevado N, Saylor L, Rompalo AM, **Cook RL**. Home screening for bacterial vaginosis (BV) to prevent sexually transmitted diseases. *Clin Infect Dis*. 2015 Nov 26. pii: civ975. PMID:26611782.
 - c. **Cook RL**, Hu X, Weber KM, Mai D, Karki M, Thomas K, Brumback B. Pharmacotherapy for hazardous drinking in women with HIV: A pilot randomized clinical trial. Presented at the 2015 Research Society on Alcoholism. San Antonio, TX. June 22, 2015; Abstract published in *Alcohol Clin Exp Research* 2015 Jun 1 (Vol. 39, pp. 77A-77A).
4. As a primary care physician, I am committed to extend our work into primary care settings. My research has studied screening measures and biomarkers that could help providers identify those in need of alcohol intervention. I have also described potential barriers that may limit primary care physician engagement in preventive activities such as alcohol intervention.
 - a. **Cook RL**, Chung T, Kelly TM, Clark DB. Alcohol screening in young persons attending a sexually transmitted disease clinic: comparison of AUDIT, CRAFFT, and CAGE instruments. *J Gen Intern Med* 2005; 20:1-6. PMID: 15693920. PMC1490040.
 - b. Korthuis PT, Berkenblit GV, Sullivan LE, Cofrancesco J, **Cook RL**, Bass M, Bashook PG, Edison M, Asch SM, Sosman JM. General internists' beliefs, behaviors, and perceived barriers to routine HIV screening in primary care. *AIDS Education and Prevention* 2011; 23(3 Suppl): 70–83. PMID: 21689038. PMC3196638. HHS295322.
 - c. Harle CA, Bauer SE, Hoang HQ, **Cook RL**, Hurley RW, Fillingim RB. Toward decision support for chronic pain care: How do primary care physicians decide when to prescribe opioids? A qualitative study. *BMC Family Practice*, 2015 Apr 14;16:48. doi: 10.1186/s12875-015-0264-3.
 5. More recently, my work has continued to describe aspects of drinking that may differ by HIV status, and to better define the relationship of alcohol consumption to chronic disease outcomes, including neurocognitive function.
 - a. Bryant VE, Whitehead NE, Burrell LE, Dotson V, **Cook RL**, Cohen RA. Depression and apathy among people living with HIV: substance use and cognitive performance differences and the implications for treatment of HIV Associated Neurocognitive Disorders. *AIDS Behav* 2015 Aug;19(8):1430-7. PubMed PMID: 25533921
 - b. McGinnis KA, Fiellin DA, Tate JP, **Cook RL**, Braithwaite RS, Bryant KJ, Edelman EJ, Gordon AJ, Kraemer KL, Maisto S, Justice AC. Number of drinks to "Feel a Buzz" varies by HIV status and viral load in men. (In press), *AIDS and Behavior*, 2015.
 - c. Kelso N, Sheps D, **Cook RL**. The association between alcohol use and cardiovascular disease among people living with HIV: A systematic review. *American Journal of Drug and Alcohol Abuse*, 2015. Aug 18:1-10 PMID: 26286352
 - d. Miguez-Burbano MJ, Espinoza L, Whitehead NE, Bryant VE, Vargas M, **Cook RL**, Quiros C, Lewis JE, Deshratan A. Brain derived neurotrophic factor and cognitive status: The delicate balance among people living with HIV, with and without alcohol abuse. *Curr HIV Res*. 2014;12(4):254-64. PMID: 25053366

My complete bibliography with over 105 peer-reviewed citations is included here:

<http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/41142419/?sort=date&direction=descending>

D. Research Support

Ongoing Research Support

Southern HIV & Alcohol Research Consortium (SHARC)

Role: Principal Investigator

Funding: NIAAA U24 AA022002

Period: 2012 – 2017

The mission of the SHARC is to improve health outcomes and reduce HIV transmission among the diverse range of populations affected by alcohol and HIV infection in the Southeastern United States.

Effects of experimentally-induced reductions in alcohol consumption on brain, cognitive, and clinical outcomes and motivation for changing drinking in older persons with HIV infection.

Role: Principal Investigator (MPI Ronald Cohen)

Funding: NIAAA U24 AA022797

Period: 2016 – 2021

This study will enroll 180 persons who drink alcohol and challenge them to stop drinking for 30- to 90-days using cash payments and a wearable alcohol biosensor to help maximize drinking reduction. Participants will receive a variety of neuroimaging and cognitive assessments to study changes in the body with alcohol reduction.

Miami Women's Interagency HIV Study (WIHS)

Role: Co-investigator (Fischl PI)

Funding: NIAID U01AA103397

Period: 2013-2018

The Miami Women's Interagency HIV Study is part of the national WIHS Cohort, which seeks to study long-term outcomes of HIV infection in women. As part of the Miami site, Dr. Cook is involved with the behavioral working group and will study other long-term outcomes in HIV.

Alcohol Consumption Patterns on Cardiovascular Health in Persons Living with HIV

Role: Primary Mentor (Kelso, PI)

Funding: NIAAA F31AA024064

Period: 2015-2017

This F31 grant will support the doctoral dissertation research for Natalie Kelso, a student obtaining a PhD in epidemiology at the University of Florida. Ms. Kelso will study the impact of alcohol consumption on carotid artery thickness and plaque within the WIHS and MACS cohorts.

Designing user-centered decision support tools for chronic pain in primary care

Role: Co-I (PI University of Florida subcontract), (Harle, PI)

Funding: AHRQ R01HS023306

Period: 2015-2017

The project seeks to understand how primary care physicians make decisions about how to treat patients with chronic pain, and to learn about what types of information they need to help make optimal decisions.

Alcohol and HIV-associated brain dysfunction – Brown University ARCH

Role: Co-I (Cohen, PI)

Funding: NIAAA P01 AA019072

Period: 2015-2019

The study is part of a series of P-grants affiliated with the Alcohol Research Center for HIV (ARCH), based at Brown University. This proposal will compare neurocognition and neuroimaging in persons with HIV according to age, HIV status, and alcohol consumption patterns.

Feasibility of SBIRT for underserved HIV+ adults age 50+ in primary care settings.

Role: Primary mentor (Whitehead, PI)

Funding: NIDA K23DA039769

Period: 2015-2019

This project is a mentored, career development award for Dr. Nicole Whitehead, a minority investigator who seeks to implement substance abuse interventions for older adults with HIV infection. Dr. Cook is the primary mentor on this award.

Previous Recent Research Support

The Impact of Long-Term Marijuana Use on the Neurocognitive Functioning of Individuals Living with HIV/AIDS

Role: Primary Mentor (Okafor, PI)

Funding: NIDA F31 DA039810

Period: 2015-2017

This F31 grant will support the doctoral dissertation research for Emeka Okafor, a minority student obtaining a PhD in epidemiology at the University of Florida. He will study the impact of cumulative marijuana use on changes in cognitive function among men participating in the MACS cohort.

Immune Dys-regulation in HIV-infected Women with Heavy Alcohol Consumption

Role: Co-investigator (Desai PI)

Funding: National Institute of Alcohol Abuse and Alcoholism

U01AA020800

Period: 09/10/11 – 08/31/15

The major goal of this project is to examine whether chronic cumulative alcohol exposure is associated with more rapid CD4 T cell decline and immune dys-functionality (microbial translocation, immune activation, inflammation and immune senescence) leading to early advent of AIDS and non AIDS co-morbidities

Southern HIV Alcohol Research Consortium Annual Meeting (SHARC)

Role: Principal Investigator

Funding: NIAAA R13 AA023167

Period: 2015

The SHARC Annual meeting focuses on research and training that is culturally relevant to the range of affected populations in Florida. The meeting showcases current research activities and helps to develop new ideas and collaborations related to SHARC.

Pharmacotherapy for alcohol consumption in HIV-infected women: Randomized trial

Role: Principal Investigator

Funding: National Institute of Alcohol Abuse and Alcoholism 1 U01 AA020797

Period: September 25, 2011 – September 24, 2016

This study is a double-blind randomized clinical trial to determine the efficacy of the medication naltrexone to reduce alcohol consumption and complications in women with HIV. (Note that AA020797 was recently renewed for 2016-2021).

Pharmacotherapy to reduce hazardous drinking in HIV-infected women

Role: Principal Investigator

Funding: NIAAA R01AA018934

Period: 2009-2012

This was a 3-year study to design and pilot-test an intervention to reduce hazardous drinking in women with HIV infection from three recruitment settings across the US.

Publications (2016)

1. *Hu X, Chen X, **Cook RL**, Chen D-G, Okafor C. Modeling drinking behavior progression in youth with cross-sectional data: solving an under-identified probabilistic discrete event system. *Curr HIV Research*. 2016;14(2):93-100. **PMID: 26511344**.
2. **Cook RL**, Kelso NE, Brumback BA, Chen X. Analytic strategies to evaluate the association of time-varying exposures to HIV-related outcomes: Alcohol consumption as an example. *Curr HIV Res*. 2016;14(2):85-92. **PubMed PMID: 26511345**.
3. *Akhtar-Khaleel W, **Cook RL**, Shoptaw S, Surkan P, Stall R, Toplin L, Plankey M. Trends and predictors of cigarette smoking among HIV seropositive and seronegative men: The Multicenter AIDS Cohort Study. *AIDS and Behavior*, 2016 Mar;20(3):622-32. doi: 10.1007/s10461-015-1099-6. **PMID: 26093780**.
4. Schwebke JR, Lee JY, Lensing S, Philip SS, Wiesenfeld W, Sena AC, Trainor N, Acevado N, Saylor L, Rompalo AM, **Cook RL**. Home screening for bacterial vaginosis (BV) to prevent sexually transmitted diseases. *Clin Infect Dis* 2016 Mar 1;62(5):531-6. doi: 10.1093/cid/civ975. Epub 2015 Nov 26. **PMID:26611782**.
5. *Akhtar-Khaleel WZ, **Cook RL**, Shoptaw S, Surkan PJ, Teplin LA, Stall R, Beyth RJ, Manini T, Plankey M. Long-term cigarette smoking trajectories among HIV-seropositive and seronegative MSM in The Multicenter AIDS Cohort Study. *AIDS Behav*. 2016 Aug; 20(8): 1713-21 **PMID:26922718**
6. Justice AC, McGinnis KA, Tate JP, Braithwaite RS, Bryant KJ, **Cook RL**, Edelman J, Fiellin LE, Freiberg MS, Gordon AH, Kraemer KL, Marshall BDL, Williams EC, Fiellin DA. Risk of mortality and physiologic injury evident with lower alcohol exposure among HIV infected compared with uninfected men. *Drug Alcohol Depend*. 2016 Apr 1; 161:95-103. doi: 10.1016/j.drugalcdep.2016.01.017. **PMID:26861883**.
7. McGinnis KA, Fiellin DA, Tate JP, **Cook RL**, Braithwaite RS, Bryant KJ, Edelman EJ, Gordon AJ, Kraemer KL, Maisto S, Justice AC. Number of drinks to "Feel a Buzz" varies by HIV status and viral load in men. *AIDS Behav*. 2016 Mar;20(3):504-11. doi: 10.1007/s10461-015-1053-7. **PMID:26936030**.
8. Harman J, Harle C, Mills J, **Cook RL**. Electronic health record availability and anxiety treatment in office based practices. *Psychiatr Serv*. 2016 Oct 1;67(10):1152-1155. **PMID: 27133721**.
9. *Hu X, Harman J, Winterstein AG, Zhong Y, Wheeler AL, Taylor TN, Plankey M, Rubtsova A, Cropsey K, Cohen MH, Adimora AA, Milam J, Adedmeji A, **Cook RL**. Utilization of alcohol treatment among HIV-positive women with hazardous drinking. *J Subst Abuse Treat*. 2016 May; 64:55-6. doi: 10.1016/j.jsat.2016.01.011. **PMID:26961420**
10. **Cook RL**, Cook CL, Karki M, Weber KM, Thoma KA, Loy CM, Goparaju L, Rahim-Williams B. Reasons for drinking and perceived consequences of alcohol consumption among women living with HIV. *BMC Public Health*, 2016 Mar 15;16(1):263. doi: 10.1186/s12889-016-2928-x. **PMID: 26975297**.

11. *Okafor CN, Zhou Z, Burrell LE, Kelso NE, Whitehead NE, Harman JS, Cook CL, **Cook RL**. Marijuana use and viral suppression in persons receiving medical care for HIV-Infection in Florida. *Am J Drug Alcohol Abuse*. 2016 Jul 11:1-8. **PMID:27398989**
12. *Okafor CN, **Cook RL**, Chen X, Surkan P, Becker J, Shoptaw S, Martin E, Plankey M. Trajectories of marijuana use among HIV-seropositive and HIV-seronegative MSM in the Multicenter AIDS Cohort Study (MACS), 1984-2014. *AIDS Behav*. 2016 Jun 3. [Epub ahead of print] **PMID:27260179**.
13. *Okafor C, Kelso NE, Bryant V, Burrell LE II, Miguez MJ, Gongvatana A, Tashima K, de la Monte S, **Cook RL**, Cohen RA. Body mass index, inflammatory biomarkers and neurocognitive impairment in HIV-infected persons. *Psychol Health Med*. 2016 Jun 20:1-14. [Epub ahead of print] **PMID: 27319430**.
14. *Kelso-Chichetto NE, Okafor CN, Zhou Z, Canidate SS, Harman JJ, Cook CL, **Cook RL**. Complementary and alternative medicine use for HIV management in the state of Florida: Medical Monitoring Project. *J Altern Complement Med*. 2016 Sep 15. [Epub ahead of print]. **PMID:27631385**.
15. Azarian T, Maraqa N, **Cook RL**, Johnson JA, Bailey C, Wheeler S, Nolan D, Rathoer MH, Morris JG, Salemi M. Genomic epidemiology of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *PLoS One*. 2016 Oct 12;11(10):e0164397. doi: 10.1371/journal.pone.0164397. **PMID: 27732618**
16. Sharpe JD, Hopkins RS, **Cook RL**, Striley C. Evaluating Google, Twitter, and Wikipedia as tools for influenza surveillance using Bayesian change point analysis: a comparative analysis. *JMIR Public Health Surveill*. 2016 Oct 20;2(2):e161. **PMID: 27765731**
17. Helian S, Brumback BA, **Cook RL**. Sparse canonical correlation analysis between phosphatidylethanol (PEth) and self-reported alcohol consumption. Accepted, 2016 *Communications in Statistics, Simulation and Computation*.
18. *Okafor CN, Chen X, Surkan P, Becker J, Shoptaw S, Martin E, Plankey M, **Cook RL**. Trends in the prevalence and predictors of marijuana use in HIV-seropositive and seronegative men in the Multicenter AIDS Cohort Study (MACS), 1984-2013. *Am J Drug Alcohol Abuse*. 2016 Nov 3:1-11. [Epub ahead of print] **PMID: 27808576**
19. *Akhtar-Khaleel WZ, **Cook RL**, Shoptaw S, Surkan PJ, Teplin LA, Stall R, Beyth RJ, Manini T, Price CE, Sactor N, Plankey M. Association of midlife smoking status with processing speed and mental flexibility among HIV-seropositive and HIV-seronegative older men: The Multicenter AIDS Cohort Study. 2016. *Journal of NeuroVirology*. Nov 26 (epub ahead of print) **PMID: 27889886**

Presentations (2016)

1. Rahim-Williams B, Kelso NE, Brumback B, Bryant K, **Cook RL**. Alcohol and Medication Use as Pain Treatment among Women living with HIV (WLHIV). Abstract accepted at 35th Annual Scientific Meeting of the American Pain Society. May 11-14, 2016. Funding support from U01 AA020797.
2. Ayala DV, Ibanez GE, **Cook RL**, Cook C, See J, Morano J, Zhou Z. Linkage to Care and HIV-Positive Individuals with an Incarceration History: The Florida Cohort Study. Poster presentation at the 2016 Annual SHARC Conference. Miami, FL. May 17-18, 2016. 1st Prize.
3. Bryant V, Woods AJ, Porges EW, **Cook RL**, Kahler CW, Tashima K, Cohen RA. Frontal Neural Correlates of Working Memory Decline in Hazardous Drinkers Living with HIV. Poster presentation at the 2016 Annual SHARC Conference. Miami, FL. May 17-18, 2016. 2nd Prize.
4. Canidate S, Cook CL, **Cook RL**. A Qualitative Assessment of HIV-Positive Women's Experiences in a Randomized Clinical Trial to Reduce Drinking. Poster presentation at the 2016 Annual SHARC Conference. Miami, FL. May 17-18, 2016.
5. Kamara M, Hart M, **Cook RL**, Lee S, Abbott S. Mobile Apps for Alcohol Use Disorder. Poster presentation at the 2016 Annual SHARC Conference. Miami, FL. May 17-18, 2016.
6. Kelso NE, **Cook RL**, Okafor C, Abraham A, Bolen R, Plankey M. Predictors of Changes in Depressive Symptoms among Persons Living with HIV. 2016 Annual SHARC Conference. Miami, FL. May 17-18, 2016.
7. Sharpe JD, **Cook RL**. Mobile Technology Use among Persons Living with HIV who Consume Alcohol. Poster presentation at the 2016 Annual SHARC Conference. Miami, FL. May 17-18, 2016.
8. **Cook RL**. Can phosphatidylethanol (PeTH) be used as a biomarker to study the relationship of alcohol consumption to clinical outcomes? Invited talk, "neuroHIV and Alcohol Abuse" – national meeting sponsored by NIAAA. Miami, FL, May 2016.

9. **Cook RL**, Zhou Z, Kelso N, Whitehead N, Cook C, Harman J, Bryant K, Poschman K, Grigg B. Does hazardous drinking contribute to gender and racial disparities in healthcare engagement and viral load suppression among persons living with HIV? Oral Presentation. 2016 Epidemiology Congress of the Americas. Miami, FL. June 21-24, 2016.
10. Kelso NE, Rahim-Williams B, Brumback B, Bryant K, **Cook RL**. (2015). The association between pain and change in alcohol consumption among women living with HIV who are hazardous drinkers. Abstract submitted for poster or oral presentation. 2016 Annual Meeting of the Society of Epidemiologic Research. Miami, FL, June 21-24, 2016.
11. Canidate S, Cook CL, **Cook RL**. A qualitative assessment of HIV-Positive Women's Experiences in a clinical trial to reduce drinking. Abstract submitted to 2016 Annual Meeting of the Research Society on Alcoholism. New Orleans, LA. June 25-29, 2016.
12. **Cook RL**, Zhou Z, Janelle J, Whitehead N, Cook CL, Cohen R, Morano J, Somboonwit C, Carter W, Bryant K. Relationship of heavy drinking and binge drinking to HIV viral suppression within a new HIV cohort in Florida. Poster - 2016 Annual Meeting of the Research Society on Alcoholism. New Orleans, LA. June 25-29, 2016.
13. Kelso NE, Miguez MJ, Okafor CN, **Cook RL**. The association between alcohol beverage type preference and metabolic risk among women living with HIV who are hazardous drinkers. Abstracted submitted for poster or oral presentation. 2016 Annual Meeting of the Research Society on Alcoholism. New Orleans, LA. June 25-29, 2016.

Steven T. DeKosky, MD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: DeKosky, Steven T.

eRA COMMONS USER NAME (credential, e.g., agency login): DeKoskyST

POSITION TITLE: Professor of Neurology

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Bucknell University, Lewisburg, PA	A.B.	1968	Psychology
University of Florida, Gainesville, FL	Grad. School	1968-70	Psychology/Neuroscience
University of Florida College of Medicine	M.D.	1974	Medicine
The Johns Hopkins Hospital, Baltimore, MD	Internship	1974-75	Internal Medicine
University of Florida College of Medicine	Residency	1975-78	Neurology
University of Virginia, Charlottesville, VA	Post Doc	1978-79	Neurochemistry

A. Personal Statement

I have worked in Alzheimer's disease (AD) and related disorders for over 30 years, studying neurochemical, neuroanatomical, genetic, and pathological changes (amyloid, neurofibrillary tangles) in AD, MCI, and normal elderly. I began clinical studies in cognitive, behavioral, neuroimaging and therapeutic interventions to translate my bench research studies, correlating imaging and cognition, trials of new medications including First in Man studies in the Pitt Alzheimer Center, and large scale (>3,000 Ss) long term (>6 years) multicenter dementia prevention trials using Ginkgo biloba; I was PI of the GEM trial. I was founding co-director (1985-1990; U. Kentucky) or director (1994-2008; Pittsburgh) of Alzheimer's Disease Research Centers (ADRCs) and serve as chair of Drug Safety Monitoring Boards. I have served as consultant/advisor for multiple pharma and biotech companies, ADRCs, and as Chair of the Alzheimer's Association Med-Sci Advisory Council, and Chair of the Med-Sci Advisory Panel of Alzheimer's Disease International. I chaired the American Academy of Neurology's Practice Parameter Workgroup on Early Detection, Diagnosis, and Treatment of Dementia, and served on or chaired multiple committees for the NIA regarding aging and dementia. I was a member of the NIH Council of Councils (overseeing the Common Fund), and served previously on the NCCAM (now NCCIH) Council. I maintained an NIH funded wet lab for over 30 years, and served as chair of the Pitt Department of Neurology for 8 years. Then, as Vice President and Dean of the University of Virginia School of Medicine (2008-2013) I developed further skills in management of large research and academic projects, and my return to research via a sabbatical year at Penn and Pitt has facilitated my re-entry into research and research administration. Returning to my graduate and medical school alma mater, the University of Florida, in July 2015, I now am Deputy Director of the McKnight Brain Institute, the center of neuroscience research and teaching at UF, as well as Associate Director of the newly NIA-funded 1Florida ADC, a collaboration among UF, Mt. Sinai Hospital in Miami Beach, and several other Florida universities.

B. Positions and Honors

Positions and Employment

- 1979-1990 Asst.to Assoc. Prof, Depts. Neurology & Anatomy/Neurobiology, Univ. Kentucky, Lexington, KY
and Staff Neurologist, Lexington VA Medical Center
- 1985-1990 Co-Director/Co-PI, Alzheimer's Disease Research Center, Univ. of Kentucky, Lexington, KY
- 1985-1987 Interim Chair, Department of Neurology, University of Kentucky, Lexington, KY
- 1985-1987 Director, Neurology Residency Training Program, University of Kentucky, Lexington, KY
- 1990-2002 Professor of Psychiatry, Neurology, and Neurobiology, University of Pittsburgh School of Medicine and Western Psychiatric Institute and Clinic (WPIC), Pittsburgh, PA
- 1990-1994 Co-Director, Alzheimer's Disease Research Center, University of Pittsburgh, Pittsburgh, PA
- 1992-2001 Director, Div. of Geriatrics & Neuropsychiatry, Dept. of Psychiatry/WPIC, Univ. of Pittsburgh
- 1994-2008 Director, ADRC, University of Pittsburgh Medical Center, Pittsburgh, PA

1997-2008	Professor, Dept. of Human Genetics, Graduate School of Public Health, University of Pittsburgh
2000-2008	Chair, Department of Neurology, University of Pittsburgh, Pittsburgh, PA
2008-present	Adjunct Professor of Neurology, University of Pittsburgh School of Medicine
2008-2013	Vice President and Dean, University of Virginia School of Medicine, Charlottesville, VA; Physician in Chief, University of Virginia Health System
2008-2014	Professor of Neurology and Psychiatry and Behavioral Sciences, UVA School of Medicine
2013-2014	Visiting Professor, Department of Medical Ethics and Health Policy, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA [Sabbatical]
2014-2015	Visiting Scholar, Department of Radiology (PET Center) and Neurology, University of Pittsburgh School of Medicine/UPMC, Pittsburgh, PA [Sabbatical]
2015-present	Professor of Neurology Emeritus, University of Virginia
2015-present	Professor of Neurology and Deputy Director, McKnight Brain Institute, University of Florida College of Medicine, Gainesville, FL
2015-present	Associate Director, 1Florida Alzheimer's Disease Center
2015-present	Deputy Director, McKnight Brain Institute, University of Florida
2015-2016	Interim Executive Director, McKnight Brain Institute, University of Florida

Other Experience and Professional Memberships

1994-2010	National Board of Directors, Alzheimer's Association, Chicago, IL; Vice-Chairman, 1998-2001
1997-2001	NIH Study Section, Neuroscience of Aging Review Committee (NIA) (Chair, 2002-2001)
1997-2001	Chair, Medical and Scientific Advisory Council, Alzheimer's Association
2002-2005	Chair, Medical and Scientific Advisory Panel, Alzheimer's Disease International
2004-2010	Member & Vice President (2010), American Board of Psychiatry & Neurology (ABPN)
2004-2007	Member, Peripheral & Central Nervous System Drugs Advisory Committee, FDA; currently
2005-present	Member, Board of Directors, American Society for Experimental NeuroTherapeutics
2008-2104	Founding Chair, ISTAART (International Society to Advance Alzheimer Research & Treatment)
2008-2013	Council of Deans, American Association of Medical Colleges (AAMC)
2009-2012	National Advisory Council for the National Center on Complementary and Alternative Medicine (NCCAM: now National Center on Complementary and Integrative Health, NCCIH)
2013-2015	Council of Councils (National Advisory Council to the NIH Director for the Common Fund)

Honors

1968-1969	Predoctoral Fellowship, Center for Neurobiological Sciences, Univ. Florida College of Medicine
1972	Alpha Omega Alpha Research Award, University of Florida College of Medicine
1974	Roger Schnell Award for Excellence in Clinical Neurology (University of Florida)
1978-1979	National Research Service Award in Developmental Neurology (Neurochemistry) NINCDS
1980-1985	Teacher-Investigator Development Award, NINCDS
1988	Presidential Award, American Neurological Association
1994-present	The Best Doctors in America
2000	Distinguished Alumnus, University of Florida College of Medicine ("Wall of Fame")
2003-present	America's Top Doctors
2003	Rita Hayworth Award, Alzheimer's Association
2005	Ronald and Nancy Reagan Research Institute Award for research/care/advocacy in AD
2006	NIH Clinical Center Great Teachers Award
2008	Alzheimer's Association Zaven Khachaturian Award
2008-2013	James Carroll Flippin Professor of Medical Science, University of Virginia
2009-present	Elected Fellow, American College of Physicians
2014	Thompson Reuters Top 1% of Cited Papers
2015	Who's Who in America (Platinum edition)
2015-present	Aerts-Cosper Professor of Alzheimer's Research, University of Florida

C. Contribution to Science (*chosen from >440 publications*)

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1XyAvtqHISWAQ/bibliographay/48450674/public/?sort=date&direction=descending>

1. Neurochemistry and synaptic plasticity in aging, MCI, and dementia

I was first to report (with Steve Scheff) the loss of synapses (by quantitative EM) in living humans with AD, that synapse counts

correlated with cognition, and that enlargement of residual synapses occurred with synaptic loss. I also demonstrated that unlike prior understanding, that cholinergic enzymes were increased in the hippocampus and frontal cortex (but not other cortical areas) during MCI—a neuroplastic attempt to compensate for neurodegeneration, which then decreased as progression to AD occurred.

- a. **DeKosky, S.T.** and Scheff, S.W. Synapse loss in frontal cortex biopsies in Alzheimer's disease: Correlation with cognitive severity. *Annals of Neurology* 27:457-464, 1990.
- b. **DeKosky, S.T.**, Harbaugh, R.E., Schmitt, F.A., Bakay, R.A.E., Chui, H.C., Knopman, D.S., Reeder, T.M., Shetter, A.G., Senter, H.J., Markesbery, W.R., and the Intraventricular Bethanecol Study Group. Cortical biopsy in Alzheimer's disease: Diagnostic accuracy and neurochemical, neuropathological and cognitive correlations. *Annals of Neurology* 32:625-632, 1992.
- c. **DeKosky, S.T.**, Ikonomic, M.D., Styren, S.D., Beckett, L., Wisniewski, S., Bennett, D., Kordower, J.H., and Mufson, E.J. Up-regulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Annals of Neurology* 51:145-155, 2002.
- d. Ikonomic, M.D., Klunk, W.E., Abrahamson, E.E., Wu, J., Mathis, C.A., Scheff, S.W., Mufson, E.J. and **DeKosky, S.T.** Precuneus amyloid burden is associated with reduced cholinergic activity in Alzheimer disease. *Neurology* 77:39-47, 2011. **PMCID: 3127332**

2. **Amyloid imaging in Alzheimer's Disease**

I held the IND, was PI of the initial Program Project Grant, and led the clinical studies of the first PET amyloid imaging compound Pittsburgh Compound B (PiB). I participated in clinical study design, assessment of the relationship of amyloid load to clinical status and cortical metabolism as indexed by FDG-PET.

- a. Mintun, M.A., LaRossa, G.N., Sheline, Y.I., Dence, C.S., Lee, S.Y., Mach, R.H., Klunk, W.E., Mathis, C.A., DeKosky, S.T., and Morris, J.C. [11C] PiB in a nondemented population: Potential antecedent marker of Alzheimer disease. *Neurology* 67:446-452, 2006.
- b. Ikonomic, MD, Klunk, WE, Abrahamson, EE, Mathis, CA, Price, JC, Tsopelas, ND, Lopresti, BJ, Ziolko, S, Bi, W, Paljug, WR, Debnath, ML, Hope, CE, Isanski, BA, Hamilton, RL and **DeKosky, ST** Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. *Brain* 131:130-1645, 2008. **PMCID:PMC2408940**
- c. Cohen, A., Price, J., Weissfeld, L., James, J., Rosario, B., Bi, W., Nebes, R., Saxton, J., Snitz, B., Aizenstein, H., Wolk, D., **DeKosky, S.T.**, Mathis, C. and Klunk, W. Basal cerebral metabolism may modulate the cognitive effects of A β in mild cognitive impairment: An example of brain reserve. *Journal of Neuroscience* 29:14770-14778, 2009. **PMCID: 2810461**
- d. Wolk, D.A., Price, J.C., Madeira, C., Saxton, J.A., Snitz, B.E., Lopez, O.L., Mathis, C.A., Klunk, W.E. and **DeKosky, S.T.** Amyloid imaging in dementias with atypical presentation. *Alzheimer's and Dementia* 8:389-398, 2012. **PMCID: 3517915**
- e. Snitz, B.E., Weissfeld, L.A., Lopez, O.L., Kuller, L.H., Saxton, J., Singhabu, D.M., Klunk, W.E., Mathis, C.A., Price, J.C., Ives, D.G., Cohen, A.D., McDade, E. and **DeKosky, S.T.** Cognitive trajectories associated with β -amyloid deposition in the oldest-old without dementia. *Neurol* 80:1378-1384, 2013. **PMCID: PMC3662268**

3. **Experimental Brain Trauma:**

I began experiments utilizing controlled traumatic brain injury as a way to study cascades that I thought were similar to Alzheimer's disease in the early 1990s (before transgenic mouse models were available). My lab demonstrated up-regulation of NGF and its control by IL1 β , elevation of APP and A β in trauma, and a number of interventions to stop elevation of A β after injury, including some applicable in human studies.

- a. **DeKosky, S.T.**, Goss, J.R., Miller, P.D., Styren, S.D., Kochanek, P.M., and Marion, D. Up-regulation of nerve growth factor following cortical trauma. *Experimental Neurology* 130:173-177, 1994.
- b. **DeKosky, S.T.**, Taffe, K.M., Abrahamson, E.A., Dixon, C.E., Kochanek, P.M., and Ikonomic, M.D. Time course analysis of hippocampal nerve growth factor and antioxidant enzyme activity following lateral controlled cortical impact brain injury in the rat. *Journal of Neurotrauma* 21:491-500, 2004.
- c. Abrahamson, E.E., Ikonomic, M.D., Ciallella, J.R., Hope, C.E., Paljug, W.R., Isanski, B.A., Flood, D.G., Clark, R.S.B., and **DeKosky, S.T.** Caspase inhibition therapy abolishes brain trauma-induced increases in A β peptide: Implications for clinical outcome. *Experimental Neurology* 197:437-450, 2006.
- d. Abrahamson, E. E., Ikonomic, M.D., Dixon, D.E. and **DeKosky, S.T.** Simvastatin therapy prevents brain trauma-induced elevations in β -amyloid peptide levels. *Annals of Neurology* 66:407-414 2009. **PMID: 19798641**

4. **Human Brain Trauma**

With Bennet Omalu I described the first case of CTE in an American football player, then 4 additional cases. Our human brain tissue studies following acute TBI confirmed rapid up-regulation of APP, A β and A β plaques (within 2 hours), a possible risk factor for subsequent cognitive decline, suggesting acute post-TBI interventions and bringing the study of AD and TBI together. We are now studying tau as a potential biomarker of CTE in living subjects.

- a. Ikonovic, M.D., Uryu, K., Abrahamson, E.E., Ciallella, J.R., Trojanowski, J.Q., Lee, V. M.-Y., Clark, R.S., Marion, D.W., Wisniewski, S.R., and **DeKosky, S.T.** Alzheimer's pathology in human temporal cortex surgically excised after severe brain injury. *Experimental Neurology* 190:192-203, 2004.
- b. **DeKosky, S.T.**, Abrahamson, E.E., Ciallella, J.R., Paljug, W.R., Wisniewski, S.R., Clark, R.S.B., and Ikonovic, M.D. Association of increased cortical soluble A β 42 levels with diffuse plaques after severe brain injury in humans. *Archives of Neurology* 64:541-544, 2007.
- c. Omalu, B.I., **DeKosky, S.T.**, Minster, R.L., Kamboh, M.I., Hamilton, R.L. and Wecht, C.H. Chronic traumatic encephalopathy in a National Football League (NFL) player. *Neurosurgery* 57:128-134, 2005.
- d. **DeKosky, S.T.**, Blennow, K., Ikonovic, M.D. and Gandy, S. Acute and chronic traumatic encephalopathies: Pathogenesis and biomarkers. *Nature Reviews Neurology* 9:192-200, 2013. **PMCID: 4006940**
- e. **DeKosky, S.T.**, Ikonovic, M.D. and Gandy, S. Traumatic brain injury: Football, warfare, and long-term effects. *New England Journal of Medicine* 363:1293-1296, 2010. **PMID: 21265421**

5. **Mild Cognitive Impairment and Prevention of Dementia**

I chaired the AAN practice parameter review that first defined MCI for research and subsequently clinical practice, showed multiple ways neuroplastic responses occurred in MCI, had a leading role in the redefinition of MCI 10 years later, and directed the first prevention trial for AD, the NIH-funded GEM Study, using Ginkgo biloba. I have published multiple studies of MCI in imaging, cognition, and behavioral symptoms.

- a. Petersen, RC, Stevens, JC, Ganguli, M et al and **DeKosky, ST** (2001) Practice parameter: Early detection of dementia: Mild cognitive impairment (an evidence-based review). *Neurology* 56:1133-1142.
- b. Albert, M.S., **DeKosky, S.T.**, Dickson, D., Dubois, B., Feldman, H.H., Fox, N.C., Gamst, A., Holtzman, D.M., Jagust, W.J., Petersen, R.C., Synder, P.J., Carrillo, M.C., Thies, B. and Phelps, C.H. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7:270-279, 2011. **PMCID: 3312027**
- c. **DeKosky ST**, Williamson JD, Fitzpatrick AL, Kronmal RA, Ives DG, Saxton JA, Lopez OL, Burke G, Carlson MC, Fried LP, Kuller LH, Robbins JA, Tracy RP, Woolard NF, Dunn L, Snitz BE, Nahin RL, Furberg CD. (2008) Ginkgo Evaluation of Memory (GEM) Study Investigators. Ginkgo biloba for prevention of dementia: a randomized controlled trial. *JAMA*. 19;300:2253-62. **PMCID: PMC2823569.**
- d. Gandy, S. and **DeKosky, S.T.** (2013) Toward the treatment and prevention of Alzheimer's disease: Rational strategies and recent progress. *Annual Review of Medicine* 64:367-383. **PMCID: PMC3625402**

D. Additional Information: Research Support

Ongoing Research Support

P50 AG047266-01A1

Golde (Director & Project Leader)

08/15/2015-05/31/2020

NIH/NIA

University of Florida and Mt. Sinai Medical Center AD Research Center

Major goals. The UF-MSMC ADRC will be focused on several activities. Clinical research activities include identification of i) markers for the earliest prodromal stages of cognitive impairment and ii) predictors of cognitive and functional decline in Hispanic and non-Hispanic individuals. The ADRC facilitates testing of novel therapies for AD and related dementias in our diverse population and will provide community and professional training and education relevant to AD and related dementias, thus having a broad state-wide educational impact. We train junior investigators & recruit trainees & investigators to participate in dementia research. Finally, the ADRC supports translational research studies to provide additional insights into AD that may lead to development of novel therapeutic approaches & novel diagnostic paradigms.

Role: Associate Director (Assoc. PL, Administrative Core)

529-13-0046-00001

Shenkman (PI)

03/01/2015-08/31/2019

Texas Health & human Serv.-HRSA

Texas External Quality Review Organization Vendor and Quality Vendor

The major goals of this project are to assess a program in which dual eligible (Medicare and Medicaid) patients are provided with resources to improve their health and fitness using flexible methods and objective follow-up of health and health care resource utilization.

Role: Co-Investigator

6AZ05

Cottler (PI)

02/23/2016-01/31/2018

FL Dept. of Health Ed & Ethel Moore Alzheimer's

Linking Older Adults from the Community in Florida to Memory Screening and Related Health Research

The major goals of this project are to determine best ways of outreach to screen and direct subjects with memory problems to a Memory Disorders Clinic and research studies.

Role: Co-Investigator

Completed Research Support

1P01 AG025204

Klunk (PI)

06/15/2011-04/30/2015

NIH

In Vivo PIB PET Amyloid Imaging: Normals, MCI & Dementia

The aim of this proposal is to define amyloid deposition in early (and pre-clinical) phases of AD and assess PIB as a surrogate marker of efficacy for anti-amyloid therapies.

Role: Site PI

5 P50 AG005133

Lopez (PI)

04/01/2010-03/31/2015

NIH

ADRC Core B: UVA Satellite Clinic

The Satellite Clinic will perform clinical and research evaluations and study entry & annual follow up with rural African American subjects with dementia & normal cognition in the Satellite Clinic at the University of Virginia.

Role: Site PI

2 P01 AG14449

Mufson (PI)

09/01/2007-03/31/2013

NIA

Neurobiology and Cognitive Impairment of the Elderly

This proposal seeks to determine what specific system impairment is reasonable for the earliest manifestations

Role: PL Project 4

Mingzhou Ding, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ding, Mingzhou

eRA COMMONS USER NAME: mingzhou_ding

POSITION TITLE: Pruitt Family Professor

EDUCATION/TRAINING:

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Peking University, China	BS	1982	Astrophysics
Institute of Theoretical Physics, China	--	1982-86	Theoretical Physics
University of Maryland, College Park, MD	PhD	1990	Physics

A. Personal Statement

The long-term objective of my laboratory is to understand the neural basis of cognition and its impairments in neurological and psychiatric disorders. Trained as a theoretical physicist/applied mathematician, my initial encounters with cognitive neuroscience occurred in the early 1990s, when I started to work with neuroscientists on a variety of problems in cognition and motor control. Subsequent appointments on various NIH study sections and NSF review panels have broadened my understanding of this fascinating field and made me aware of its potential in helping address cognitive impairments in brain disorders. Currently, by utilizing advanced signal processing methods to model and understand multimodal neural data, including electroencephalogram (EEG), functional magnetic resonance imaging (fMRI), and simultaneous EEG-fMRI, we investigate basic and clinical neuroscience questions in attention, working memory, emotion and cognitive control.

B. Positions and Honors

Positions

1990-2004 Assistant, associate and full Professor, Center for Complex Systems and Brain Sciences and Department of Mathematical Sciences, Florida Atlantic University, Boca Raton, Florida

2004-present Professor, J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida

2008-present J. Crayton Pruitt Family Professor, J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida

Honors

1989 University of Maryland Dissertation Fellowship

1992 First Prize for Natural Sciences, Chinese Academy of Sciences, China

1993 State Award of Second Rank in Natural Sciences, State Council, China

1998 Florida Atlantic University Researcher of the Year Award

1995-1998 Member, NIMH Study Section on Cognitive Functional Neuroscience (CFN)

2002-2006 Member, NIH Study Section on Cognitive Neuroscience (COG)

2003-2005 Associate Editor, Mathematical Biosciences and Engineering

2004-2005 Editor, *Physica D*

2005, 2006, 2012 Member, NIMH Study Section for the Conte Center for Neuroscience Research

2008 Fellow, American Institute for Medical and Biological Engineering (AIMBE)

2008-2012 Member, NIH Study Section on Cognitive Neuroscience (COG)

1995-2015 ad hoc member, numerous NIH study sections

2012-2015 ad hoc member, numerous NSF panels

2013-2016 University of Florida Research Foundation Professorship

2015-present Member of Editorial Board, *Scientific Reports*

2016-present Associate Editor, *Journal of Neuroscience*

C. Contribution to Science

1. *Analyzing information flow in neural networks*: Neural interactions, being mediated by the synaptic transmission of action potentials, are directional. Our ability to assess the directionality of neural interactions and information flow in brain networks holds the key to understanding the cooperative nature of neural computation and its breakdown in disease. Research over the last few years has proven that Granger causality is a statistical technique that furnishes this capability. My lab has pioneered the application of Granger causality to neuroscience by demonstrating its effectiveness in recordings from multiple species and experimental paradigms and developing the software package used by hundreds of labs around the world.
 - a. Wen, X., Liu, Y., Yao, L., & **Ding, M.** (2013). Top-down regulation of default mode activity in spatial visual attention. *Journal of Neuroscience*, 33(15), 6444-6453. **PMCID: PMC3670184.**
 - b. Dhamala, M., Rangarajan, G. & **Ding, M.** (2008). Analyzing information flow in brain networks with nonparametric Granger causality. *NeuroImage*, 41(2), 354-362. **PMCID: PMC2685256.**
 - c. Brovelli, A., **Ding, M.**, Ledberg, A., Chen, Y., Nakamura, R., & Bressler, S.L. (2004). Beta oscillations in a large-scale sensorimotor cortical network: Directional influences revealed by Granger causality. *Proceedings of the National Academy of Sciences of the United States of America*, 101(26), 9849-9854. **PMCID: PMC470781.**
2. *Single trial analysis of event-related signals*: Neural data following the onset of a stimulus is comprised of an event-related component that is relatively time-locked to stimulus onset and ongoing brain activity. These two types of signals, generated by possibly different neural mechanisms, may reflect different aspects of cognitive information processing. Estimation of the two, however, remains a major challenge. My lab has developed methods that are capable of separating the two signals on a trial-by-trial basis and demonstrated their effectiveness in numerous experimental preparations and paradigms. These methods are currently being used to answer questions in areas ranging from network basis of decision-making to improved target detection in cognitive brain machine interface to determination of the time course of emotional conditioning.
 - a. Knuth, K.H., Shah, A.S., Truccolo, W.A., **Ding, M.**, Bressler, S.L., & Schroeder, C.E. (2006). Differentially Variable Component Analysis (dVCA): Identifying Multiple Evoked Components Using Trial-to-Trial Variability. *Journal of Neurophysiology*, 95, 3257-3276.
 - b. Xu, L., Stoica, P., Li, J., Bressler, S.L., Shao, X., & **Ding, M.** (2009). ASEO: A Method for the Simultaneous Estimation of Single-trial Event-Related Potentials and Ongoing Brain Activities. *IEEE Transactions on Biomedical Engineering*, 56, 111-121.
 - c. Liu, Y., Keil, A., and **Ding, M.** (2012). Effects of emotional conditioning on early visual processing: Temporal dynamics revealed by ERP single-trial analysis. *Human Brain Mapping*, 33, 909-919.
3. *Neuronal oscillations*: Electrophysiological recordings of neural activity are replete with oscillatory components. Characterizing these neuronal oscillations is important for understanding both normal brain function and its impairments in brain disorders. My lab has contributed significantly to this field. We are the first to provide a thorough laminar analysis of alpha oscillations in multiple visual areas in awake-behaving macaque monkeys. We are also the first to demonstrate that theta oscillations mediate the interaction between prefrontal cortex and medial temporal lobe in human memory processes. More recently, applying novel methods to spike and LFP mixed recordings, we addressed the relation between medial septum and hippocampus during theta and non-theta behavioral states.
 - a. Bollimunta, A., Chen, Y., Schroeder, C.E., & **Ding, M.** (2008). Neuronal Mechanisms of Cortical Alpha Oscillations in Awake-behaving Macaques. *Journal of Neuroscience*, 28, 9976-9988
 - b. Anderson, K.L., Rajagovindan, R., Ghacibeh, G.A., Meador, K.J., & **Ding, M.** (2010). Theta Oscillations Mediate Interaction Between Prefrontal Cortex and Medial Temporal Lobe in Human Memory. *Cerebral Cortex*, 20, 1604-1612
 - c. Kang, D., **Ding, M.**, Topchiy, I., Shifflett, L., & Kocsis, B. (2015). Theta-rhythmic drive between medial septum and hippocampus in slow-wave sleep and microarousal: a Granger causality analysis. *Journal of Neurophysiology*, 114, 2797-2803
4. *Simultaneous recording of EEG and fMRI*: EEG and fMRI are the two major methods for imaging human brain function. EEG is known for its excellent temporal resolution (millisecond) but poor spatial resolution (centimeter) whereas fMRI is known for its good spatial resolution (millimeter) but poor temporal resolution (second). Simultaneous EEG-fMRI, in which EEG is recorded together with fMRI inside the MRI scanner, is an emerging technique that promises to combine the strengths of the two methods and overcome their shortcomings. My lab is at the forefront of applying this cutting-edge technology to address important neuroscience problems.
 - a. Liu, Y., Huang, H., McGinnis, M., Keil, A., & **Ding, M.** (2012). Neural substrate of the late positive potential in emotional processing. *Journal of Neuroscience*, 32, 14563-14572

- b. Mo, J., Liu, Y., Huang, H., & **Ding, M.** (2013). Coupling Between Visual Alpha Oscillations and Default Mode Activity. *NeuroImage*, 68,112-118
 - c. Liu, Y., Bengson, J., Huang, H., Mangun, G.R., & **Ding, M.** (2016). Top-down Modulation of Neural Activity in Anticipatory Visual Attention: Control Mechanisms Revealed by Simultaneous EEG-fMRI. *Cerebral Cortex*, 26, 517-529
5. *Cognitive fatigue and fatigability*: Fatigue is the primary reason community-dwelling older adults restrict their activities and is associated with disability, diminished quality of life and increased mortality. Our understanding of the causes of fatigue in older adults is quite limited and we have no proven treatments. The construct of fatigue can be divided into perceived fatigue and performance fatigability with the former referring to subjective perceptions of exhaustion and the latter objective decrements in performance associated with prolonged exertion. My lab has made original contributions to the measurement of cognitive fatigability, its relation to perceived fatigue and the underlying neuronal mechanisms.
- a. Wang, C., **Ding, M.**, & Kluger, B.M. (2014). Change in Intraindividual Variability over Time as a Key Metric for Defining Performance-Based Cognitive Fatigability. *Brain and Cognition*, 85, 251-258
 - b. Wang, C., Trongnetrpunya, A., Samuel, I.B.H., **Ding, M.**, & Kluger, B.M. (2016). Compensatory Neural Activity in Response to Cognitive Fatigue. *Journal of Neuroscience*, 36, 3919-3924

D. Research Support

R01 MH097320 NIMH <i>Acquisition and Extinction of Affective Bias in Perception: A Single Trial Approach</i> Objective: To characterize and quantify – on a trial by trial basis – the temporal evolution of neural changes in the human visual system that accompanies the acquisition and extinction of conditioned fear. Overlap: None	Ding/Keil (PI)	04/02/12-02/28/17
R01 MH100820 NIMH <i>Spatiotemporal Network Dynamics in a Rat Model of Schizophrenia</i> Objective: To study the spectral structure, anatomy, physiology and pharmacology in normal rats and pharmacological rat models of schizophrenia. Overlap: None	Kocsis/Ding (PI)	04/01/14-03/31/19
R21 AG044862 NIA <i>Measuring Cognitive Fatigability in Older Adults</i> Objective: To examine the relationship between an objective measure of cognitive performance fatigability and activity levels in older adults. Overlap: None.	Ding/Kluger (PI)	09/15/14-04/30/17
BCS-1439188 NSF <i>Mechanisms of anticipatory attention</i> Objective: To study the neural basis of anticipatory attention in both humans and monkeys using electrophysiology and advanced computational methods. Overlap: None	Ding (PI)	09/01/14–08/31/17
R01 MH094386 NIMH <i>Anxiety, comorbidity, negative affect, and fear circuit activation</i> Objective: To identify the pathophysiology underlying anxiety, fear and emotional dysregulation using multimodal brain imaging. Overlap: None	Lang (PI)	04/04/12-03/31/17
R01 NR014181 NINR <i>Neuroimaging biomarkers for post-operative cognitive decline in older adults</i> Objective: To develop imaging biomarkers to predict cognitive outcomes in older adults undergoing orthopedic surgery. Overlap: None	Price (PI)	09/26/12-05/31/17

R01 NS076665 NINDS <i>Characterizing and predicting drug effects on cognition</i> Objective: To study the adverse effects of antiepileptic drug topiramate on cognition using behavior, genetics and electrophysiological methods. Overlap: None	Marino (PI)	09/27/12-06/30/17
R01 NS082386 NINDS <i>White Matter Connectivity and PD Cognitive Phenotypes</i> Objective: To examine white matter connectivity in PD patients and develop biomarkers for different cognitive phenotypes. Overlap: None	Price (PI)	09/25/13-08/31/18
BCS-1344285 NSF <i>INSPIRE Track 1: Crowd-sourcing neuroscience: Neural oscillations and human social dynamics</i> Objective: To study the neural basis of interpersonal communication using electrophysiology and advanced computational approaches. Overlap: None	Poeppel (PI)	09/15/13-08/31/18
N/A Facial Pain Research Foundation <i>Mapping Towards a Cure – Identification of Neurophysiologic Signatures of Trigeminal Neuralgia Pain</i> Objective: To study the cause of trigeminal neuralgia pain using a translational approach by combining both humans and animals models. Overlap: None	Neubert (PI)	02/01/15-01/31/18
R56HL127175 NHLBI <i>Brain and cognition effects of cardio resynchronization therapy in heart failure</i> Objective: The goal of this study is to evaluate cognitive and brain consequences of cardiac resynchronization therapy in heart failure patients using functional neuroimaging, magnetic resonance spectroscopy, & arterial spin labeling. Role: Co-I Overlap: None	Williamson (PI)	09/08/15-08/31/16
1R01AG054077-01 NIH <i>Augmenting Cognitive Training in Older Adults: The ACT Grant</i> Objective: The goal of this randomized clinical trial is to examine the effect of augmenting cognitive training with transcranial direct current stimulation to maximize cognitive and functional outcomes in older adults experiencing age-related cognitive decline.	Woods (PI)	9/1/16 – 4/30/21
2P01AA019072 NIH <i>Alcohol and HIV: Biobehavioral Interactions and Interventions</i> Objective: The goal of this project is to contribute to efforts to improve the health of those living with HIV and to reduce the spread of HIV by increasing our understanding of how excessive alcohol consumption and interventions to reduce excessive drinking can impact HIV-related health outcomes.	Monti (PI)	09/30/10 – 05/31/20
Completed Research Support RX4235406 NSF <i>Brain activity maps of novelty detection</i> Objective: To study the neural basis of novelty detection using multi-modal neuroimaging and advanced computational approaches.	Wu (PI)	09/07/13-02/06/15

Invited Talks:

"Assessing Causal Relations in Neural Circuits with Granger Causality," Department of Bioengineering, University of Pittsburgh, October, 2016

"Applications of Granger Causality to Neuroscience," Workshop on A Hands-on Approach to Neural Connectivity Inference Methods, 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Orlando, August, 2016

"Is Granger causality a viable technique for analyzing fMRI data?" Minisymposium on Advances in Brain Connectivity Analysis: Perspectives and Pitfalls, 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Orlando, August, 2016

"Imaging Human Brain Function with Simultaneous EEG-fMRI," International Symposium on Nonlinear Sciences and Applications, Shanghai, July, 2016

"Analyzing Brain Networks with Granger Causality," Kavli Summer Institute in Cognitive Neuroscience, Santa Barbara, June, 2016

"Imaging Human Brain Function with Simultaneous EEG-fMRI," Neuroimaging Session I, The 13th Annual Conference of Society for Brain Mapping & Therapeutics, Miami, Florida, April, 2016

"Novel Techniques for fMRI Data analysis," Life Sciences Symposium: Seeing the Heart & Mind: Modern Imaging Techniques, Florida State University, Tallahassee, February, 2016

Publications:

Liu, Y., Hong, X., Bengson, J., **Ding, M.**, Mangun, G.R. (2016). Deciding Where to Attend: Large-scale Network Mechanisms Revealed by Graph-theoretic Analysis Visual Attention: Control Mechanisms Revealed by Simultaneous EEG-fMRI, *NeuroImage*, under review

Wang, C., Burtis, D.B., **Ding, M.**, Mo, J., S.W., Williamson, J.B., Heilman, K.M. (2016). The Effects of Left and Right Monocular Viewing on Hemispheric Activation, *Brain and Cognition*, under review

Kang, D., Liu, Y., Miskovic, V., Keil, A., **Ding, M.** (2016). Affective Scene Processing: Large-scale Functional Interactions Revealed by Beta-series Connectivity Analysis, *Psychophysiology* 53, 1627–1638

Yin, S., Liu, Y., **Ding, M.** (2016). Amplitude of sensorimotor mu rhythm is correlated with BOLD from multiple brain regions: A simultaneous EEG-fMRI study. *Frontiers in Human Neuroscience* 10:364 doi: 10.3389/fnhum.2016.00364

DeAndrade, M.P., Trongnetrpunya, A., Yokoi, F., Cheetham, C.C., Peng, N., Wyss, J.M., **Ding, M.**, and Li, Y (2016). Electromyographic evidence in support of a knock-in mouse model of DYT1 dystonia, *Movement Disorders* DOI: 10.1002/mds.26677

Wang, C., Trongnetrpunya, A., Samuel, I.B.H., **Ding, M.**, Kluger, B.M. (2016). Compensatory Neural Activity in Response to Cognitive Fatigue. *Journal of Neuroscience* 36 (14), 3919-3924

Zhang, Y., Li, W., Wen, X., Cai, W., Li, G., Tian, J., Zhang, Y.E., Liu, J., Yuan, K., Zhao, J., Wang, W., Zhou, Z., **Ding, M.**, Gold, M.S., Liu, Y., Wang, G.J. (2016), "Granger causality reveals a dominant role of memory circuit in chronic opioid dependence," *Addiction Biology* doi:10.1111/adb.12390, 1-13

Jiang, Y., Huang, H., Abner, E., Broster, L.S., Jicha, G.A., Schmitt, F.A., Kryscio, R., Andersen, A., Powell, D., Van Eldik, L., Gold, B.T., Nelson, P.T., Smith, C, **Ding, M.** (2016), "Alzheimer's Biomarkers are Correlated with Brain Connectivity in Older Adults Differentially during Resting and Task States," *Frontiers in Aging Neuroscience* 8:15 doi: 10.3389/fnagi.2016.00015

Huang, H., **Ding, M.** (2016), "Linking functional connectivity and structural connectivity quantitatively: A comparison of methods," *Brain Connectivity* 6(2): 99-108

Trongnetrpunya, A., Nandi, B., Kang, D., Kocsis, B., Schroeder, C.E., **Ding, M.** (2016), "Assessing Granger causality in electrophysiological data: the importance of bipolar derivations," *Frontiers in Systems Neuroscience* 9:189 doi: 10.3389/fnsys.2015.00189

Wang, C., Rajagovindan, R., Han, S., **Ding, M.** (2016), "Top-Down Control of Visual Alpha Oscillations: Sources of Control Signals and Their Mechanisms of Action," *Frontiers in Human Neuroscience* 10:15 doi: 10.3389/fnhum.2016.00015

Liu, Y., Bengson, J., Huang, H., Mangun, G.R., **Ding, M.** (2016), "Top-down Modulation of Neural Activity in Anticipatory Visual Attention: Control Mechanisms Revealed by Simultaneous EEG-fMRI," *Cerebral Cortex* 26:517-529

Vonetta Dotson, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Vonetta M. Dotson		POSITION TITLE Associate Professor	
eRA COMMONS USER NAME (credential, e.g., agency login) dotsonv			
EDUCATION/TRAINING <i>(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)</i>			
INSTITUTION AND LOCATION	DEGREE <i>(if applicable)</i>	MM/YY	FIELD OF STUDY
St. Mary's University	B.A.	5/99	Psychology
University of Florida	M.S., Ph.D.	5/02, 8/06	Psychology (Clinical)
James A. Haley Veterans Hospital	N/A	7/06-8/06	Predoctoral Internship
NIA Intramural Research Program	Postdoctoral	8/06-7/09	Cognitive Neuroscience of Aging and Depression

A. Personal Statement

Vonetta Dotson is an Associate Professor in the Department of Clinical and Health Psychology (CHP) at the University of Florida, with a joint appointment in the Department of Neuroscience at the University of Florida. She received her Ph.D. from CHP in 2006 with a specialization in neuropsychology and a certificate in gerontology. She completed her postdoctoral training in the Laboratory of Personality and Cognition in the National Institute on Aging Intramural Research Program under the mentorship of Drs. Susan Resnick and Alan Zonderman. Her research focuses on studying the interaction of psychological disorders such as depression with cognitive and brain aging using both neuroimaging and behavioral techniques. Her more recent work focuses on the impact of aerobic exercise on depression-related cognitive and brain changes in older adults.

B. Positions and Honors

Positions

2006-2009	Postdoctoral Fellow, Laboratory of Personality and Cognition, National Institute on Aging Intramural Research Program, Baltimore, MD
8/2009-7/2016	Assistant Professor, Department of Clinical and Health Psychology, University of Florida, Gainesville, FL Affiliate Faculty, Department of Neuroscience, University of Florida, Gainesville, FL
7/2016-present	Associate Professor, Department of Clinical and Health Psychology, University of Florida, Gainesville, FL Affiliate Faculty, Department of Neuroscience, University of Florida, Gainesville, FL

Honors

1997	ACCD Foundation Scholars Award
1997-1999	Dean's List, St. Mary's University
1998-1999	The National Dean's List
2000-2004	University of Florida Graduate Minority Fellowship
2003-2005	University of Florida Institute on Aging Trainee
2004-2005	National Institute on Aging funded Predoctoral Fellow
2004	Recipient of National Institute on Aging Technical Assistance Workshop travel fellowship
2005	Accepted into the Society for Neuroscience's Neuroscience Scholars Program
2006	Accepted to attend the American Psychological Association's Advanced Training Institute on Functional Magnetic Resonance Imaging
2006	Recipient of the Institute for Learning in Retirement Graduate Aging Research Award
2007	Accepted to attend the American Psychological Association's Advanced Training Institute on Structural Equation Modeling for Longitudinal Research
2007	Recipient of National Institute on Aging Summer Institute on Aging Research travel fellowship
2010	Claude D. Pepper Affiliated Scholar
2012-2015	Claude D. Pepper Scholar

Licensure: Licensed psychologist, State of Florida, License No. PY 8055

Professional Memberships: Society for Neuroscience, International Neuropsychological Society, American Psychological Association

C. Peer-reviewed publications or manuscripts in press (in chronological order)

1. Perlstein, W.M., Larson, M.J., **Dotson, V.M.**, & Kelly, G.K. (2006). Temporal dissociation of components of cognitive control dysfunction in severe TBI: ERPs and the cued-Stroop task. *Neuropsychologia*, 44(2), 260-274. **PMID: 15979655**
2. Larson, M.J., Perlstein, W.M., Stigge-Kaufmann, D., Kelly, G.K., & **Dotson, V.M.** (2006). Affective context induced modulation of the error-related negativity. *Neuroreport*, 17(3), 329-33. **PMID: 16462607**
3. **Dotson, V.M.**, Singletary, F.S., Fuller, R., Koehler, S., Bacon Moore, A., Rothi, L.J.G., & Crosson, B. (2008). Treatment of word-finding deficits in fluent aphasia through the manipulation of spatial attention: Preliminary findings. *Aphasiology*, 22(1), 103–113.
4. **Dotson, V.M.**, Schinka, J.A., Brown, L., Borenstein, A.R., & Mortimer, J.A. (2008). Characteristics of the Florida Cognitive Activities Scale in older African Americans. *Assessment*, 15(1), 72-77. **PMID: 18258733**
5. **Dotson, V.M.**, Resnick, S.M., & Zonderman, A.B. (2008). Differential Association of Baseline, Concurrent, and Chronic Depressive Symptoms with Cognitive Decline in Older Adults. *American Journal of Geriatric Psychiatry*, 16, 318-330. **PMID: 18378557**
6. **Dotson, V.M.**, Kitner-Triolo, M., Evans, M.K., & Zonderman, A.B. (2008). Literacy-based normative data for low socioeconomic status African Americans. *The Clinical Neuropsychologist*, 22, 989–1017. **PMID: 18609322**
7. Pedraza, O., **Dotson, V.M.**, Willis, F.B., Graff-Radford, N.R., and Lucas, J.A. (2009). Internal Consistency and Test-Retest Reliability of the Geriatric Depression Scale-Short Form in African American Older Adults. *Journal of Psychopathology and Behavioral Assessment*, 31(4), 412-416. **PMID: 20161488**
8. **Dotson, V.M.**, Kitner-Triolo, M., Evans, M.K., & Zonderman, A.B. (2009). Effects of Race and Socioeconomic Status on the Relative Influence of Education and Literacy on Cognitive Functioning. *JINS*, 15, 580-589. **PMID: 19573276**
9. **Dotson, V.M.**, Beason-Held, L., Kraut, M.A., & Resnick, S.M. (2009). Longitudinal Study of Chronic Depressive Symptoms and Regional Cerebral Blood Flow in Older Men and Women. *International Journal of Geriatric Psychiatry*, 24(8), 809-19. **PMID: 19484709**
10. **Dotson, V.M.**, Davatzikos, C., Kraut, M.A., & Resnick, S.M. (2009). Depressive Symptoms and Brain Volumes in Older Adults: A Longitudinal MRI Study. *Journal of Psychiatry and Neuroscience*, 34(5), 367-375. **PMID: 19721847**
11. **Dotson, V.M.**, Zonderman, A.B., Davatzikos, C., Kraut, M.A., & Resnick, S.M. (2009). Frontal Atrophy and Immediate Memory Deficits in Older Adults with a History of Elevated Depressive Symptoms. *Brain Imaging and Behavior*, 3, 358–369. **PMID: 20161651**
12. **Dotson, V.M.**, Baydoun, M.A., & Zonderman, A.B. (2010). Recurrent depressive symptoms and the incidence of dementia and MCI. *Neurology*, 75, 27-34. **PMID: 20603482**
13. Sutin, A. R., Beason-Held, L. L., **Dotson, V. M.**, Resnick, S. M., & Costa, P. T. (2010). The neural correlates of neuroticism differ by sex and prospectively mediate depressive symptoms among older women. *Journal of Affective Disorders*, 127, 241-7. **PMID: 20599276**
14. Goveas, J.S., Espeland, M.A., Hogan, P., **Dotson, V.**, Tarima, S., Coker, L.H., Ockene, J., Brunner, R., Woods, N.F., Wassertheil-Smoller, S., Kotchen, J.M., Resnick, S. (2011). Depressive symptoms, brain volumes and subclinical cerebrovascular disease in postmenopausal women: the Women’s Health Initiative MRI Study. *Journal of Affective Disorders*, 132, 275–284. **PMID: 21349587**
15. **Dotson, V.M.**, Zonderman, A.B., Kraut, M.A., & Resnick, S.M. (2013). Temporal Relationships between Depressive Symptoms and White Matter Hyperintensities in Older Men and Women. *International Journal of Geriatric Psychiatry*, 28, 66–74. doi: 10.1002/gps.3791.
16. **Dotson, V.M.**, Sozda, C.N., Marsiske, M., & Perlstein, W.M. (2013). Within-session Practice Eliminates Age Differences in Cognitive Control. *Aging, Neuropsychology and Cognition: A Journal on Normal and Dysfunctional Development*, 20 (5), 522-531. doi:10.1080/13825585.2012.736469.

17. Kirton, J. W., Resnick, S. M., Davatzikos, C. Kraut, M. A. & **Dotson, V. M.** (2013). Depressive Symptoms, Symptom Dimensions and White Matter Lesion Volume in Older Adults: A Longitudinal Study. *American Journal of Geriatric Psychiatry*. doi: 10.1016/j.jagp.2013.10.005.
18. **Dotson, V.M.**, Szymkowicz, S.M., Kirton, J.W., McLaren, M.E., Green, M., & Rohani, J.Y. (2014). Unique and interactive effect of anxiety and depressive symptoms on cognitive and brain function in young and older adults. *Journal of Depression and Anxiety*. doi: 10.4172/2167-1044.S1-003
19. Bryant, V.E., Whitehead, N.E., Burrell, L.E., **Dotson, V.M.**, Cook, R.L., Malloy, P., Devlin, K., & Cohen, R.A. (2014). Depression and apathy among people living with HIV: Implications for treatment of HIV associated neurocognitive disorders. *AIDS and Behavior*. doi: 10.1007/s10461-014-0970-1
20. McLaren, M.E., Szymkowicz, S.M., Kirton, J.W., & **Dotson, V.M.** (2015). Impact of education on memory deficits in subclinical depression. *Archives of Clinical Neuropsychology*, 30, 387–393. doi: 10.1093/arclin/acv038
21. O’Shea, D.M., **Dotson, V.M.**, Fieo, R.A., Angeliki, T., Zahodne, L. & Stern, Y. (2015). Older adults with poor self-rated memory have less depressive symptoms and better delayed memory performance when perceived self-efficacy is high. *International Journal of Geriatric Psychiatry*. doi: 10.1002/gps.4392
22. Anton, S.D., Woods, A.J., Ashizawa, T., Barb, D., Buford, T.W., Carter, C.S., Clark, D.J., Cohen, R.A., Corbett, D.B., Cruz-Almeida, Y., **Dotson, V.M.**, et al. (2015). Successful aging: Advancing the science of physical independence in older adults. *Ageing Research Reviews*, 24, 304-327. doi: 10.1016/j.arr.2015.09.005. **PMCID: PMC4661112**
23. Kirton, J.W. & **Dotson, V.M.** (2016). The interactive effects of age, education, and BMI on cognitive functions in community dwelling adults. *Aging, Neuropsychology and Cognition*, 23(2), 253-262. doi: 10.1080/13825585.2015.1082531. **PMCID: PMC4683610**
24. **Dotson, V.M.**, Hsu, F.C., Langaee, T.Y., McDonough, C.W., King, A.C., Cohen, R.A., Newman, A.B., Kritchevsky, S.B., Myers, V., Manini, T.M., Pahor, M., & LIFE Study Group (2016). Genetic moderators of the impact of physical activity on depressive symptoms. *Journal of Frailty and Aging*, 5(1), 6-14. doi: 10.14283/jfa.2016.76
25. **Dotson, V.M.**, Szymkowicz, S.M., Sozda, C.N., Kirton, J.W., Green, M., O’Shea, A., McLaren, M.E., Anton, S.D., Manini, T.M. & Woods, A.J. (2016). Age differences in prefrontal surface area and thickness in middle aged to older adults. *Frontiers in Aging Neuroscience*, 7, 250. doi: 10.3389/fnagi.2015.00250
26. McLaren, M.E., Szymkowicz, S.M., O’Shea, A., Woods, A.J., Anton, S.D., & **Dotson, V.M.** (2016). Symptom dimensions of subthreshold depression and cingulate volumes in older adults. *Translational Psychiatry*
27. Szymkowicz, S.M., McLaren, M.E., Kirton, J.W., O’Shea, A., Woods, A.J., Manini, T.M., Anton, S.D., & **Dotson, V.M.** (2016). Depressive symptom severity is associated with increased cortical thickness in older adults. *International Journal of Geriatric Psychiatry*, 31, 325–333. doi: 10.1002/gps.4324. **PMCID: PMC4724336**
28. Stigge-Kaufmann, D., Sozda, C.N., **Dotson, V.M.**, & Perlstein, W.M. (2016). An event-related potential investigation of the effects of age on alerting, orienting, and executive function. *Frontiers in Aging Neuroscience*, 8, 99. doi: 10.3389/fnagi.2016.00099
29. De Wit, L., Kirton, J.W., O’Shea, D. M., Szymkowicz, S. M., McLaren, M. E., & **Dotson, V. M.** (2016). Effects of body mass index and years of education on verbal and nonverbal memory. *Aging, Neuropsychology and Cognition*. doi: 10.1080/13825585.2016.1194366
30. O’Shea, D. M., De Wit, L., Szymkowicz, S. M., McLaren, M. E., & **Dotson, V. M.** (2016). Anxiety modifies the association between fatigue and verbal fluency in cognitively normal adults. *Archives of Clinical Neuropsychology*. doi: 10.1093/arclin/acw045
31. Szymkowicz, S.M., McLaren, M.E., O’Shea, A., Woods, A.J., Anton, S.D., & **Dotson, V.M.** (2016). Depressive symptoms moderate age effects on hippocampal subfields. *Geriatrics & Gerontology International*. doi: 10.1111/ggi.12901
32. O’Shea, D.M., **Dotson, V.M.**, & Fieo R.A. (2016). Aging perceptions and self-efficacy mediate the association between personality traits and depressive symptoms in non-demented older adults. *International Journal of Geriatric Psychiatry*. doi: 10.1002/gps.4584
33. **Dotson, V.M.** (2016). Variability in Depression: What Have We Been Missing? *American Journal of Geriatric Psychiatry*. doi: 10.1016/j.jagp.2016.10.005
34. McLaren, M.E., Szymkowicz, S.M., O’Shea, A., Woods, A.J., Anton, S.D., & **Dotson, V.M.** (2016). Vertex-wise examination of symptom dimensions of subthreshold depression and brain volumes. doi: 10.1016/j.psychres.2016.12.008

D. Research Support

Ongoing Research Support

R03 MH109336-01A1 **Dotson (PI)** 8/14/15-4/30/17

National Institute of Mental Health

Dissociating Components of Anhedonia: Pilot Behavioral and fMRI Data for the Effort Expenditure for Rewards Task

The study purpose is to dissociate functional brain activity underlying anticipatory and consummatory components of anhedonia in young and older adults.

Role: PI

Completed Research Support

5T32AG020499-07 Marsiske (PI) 05/01/03-04/30/08

National Institute on Aging

Physical, Cognitive and Mental Health in Social Context

The major goals of this project were to train pre-doctoral researchers in the behavioral theories, methodologies and analyses needed to address questions of health, independence and functioning in older adults.

Role: Research Fellow/Trainee (2004-2005)

R03 AG024539-01 **Dotson (PI)** 09/30/04-06/30/06

National Institute on Aging

Double Jeopardy: Cognitive Decline in Depression and Aging

The major goals of this project were to determine whether the combined effect of aging and depression were associated with additive or synergistic effects on executive functioning and brain activity measured by event-related potentials.

Role: PI

No number assigned **Dotson (PI)** 03/01/11-8/31/13

McKnight Brain Research Foundation

Effect of Exercise on Memory in Geriatric Depression: An fMRI Pilot Study

The major goals of this project were to determine whether aerobic exercise leads to improved memory and changes in memory-related brain activity in older depressed adults.

Role: PI

U01 AG022376 Pahor (PI) 2/01/12-11/30/13

National Institute on Aging

Diversity Supplement to the Lifestyle Interventions and Independence for Elders (LIFE) Study (PI)

The major goals of this project were to examine the impact of physical activity on depressive symptoms in older adults and to determine if genetic variation moderated the effect.

Role: PI for diversity supplement

E. Publications and Presentations in 2016

Publications:

1. Kirton, J.W. & **Dotson, V.M.** (2016). The interactive effects of age, education, and BMI on cognitive functions in community dwelling adults. *Aging, Neuropsychology and Cognition*, 23(2), 253-262. doi: 10.1080/13825585.2015.1082531.
PMCID: PMC4683610
2. **Dotson, V.M.**, Hsu, F.C., Langaee, T.Y., McDonough, C.W., King, A.C., Cohen, R.A., Newman, A.B., Kritchevsky, S.B., Myers, V., Manini, T.M., Pahor, M., & LIFE Study Group (2016). Genetic moderators of the impact of physical activity on depressive symptoms. *Journal of Frailty and Aging*, 5(1), 6-14. doi: 10.14283/jfa.2016.76
3. **Dotson, V.M.**, Szymkowicz, S.M., Sozda, C.N., Kirton, J.W., Green, M., O'Shea, A., McLaren, M.E., Anton, S.D., Manini, T.M. & Woods, A.J. (2016). Age differences in prefrontal surface area and thickness in middle aged to older adults. *Frontiers in Aging Neuroscience*, 7, 250. doi: 10.3389/fnagi.2015.00250
4. McLaren, M.E., Szymkowicz, S.M., O'Shea, A., Woods, A.J., Anton, S.D., & **Dotson, V.M.** (2016). Symptom dimensions of subthreshold depression and cingulate volumes in older adults. *Translational Psychiatry*
5. Szymkowicz, S.M., McLaren, M.E., Kirton, J.W., O'Shea, A., Woods, A.J., Manini, T.M., Anton, S.D., & **Dotson, V.M.** (2016). Depressive symptom severity is associated with increased cortical thickness in older adults. *International Journal of Geriatric Psychiatry*, 31, 325-333. doi: 10.1002/gps.4324. **PMCID: PMC4724336**

6. Stigge-Kaufmann, D., Sozda, C.N., **Dotson, V.M.**, & Perlstein, W.M. (2016). An event-related potential investigation of the effects of age on alerting, orienting, and executive function. *Frontiers in Aging Neuroscience*, 8, 99. doi: 10.3389/fnagi.2016.00099
7. De Wit, L., Kirton, J.W., O'Shea, D. M., Szymkowicz, S. M., McLaren, M. E., & **Dotson, V. M** (2016). Effects of body mass index and years of education on verbal and nonverbal memory. *Aging, Neuropsychology and Cognition*. doi: 10.1080/13825585.2016.1194366
8. O'Shea, D. M., De Wit, L., Szymkowicz, S. M., McLaren, M. E., & **Dotson, V. M.** (2016). Anxiety modifies the association between fatigue and verbal fluency in cognitively normal adults. *Archives of Clinical Neuropsychology*. doi: 10.1093/arclin/acw045
9. Szymkowicz, S.M., McLaren, M.E., O'Shea, A., Woods, A.J., Anton, S.D., & **Dotson, V.M.** (2016). Depressive symptoms moderate age effects on hippocampal subfields. *Geriatrics & Gerontology International*. doi: 10.1111/ggi.12901
10. O'Shea, D.M., **Dotson, V.M.**, & Fieo R.A. (2016). Aging perceptions and self-efficacy mediate the association between personality traits and depressive symptoms in non-demented older adults. *International Journal of Geriatric Psychiatry*. doi: 10.1002/gps.4584
11. **Dotson, V.M.** (2016). Variability in Depression: What Have We Been Missing? *American Journal of Geriatric Psychiatry*. doi: 10.1016/j.jagp.2016.10.005
12. McLaren, M.E., Szymkowicz, S.M., O'Shea, A., Woods, A.J., Anton, S.D., & **Dotson, V.M.** (2016). Vertex-wise examination of symptom dimensions of subthreshold depression and brain volumes. doi: 10.1016/j.psychresns.2016.12.008

Presentations:

1. **Dotson, V.M.** (2016). Symptom dimensions in late-life subthreshold depression: Evidence from cognitive, neuroscience, and exercise intervention studies. Paper presented at the 44th annual International Neuropsychological Society (INS) meeting, Boston, MA
2. O'Shea, D.M., De Wit, L., Szymkowicz, S. M., McLaren, M. E., Talty F & **Dotson, V. M.** (2016). Anxiety but not depressive symptoms modifies the association between fatigue and cognition in healthy adults. Posted to be presented at 14th Annual Meeting of the American Academy of Clinical Neuropsychology, Chicago, Illinois
3. Szymkowicz, S. M., McLaren, M. E., De Wit, L., O'Shea, A., Woods, A. J., Anton, S. D., & **Dotson, V. M.** (2016). Structural abnormalities in cortical thickness, surface area, and volume of the precuneus in older adults with depressive symptoms. Poster presented at the 44th annual International Neuropsychological Society (INS) meeting, Boston, MA
4. McLaren, M.E., Szymkowicz, S.M., O'Shea, A., Woods, A.J., Anton, S.D. & **Dotson, V.M.** (2016) Symptom Dimensions of Depression and Age Impact Subfield Hippocampal Volume. Poster presented at the 44th annual International Neuropsychological Society (INS) meeting, Boston, MA
5. Kirton, J.W., Seider, T., O'Shea, A., Lamb, D., Woods, A.J., Cohen, R.A., & **Dotson, V.M.** (2016). Regional white matter lesion volume and depressive symptom dimensions. Poster presented at the 44th annual International Neuropsychological Society (INS) meeting, Boston, MA
6. McLaren, M.E., Szymkowicz, S.M., O'Shea, A., Woods, A.J., Anton, S.D., & **Dotson, V.M.** (2016). Symptom Dimensions of Depression and Age Impact Subfield Hippocampal Volume. Poster presented at the 7th annual Spotlight on Aging Research exhibition, University of Florida, Gainesville, FL
7. Szymkowicz, S. M., McLaren, M. E., De Wit, L., O'Shea, A., Woods, A. J., Anton, S. D., & **Dotson, V. M.** (2016). Structural abnormalities in cortical thickness, surface area, and volume of the precuneus in older adults with depressive symptoms. Poster presented at the 7th annual Spotlight on Aging Research exhibition, University of Florida, Gainesville, FL

Kenneth M. Heilman, PhD

BIOGRAPHICAL SKETCH

NAME: Heilman, Kenneth M.

eRA COMMONS USER NAME (credential, e.g., agency login): kmheilman

POSITION TITLE: Distinguished Professor of Neurology, University of Florida; Neurologist, NF/SG VAMC

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Virginia, College of Medicine, Charlottesville VA	MD	06/1963	Medicine
Cornell Medical Div. Bellevue Hospital, NY	Intern and Assistant Resident (PGY 1,2)	06/1965	Internal Medicine
Harvard Neurological Unit, Boston City Hospital, Boston MA	Resident, Chief Resident and Fellow (PGY 3,4,5)	06/1970	Neurology

A. Personal Statement

I am a Behavioral Neurologist who provides training for clinicians and researchers, provides clinical care for patients with neurological disorder and performs research. In regard to research, since joining the faculty at the University of Florida in 1970, and the Malcom Randall VAMC in 1977, I have established and maintained a productive research program that have been funded by the National Institutes of Health and the Veteran Affairs Research Office (Merit Reviewed proposals). Research in my laboratory includes studies on the neurological basis and disorders of movement programming (apraxia), attention (unilateral neglect), emotion (disorders of emotional communication), language (aphasia, agraphia and alexia), frontal-action-intentional systems (executive disorders), episodic memory (amnesia), and creativity. I have been an author-co-author of over 600 published journal articles, editorials and letters, and in past year the author of about 19 journal publications. I have also been author of more than 100 chapters as well as editor-author of 16 books. In regard to programming disorders and Parkinson's disease (PD), according to Pubmed I have about 100 papers written about disorders of movement programming including apraxia and action-intentional disorders, and 45 papers about PD. The following are three example articles of some of the papers that I have written about motor programming disorders associated with PD:

1. Cohen ML, Schwab NA, Price CC, **Heilman KM**. Impaired Switching from Self-Prepared Actions in Mild Parkinson Disease. *J Parkinsons Dis*. 2015;5(4):961-70
2. Quencer K, Okun MS, Crucian G, Fernandez HH, Skidmore F, **Heilman KM**. Limb-kinetic apraxia in Parkinson disease. *Neurology*. 2007 Jan 9;68(2):150-1
3. Falchook AD, Decio D, Williamson JB, Okun MS, Malaty IA, Rodriguez RL, **Heilman KM**. Alternate but do not swim: a test for executive motor dysfunction in Parkinson disease. *J Int Neuropsychol Soc*. 2011 Jul;17(4):702-8

B. Positions and Honors

Positions and Employment

1965-1967	Captain, Air Force, and Chief of Medicine, NATO Hospital, Izmir, Turkey
1970-1973	Assistant Professor of Medicine, Division of Neurology, University of Florida College of Medicine
1973-1975	Associate Professor of Neurology, University of Florida College of Medicine
1973-1977	Associate Professor of Clinical Psychology, University of Florida College of Medicine
1975-1998	Professor, Department of Neurology, University of Florida, Gainesville, Florida
1977-present	Professor, Department of Clinical Psychology, University of Florida Gainesville, Florida
1977-1996	Staff Physician, Malcom Randall VA Medical Center, Gainesville, Florida
1984-present	Director, Center for Neuropsychological Studies, University of Florida College of Medicine
1988-present	Director, Cognitive and Memory Disorder Clinic, University of Florida
1990-2008	James E. Rooks Jr., Professor of Neurology, University of Florida
2009-present	James E. Rooks Jr., Professor of Neurology
1996-2008	Chief of Neurology, Malcom Randall Veterans Affairs Medical Center
1998-present	Distinguished Professor, University of Florida College Medicine, Gainesville, Florida
2009-present	Staff Physician and Member of GRECC, Malcom Randall VA Medical Center

Board Certification

1973-present	American Board of Psychiatry and Neurology
1994-2004	American Society of Neurorehabilitation
2006-present	United Council for Neurologic Subspecialties-Behavioral Neurology

Advisory

1976	Presentation to the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research – Psychosurgery
1976-1977	University of Florida Senate
1981-1984	National Institutes of Health: Study Sections: Neurobiology Review Group (Study Section)
1984-1985	University of Florida Senate
1986-1992	American Board of Psychiatry and Neurology, Part I Neurology Committee (Minor Subcommittee)

Memberships and Honors

1970-present	Alachua County Medical Society
1970-present	American Academy of Neurology (Fellow from 1975-present)
1974-1977	International Neuropsychology Society (Member, Executive Committee)
1976-2008	American Neurological Association Active Member
1982	Alpha Omega Alpha
1982	Sigma Xi
1982-1983	Society for Behavioral and Cognitive Neurology; President
1982-1983	International Neuropsychology Society (President)
1984	International Society for Research of Emotion; Board of Directors, Phi Kappa Phi
1987-1990	American Academy of Aphasia (Governing Board)
1997-1999	University of Florida Research Foundation Professorship (Award)
1989	National Aphasia Association; Advisory Board
1993	Faculty Research Award in Clinical Science, College of Medicine, University of Florida
1994-2004	American Society of Neurorehabilitation
1996	Society for Behavioral and Cognitive Neurology; Outstanding Achievement Award Aphasia Research Group of the World Federation of Neurology
2003	The Dana Foundation Alliance
2003	The American Speech and Hearing Association, Distinguished Service Award for Scientific and Educational Contributions
2005-2007	University of Florida Research Foundation Professorship (Award)
2008-present	American Neurological Association Honorary Member
2008	University of Florida, College of Medicine, Lifetime Achievement Award
2009	International Neuropsychology Society Lifetime Achievement Award
2009	American VA Speech and Language Pathologists' President's Award
2009	American Academy of Neurology, Wartenberg Award and Keynote Lecture

C. Some of the Contributions to Science

1. **Programing Disorders of Purposeful Skilled Movements:** In order to perform activities of daily living and instrumental activities, people have to be able to use their upper limbs to perform skilled purposeful movements. A loss of these skills is called apraxia. There are several forms of apraxia, and we have published more than 70 papers that have attempted to understand the pathophysiology of these disorders. Four of the more highly quoted paper include:
 - a. **Heilman KM, Rothi LJ, Valenstein E.** "Two forms of ideomotor apraxia." *Neurology* 32(4) (1982):342-6
 - b. **Watson RT, Heilman KM.** "Callosal apraxia." *Brain*. Jun;106 (Pt 2) (1983):391-403
 - c. **Hanna-Pladdy B, Mendoza JE, Apostolos GT, Heilman KM.** "Lateralised motor control: hemispheric damage and the loss of dexterity." *J Neurol Neurosurg Psychiatry*. 73(5) (2002):574-7
 - d. **Heilman KM, Maher LM, Greenwald ML, Rothi LJ.** "Conceptual apraxia from lateralized lesions." *Neurology*. 49(2) (1997): 457-64
2. **New Disorders and Diseases:** Throughout my career I have identified previously unrecognized neurological diseases, creating new opportunities for research and treatment. The following are four examples of new neurological diseases that my coworkers and I have reported:
 - a. **Heilman, KM** Orthostatic tremor. *Archives of Neurology* 41, no. 8 (1984): 880-881
 - b. **Heilman, Kenneth M.,** and Waldo R. Fisher. Hyperlipidemic Dementia. *Archives of Neurology* 31, no. 1 (1974): 67-68
 - c. **Milano NJ, Heilman KM.** Primary Progressive Speech Abulia: "Primary Progressive Speech Abulia." *Journal of Alzheimer's Disease* (2015): 46(3):737-45
 - d. **Milano, NJ, and Heilman, KM.** Progressive affective aprosodia and prosoplegia. Ghacibeh, GA, and **Heilman, KM.** *Neurology* 60, no. 7 (2003):1192-1194
3. **Memory Disorders:** Disorders of memory of one of the most common and disabling neurobehavioral disorders that can be associated with a variety of diseases. A search on PubMed with the terms memory and Heilman K has revealed 114 papers published on this topic. In regard to helping define the anatomy of amnesia, we were one of the first to demonstrate that lesions in several areas of the brain, beside the hippocampus, can cause disorders of episodic memory including:
 - a. **Heilman KM, Sybert GW.** Korsakoff's syndrome resulting from bilateral fornix lesions. *Neurology*. 1977 May;27(5):490-3
 - b. **Valenstein E, Bowers D, Verfaellie M, Heilman KM, Day A, Watson RT.** Retrosplenial amnesia. *Brain*. 1987 Dec;110 (Pt 6):1631-46).
 - c. **Heilman KM.** Transient memory impairment and hallucinations associated with tolterodine use. *N Engl J Med*. 2003. 349(23):2274-5;
 - d. **Ghacibeh GA, Shenker JI, Shenal B, Uthman BM, Heilman KM.** The influence of vagus nerve stimulation on memory. *Cogn Behav Neurol*. 2006 Sep;19(3):119-22).
4. **Unilateral Neglect:** Patients with primarily right-sided strokes and some patients with degenerative diseases present with a very disabling disorder called unilateral neglect, where they are unaware of items or parts of their body in the contra-lesional portion of space. Spatial neglect has been a primary research interest throughout my career, and I have published more than 150 peer-reviewed papers on this topic. The following are just a sample of these papers:
 - a. **Heilman, KM, Valenstein, E.** "Frontal lobe neglect in man." *Neurology*, 22 (1972): 660-664
 - b. **Watson, RT, Heilman, KM.** "Thalamic neglect." *Neurology*, 29 (1979): 690-694
 - c. **Heilman, KM, Van den Abell, R.** "Right hemisphere dominance for attention: The mechanism underlying hemispheric asymmetries of inattention." *Neurology*, 30 (1980): 327-330
 - d. **Heilman KM,** "Intentional neglect." *Frontiers in Bioscience* (2004): (9):694-705
5. **Emotional Communication:** Since the time of Paul Broca, it has been known that the left hemisphere mediates propositional speech, reading and writing. We were one of the first laboratories to reveal that the right hemisphere mediates emotional communication, and that disorders of the right hemisphere can impair both the expression and comprehension of emotional speech prosody and emotional facial expressions. We have written more than 65 papers on the neuropsychology of emotions and the following are 4 examples of papers we have written on this topic:

- a. Bowers, D., Coslett, HB, Speedie, LJ, **Heilman, KM**. "Comprehension of emotional prosody following unilateral hemispheric lesions; Processing defects vs. distraction defects." *Neuropsychologia*, 25 (1987): 317-328
- b. Harciarek M, **Heilman KM**. "Contribution of anterior and posterior regions of the right hemisphere to the recognition of emotional faces." *J Clin Exp Neuropsychol*. 31(3) (2009): 322-30
- c. Blonder, LX, Bowers, D, **Heilman, KM**. "The role of the right hemisphere on emotional communication." *Brain*, 114 (1991): 1115-1127
- d. Bowers, D, Bauer, RM, Coslett, HB, **Heilman, KM**. "Processing of faces by patients with unilateral hemispheric lesions. I. Dissociation between judgments of facial affect and facial identity." *Brain and Cognition*, 4 (1985): 258-272

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1PgQ2XC8Hg55o/bibliography/44852418/public/?sort=date&direction=descending>

D. Current Research Support

I01 CX000744 VA Clinical Science Research & Development-Merit Review

Vertical Neglect 10/1/2012 – 9/30/2016

This grant provides support for research that is attempting to understand some of the neuropsychological mechanisms that may account for the signs of the 'neglect syndrome.'

Role: PI

XZ302 DOEA State of Florida, Department of Elder Affairs Memory Disorder Clinics

Alzheimer's Disease Initiative 7/01/88 – 6/30/16

This support allows us to develop new assessments and behavioral treatments for the cognitive disorders associated with dementing diseases. It also provides funding for the training of neurologists, psychologist and speech pathologists in the care of patients with dementia.

Role: PI

09/30/NIH 1R21AG04449-01A1 2013-12/31/2016 1.20 Calendar Months

Disorders of Emotional Communication in Patient with Cerebellar Dysfunction

The major goal of this proposal is to test the hypothesis that cerebellar damage is associated with emotional communication deficits.

Role: PI

RX000707-01 Williamson (PI) 04/01/2012-03/31/2017 0.80 Calendar Months

VA Career Development Award *White Matter Changes and Mild TBI: Emotional and Autonomic Consequences*

Research on the brain mechanisms that may induce the behavioral deficits caused by mild TBI is important for several reasons. Perhaps the most important reason is the frequency of these injuries and the disability suffering caused by TBI. Understanding the pathophysiology of a disorder is often an important initial step in finding a successful treatment.

Role: Mentor

RX000958-01A2 Leon (PI) 01/01/2014-12/31/2016 0.60 Calendar Months

VA Career Development Award

Treatment of Emotional Prosodic Disorders in Parkinson's Disease

The goal of this CDA1 proposal is to determine effect size for treating individuals with Parkinson's disease (PD) who have deficits in the production of emotional prosodic speech.

Role: Mentor

N/A Stamps (PI) 12/01/2014-11/31/2015 0.00 Calendar Months

Alzheimer's Art Quilt Initiative \$44,473

A brief, clinical test of odor detection for diagnosing early Alzheimer's disease – The purpose of this study is to replicate and build upon the findings of our preliminary study that demonstrated the left nostril was significantly worse at detecting an odor than the right nostril of individuals with early Alzheimer's disease.

Role: Primary Mentor

P50AG047266-01A1 (PIT. Golde) 08/15/2015-05/31/2020

Alzheimer's Disease Research Center (ADRC) – NIA

University of Florida – Mt. Sinai Medical Center AD Research Center

Role: Co-Investigator

PUBLICATIONS (2016)

The publications, including articles, chapters and books, in which Kenneth M. Heilman is an author or co-author have been cited more than 40,000 times.

Books (Author, Editor, Co-Editor)

1. Minagar A, Finney GR, **Heilman KM**. *Neurobehavioral Manifestations of Neurological Diseases: Diagnosis and Treatment*. *Neurologic Clinics*. Volume 34, February 2016.

Chapters:

1. **Heilman KM**, Salardini A, Falchook AD, *Action-Intentional Disorders in Neurodegenerative Diseases*. In: *Progressive Cognitive Impairment and its Neuropathologic Correlates*. Ed: Gediminas Peter Gliebus. Hauppauge NY Nova Science Publishers, Inc. 2016

Articles, Letters and Editorials Published in Journals

1. Good AJ, Harris MK, Falchook AD, Watson RT, **Heilman KM**. Callosal disconnection neglect: reassessment after 34 years. *Neurocase*. 2016 Nov 21:1-4. [Epub ahead of print]
2. Cimino-Knight AM, Gonzalez Rothi LJ, He Y, **Heilman KM**. Callosal ideomotor apraxia in Alzheimer's disease. *J Clin Exp Neuropsychol*. 2016 Nov 10:1-8. [Epub ahead of print]
3. Lamb DG, Correa LN, Seider TR, Mosquera DM, Rodriguez JA Jr, Salazar L, Schwartz ZJ, Cohen RA, Falchook AD, **Heilman KM**. The aging brain: Movement speed and spatial control. *Brain Cogn*. 2016 Sep 19; 109:105-111. doi: 10.1016/j.bandc.2016.07.009. [Epub ahead of print].
4. Na HK, Kang DR, Kim S, Seo SW, **Heilman KM**, Noh Y, Na DL. Malignant progression in parietal-dominant atrophy subtype of Alzheimer's disease occurs independent of onset age. *Neurobiol Aging*. 2016 Aug 12;47 :149-156. doi: 10.1016/j.neurobiolaging.2016.08.001. [Epub ahead of print].
5. Zhang L, McFarland KN, Subramony SH, **Heilman KM**, Ashizawa T. SPG7 and Impaired Emotional Communication. *Cerebellum*. 2016 Aug 24. [Epub ahead of print]
6. Tacik P, DeTure MA, Yari C, Lin WL, Murray ME, Baker MC, Josephs KA, Boeve BF, Wszolek ZK, Graff-Radford NR, Parisi JE, Petrucelli 2, Rademakers R, Isaacson RS, **Heilman KM**, Petersen RC, Dickson DW, Kouri N. FTDP-17 with pick body-like inclusions associated with a novel tau mutation, p. E372G. *Brain Pathol*. 2016 Aug 16. doi: 10.1111/bpa.12428. [Epub ahead of print]
7. Harciarek M, Michałowski J, Biedunkiewicz B, Williamson J, Dębska-Ślizień A, Rutkowski B, **Heilman KM**. Disorders of the anterior attentional-intentional system in patients with end stage renal disease: Evidence from reaction time studies. *Brain Cogn*. 2016 Aug; 107:1-9. doi: 10.1016/j.bandc.2016.05.005. Epub 2016 Jun 27.
8. Michałowski JM, Harciarek M, Biedunkiewicz B, Williamson J, Dębska-Ślizień A, Rutkowski B, **Heilman KM**. Slowing with end-stage renal disease: Attentive but unprepared to act. *Int J Psychophysiol*. 2016 Jun 7. pii: S0167-8760(16)30105-2. doi: 10.1016/j.ijpsycho.2016.06.002. [Epub ahead of print]
9. **Heilman KM** Possible Brain Mechanisms of Creativity. *Arch Clin Neuropsychol*. 2016 Mar 21. pii: acw009. [Epub ahead of print].
10. **Heilman KM**. Jews, Creativity and the Genius of Disobedience. *J Relig Health*. 2016 Feb;55(1):341-9. doi: 10.1007/s10943-015-0153-z
11. Kesayan T, Williamson JB, Falchook AD, Skidmore FM, **Heilman KM**. Allocentric but Not Egocentric Pseudoneglect of Peripersonal Space. *Cogn Behav Neurol*. 2016 Mar;29(1):18-23.
12. Anderson-Mooney AJ, Schmitt FA, Head E, Lott IT, **Heilman KM**. Gait dyspraxia as a clinical marker of cognitive decline in Down syndrome: A review of theory and proposed mechanisms. *Brain Cogn*. 2016 Feb 27; 104:48-57. doi: 10.1016/j.bandc.2016.02.007. [Epub ahead of print].
13. Falchook AD, Watson RT, **Heilman KM**. Callosal apraxia: a 34-year follow-up study. *Neurocase*. 2016 Feb 29:1-6. [Epub ahead of print]
14. Minagar A, Finney GR, **Heilman KM**. *Neurobehavioral Manifestations of Neurological Diseases: Diagnosis and Treatment*. *Neurol Clin*. 2016 Feb;34(1):xiii-xiv. doi: 10.1016/j.ncl.2015.10.001
15. Finney GR, Minagar A, **Heilman KM**. Assessment of Mental Status. *Neurol Clin*. 2016 Feb;34(1):1-16. doi: 10.1016/j.ncl.2015.08.001.

16. Kim GH, Seo SW, Jung K, Kwon OH, Kwon H, Kim JH, Roh JH, Kim MJ, Lee BH, Yoon DS, Hwang JW, Lee JM, Jeong JH, You H, **Heilman KM**, Na DL The neural correlates of motor intentional disorders in patients with subcortical vascular cognitive impairment. *J Neurol*. 2016 Jan;263(1):89-99. doi: 10.1007/s00415-015-7946-6.
17. Zilli EM, **Heilman KM**. Spatial neglect in a patient with logopenic progressive aphasia. *Neurocase*. 2016;22(1):30-9.

Paper-Poster Presentations and Abstracts (2016)

- Abhishek Lunagariya, Achint Patel, Jillianne Grayson, **Kenneth Heilman**. Ethical Dilemma: A National Perspective on Utilization of Do-Not-Resuscitate Orders in Status Epilepticus. Presented at the 68th Annual Meeting of the American Academy of Neurology, April 16, 2016, Vancouver BC.
- Tigran Kesayan, Damon Lamb, John Williamson, Adam Falchook, **Kenneth Heilman** Perceptual Pseudoneglect: Laterality and the Perception of Tactile Pressure. Presented at the 68th Annual Meeting of the American Academy of Neurology, April 19, 2016, Vancouver BC.
- Tigran Kesayan, Damon Lamb, John Williamson, Adam Falchook, **Kenneth Heilman**. Reduced Tactile Pressure Perception with Age. Presented at the 68th Annual Meeting of the American Academy of Neurology, April 21, 2016, Vancouver BC.
- Nicholas Milano, Annika Goldman, Adam Woods, John Williamson, Lealani Acosta, Damon Lamb, Han Zhang, **Kenneth Heilman**. The Influence of Right and Left Frontotemporal Stimulation on Visuospatial Creativity. Presented at the 68th Annual Meeting of the American Academy of Neurology, April 19, 2016, Vancouver BC.
- M. Harciarek, J. Michałowski, B. Biedunkiewicz, J Williamson, A. Dębska-Ślizień, B. Rutkowski, **K.M. Heilman**. Attentional-intentional deficits associated with end stage renal disease and dialysis are normalized with kidney transplantation. Paper presented at the International Neuropsychological Society 2016 Mid-Year Meeting; 6-8.07.2016 London (Great Britain).
- A.Wojtowicz, M. Harciarek, J. Williamson, **K.M. Heilman**. The influence of right and left deviations of spatial attention on emotional picture recognition. Poster presented at the International Neuropsychological Society 2016 Mid-Year Meeting; 6-8.07.2016 London (Great Britain).
- A.Cimino-Knight L/ Gonzales-Rothi L, KM Heilman Callosal Ideomotor Apraxia in Alzheimer's Disease. Presented at the Annual Meeting, International Neuropsychological Society, February 2016, Boston, MA. (*Journal of the International Neuropsychological Society*).
- K.T Balavage, D. Lamb, L. Knight, D. Bielick, K.J. Kincaid, **KM Heilman**. The Effects of the Allocation of Focal Attention and Habituation on the Line Bisection Task. Presented at the Annual Meeting, International Neuropsychological Society, February 2016, Boston, MA. (*Journal of the International Neuropsychological Society*).
- D. Bielick, D. Lamb, K.J. Kincaid, K.T Balavage, L. Knight, **K.M. Heilman**. Hemispheric Lateralization of Attentional Background Distraction. Presented at the Annual Meeting, International Neuropsychological Society, February 2016, Boston, MA. (*Journal of the International Neuropsychological Society*).
- K.J. Kincaid, D. Lamb, D. Bielick, L. Knight, K.T Balavage, **K.M. Heilman**. Influence of Viewing Eye on Altitudinal Attentional Bias. Presented at the Annual Meeting, International Neuropsychological Society, February 2016, Boston, MA. (*Journal of the International Neuropsychological Society*).
- L. Knight, D. Lamb, K.T Balavage, D. Bielick, K.J. Kincaid, **K.M. Heilman**. Effects of Focal and Global Spatial Attention on the Compound Line Bisection Tasks. Presented at the Annual Meeting, International Neuropsychological Society, February 2016, Boston, MA. (*Journal of the International Neuropsychological Society*).

Christiaan Leeuwenburgh, PhD

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Christiaan Leeuwenburgh

eRA COMMONS USER NAME (credential, e.g., agency login): cleeuwen

POSITION TITLE: Professor and Chief

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Florida, Gainesville	BS	05/88	Applied Physiology
University of Florida, Gainesville	MS	05/90	Applied Physiology
University of Illinois, Urbana-Champaign	PhD	10/95	Biochemistry and Aging
University of Wisconsin, Madison	Pre-Fellow	10/95	Biochemistry and Aging
Washington Univ. School of Medicine, St Louis	Post- Fellow	12/98	Geriatrics & Gerontology

A. Personal Statement

I received my PhD from the University of Illinois, Urbana-Champaign in 1995 which focused on the regulation of glutathione (major intracellular antioxidant) and antioxidant enzymes in young and old animals. I completed my postdoctoral studies in Internal Medicine, Division of Geriatrics and Gerontology and Division of Atherosclerosis, Nutrition and Lipid Research at Washington University School of Medicine, Saint Louis with John Holloszy (MD) and Jay Heinecke (MD) as primary mentors. My major research focus is to better understand the molecular mechanisms of mitochondrial dysfunction, autophagy, inflammation, oxidative stress and programmed cell death (apoptosis) with age and age-related diseases. I have participated in various NIH study sections, NIH workshops focused on the biology of aging, mitochondrial biology and geriatric research and have published papers in *Cell*, *Science*, *Aging Cell*, *The Journal of Biological Chemistry*, *American Journal of Physiology*, *PLoS One*, *Journal of Gerontology*, *FASEB Journal*, *Experimental Gerontology*, *Neurobiology of Aging*, *Rejuvenation Research*, *Journal of Clinical Investigation* and *PNAS*. Our work on the assessment of mitochondrial biology, oxidative stress, inflammation, autophagy and mitochondrial mediated apoptosis in aging and disease has been increasingly recognized and appreciated by scientists. In summary, as a basic and translational scientist, my goal is to bridge the gap between basic and clinical sciences, focusing on biological mechanisms of aging and disease and testing translational interventions.

B. Positions and Honors

Positions and Employment

1995-1998	Washington University School of Medicine, St. Louis, Department of Internal Medicine, Divisions of Geriatrics and Gerontology, and Atherosclerosis, Nutrition and Lipid Research Postdoctoral Fellow in Internal Medicine and Geriatrics and Gerontology; Research Associate in Medicine; Mentors: John O. Holloszy, MD and Jay W. Heinecke, MD
1998-2002	Assistant Professor, Director of the Biochemistry of Aging Laboratory, University of Florida
2002-2005	Associate Professor and Director of the Biochemistry of Aging Laboratory, University of Florida
2005-2007	Associate Professor, College of Medicine, Department of Aging and Geriatric Research
2005-	Director, Metabolism and Translational Science Core of the University of Florida Institute on Aging
2006-	Chief, Division of Biology of Aging, Department of Aging and Geriatric Research
2007-	Professor, College of Medicine, Department of Aging and Geriatric Research, Division of Biology of Aging
2015-	Vice Chair of Research, Department of Aging and Geriatric Research, College of Medicine

Other Experience and Professional Memberships

1995-2008	Society for Free Radical Biology and Medicine
1995-2008	International Society for Free Radical Research
2004-2012	NIH Peer Review Committees; Special Emphasis Panels
1997-present	The American Physiological Society
2003-present	Member, American Aging Association
2003-present	Member, Gerontological Society of America
2008-present	Editor, <i>Experimental Gerontology</i>

Honors

1993-1995	American Heart Association, Pre-doctoral Fellowship, Illinois Affiliate
1996	Young Investigator Award, Oxygen Society, Intern. Soc. Free Rad. Res., Miami, FL
1997-1998	National Research Service Award, NRSA-NIH, National Institute of Aging
1999-2000	Merck Geriatric Cardiology Research Award, Society of Geriatric Cardiology
2000-2002	American Heart Association, Young Investigator Award, FL
2004-2005	University of Florida Research Foundation, Professor Award
2004	Nathan W. Shock Lecture Award Winner, National Institute on Aging
2010	Exemplary Teacher Award, College of Medicine
2011-2013	University of Florida Research Foundation Professor

C. Contribution to Science

1. In the context of this specific grant Drs Kim and Leeuwenburgh have published several key papers together showing the increased vulnerability of the aged liver to injury and the specific biological pathways implicated. Autophagy suppresses age-dependent ischemia and reperfusion injury in livers of mice. Wang JH, Ahn IS, Fischer TD, Byeon JI, Dunn WA Jr, Behrns KE, **Leeuwenburgh C**, Kim JS. *Gastroenterology*. 2011. Critical role of autophagy in ischemia/reperfusion injury to aged livers. Wang JH, Behrns KE, **Leeuwenburgh C**, Kim JS. *Autophagy*. 2012. The emerging role of iron dyshomeostasis in the mitochondrial decay of aging. Xu J, Marzetti E, Seo AY, Kim JS, Prolla TA, **Leeuwenburgh C**. *Mech Ageing Dev*. 2010. Upregulated autophagy protects cardiomyocytes from oxidative stress-induced toxicity. Dutta D, Xu J, Kim JS, Dunn WA Jr, **Leeuwenburgh C**. *Autophagy*. 2013. Autophagy in the liver: cell's cannibalism and beyond., Flores-Toro JA, Go KL, Leeuwenburgh C, Kim JS., *Arch Pharm Res*. 2016 Aug;39(8):1050-61
2. What's more, in 2005 we published a publication in *Science* (Kujoth, *Science* 2005; cited 1095 times) using transgenic mice to show that for the first time an accumulation of mtDNA mutations can promote apoptosis and is a central mechanism driving mammalian aging. At that time it was unknown if mutations in mitochondrial DNA (mtDNA) accumulate in tissues of mammalian species drive aging, although it was shown that they were associated with aging no causal relationship was established. We showed experimentally that mice expressing a proofreading-deficient version of the mitochondrial DNA polymerase γ (POLG) accumulate mtDNA mutations also displayed significant features of accelerated aging. Accumulation of mtDNA mutations was also causal to the induction of apoptosis, particularly in tissues characterized by rapid cellular turnover. The levels of apoptotic markers were also found to increase during aging in normally aging mice.

Kujoth, G.C., Hiona, A., Pugh, T.D., Someya, S., Panzer, K., Wohlgemuth, S.E., Hofer, T., Seo, A.Y., Sullivan, R., Jobling, W.A., et al. 2005. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* 309:481-484.
PMID: 16020738
3. In 2010, we published a paper in the journal *Cell* "Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction" (Someya, *Cell* 2010; cited over 400 times) to demonstrate for the first time that one of the sirtuins (Sir3) had an important role in maintaining an important physiological function (hearing loss) with aging. It was already known that caloric restriction (CR) extends the life span and health span of a variety of species and slows the progression of age-related hearing loss (AHL), a common age-related disorder associated with oxidative stress. However, in this report we showed that CR reduces oxidative DNA damage in multiple tissues and prevents AHL in wild-type mice but fails to modify these phenotypes in mice lacking the mitochondrial deacetylase Sirt3, a member of the sirtuin family. In response to CR, Sirt3 also directly deacetylates and activates mitochondrial isocitrate dehydrogenase 2 (Idh2), leading to increased NADPH levels and an increased ratio of reduced-to-oxidized glutathione in mitochondria. In addition using cultured cells, overexpression of Sirt3 and/or Idh2 increases NADPH levels protects cells from oxidative stress-induced cell death. Therefore, these findings identify for the first time that Sirt3 is an essential player in enhancing the mitochondrial glutathione antioxidant defense system during CR and shows that Sirt3-dependent mitochondrial adaptations are a central mechanism of aging retardation in mammals.

R01 DC014437 Someya (PI) 04/1/2015-03/31/2020

Cochlear detoxification system

The overall goal of our research proposal is to provide new basic knowledge of the molecular basis for the cochlear detoxification system and its role in the elimination of foreign chemicals throughout the lifespan.

Role: Co-I

R01GM113945 Efron (PI) 04/01/2015-01/31/2017

Hematopoietic Stem Cell Dysfunction in the Elderly after Severe Injury

This work will elucidate the cause of increased susceptibility and worsened outcomes to trauma in the aged and illustrates promise for areas of intervention.

Role: Co-I

Completed Projects

U01-AG022376 Pahor (PI) 09/1/2009-11/30/2015

Physical Exercise to Prevent Disability – LIFE Study

We propose conducting a Phase 3, single-masked multicenter randomized controlled trial to compare a moderate-intensity physical activity program to a successful aging health education program in sedentary older persons who are at risk of disability.

Role: Co-I

Osato Research Institute Anton-Leeuwenburgh (PI's) 07/1/2013-12/31/2015

Efficacy of fermented papaya preparation (FPP) in improving health and physical function in older adults with mild functional limitations

This pilot study will evaluate the effects of supplementation with FPP (dosage = 9 grams per day) for one month on markers of systemic inflammation, physical performance, tissue oxygenation, fatigue, and health related quality of life, in generally healthy, older adults (age > 65 years) with elevated levels of systemic inflammation (C-reactive protein levels > 1.0) and moderate functional limitations (Short Physical Performance Battery Score < 10).

Role: Co-PI

Publications 2016

Aubertin-Leheudre M, Anton S, Beavers DP, Manini TM, Fielding R, Newman A, Church T, Kritchevsky SB, Conroy D, McDermott MM, Botosaneanu A, Hauser ME, Pahor M; LIFE Research Group. Dynapenia and Metabolic Health in Obese and Nonobese Adults Aged 70 Years and Older: *The LIFE Study*. *J Am Med Dir Assoc*. 2016 Nov 30. pii: S1525-8610(16)30468-6. doi: 10.1016/j.jamda.2016.10.001. [Epub ahead of print]

Calvani R, Marini F, Cesari M, Buford TW, Manini TM, Pahor M, **Leeuwenburgh C**, Bernabei R, Landi F, Marzetti E. Systemic inflammation, body composition, and physical performance in old community-dwellers. *J Cachexia Sarcopenia Muscle*. 2016 Aug 8. doi: 10.1002/jcsm.12134. [Epub ahead of print]

Han C, Ding D, Lopez MC, Manohar S, Zhang Y, Kim MJ, Park HJ, White K, Kim YH, Linser P, Tanokura M, **Leeuwenburgh C**, Baker HV, Salvi RJ, Someya S. Effects of Long-Term Exercise on Age-Related Hearing Loss in Mice. *J Neurosci*. 2016 Nov 2;36(44):11308-11319.

Aydemir TB, Troche C, Kim J, Kim MH, Teran OY, **Leeuwenburgh C**, Cousins RJ. Aging amplifies multiple phenotypic defects in mice with zinc transporter Zip14 (Slc39a14) deletion. *Exp Gerontol*. 2016 Dec 1;85:88-94. doi: 10.1016/j.exger.2016.09.013.

Picca A, Pesce V, Sirago G, Fracasso F, **Leeuwenburgh C**, Lezza AM. "What makes some rats live so long?" The mitochondrial contribution to longevity through balance of mitochondrial dynamics and mtDNA content. *Exp Gerontol*. 2016 Dec 1;85:33-40. doi: 10.1016/j.exger.2016.09.010.

Mankowski RT, Ahmed S, Beaver T, Dirain M, Han C, Hess P, Martin T, Smith BK, Someya S, **Leeuwenburgh C**, Martin AD. Intraoperative hemidiaphragm electrical stimulation reduces oxidative stress and upregulates autophagy in Surgery patients undergoing mechanical ventilation: exploratory study. *J Transl Med*. 2016 Oct 26;14(1):305.

Extermann M, **Leeuwenburgh C**, Samiiian L, Sehovic M, Xu J, Cubitt C, Jacobsen PB, Pahor M, Grobmyer SR, Manini TM. Impact of chemotherapy on medium-term physical function and activity of older breast cancer survivors, and associated biomarkers. *J Geriatr Oncol*. 2016 Oct 13. pii: S1879-4068(16)30116-3. doi: 10.1016/j.jgo.2016.09.004. [Epub ahead of print]

White SH, McDermott MM, Sufit RL, Kosmac K, Bugg AW, Gonzalez-Freire M, Ferrucci L, Tian L, Zhao L, Gao Y, Kibbe MR, Criqui MH, **Leeuwenburgh C**, Peterson CA. Walking performance is positively correlated to calf muscle fiber size in peripheral artery disease subjects, but fibers show aberrant mitophagy: an observational study. *J Transl Med*. 2016 Sep 29;14(1):284.

- Layne AS, Hsu FC, Blair SN, Chen SH, Dungan J, Fielding RA, Glynn NW, Hajduk AM, King AC, Manini TM, Marsh AP, Pahor M, Pellegrini CA, Buford TW; LIFE Study Investigators.. Predictors of Change in Physical Function in Older Adults in Response to Long-Term, Structured Physical Activity: *The LIFE Study. Arch Phys Med Rehabil.* 2017 Jan;98(1):11-24.e3. doi: 10.1016/j.apmr.2016.07.019.
- Marzetti E, D'Angelo E, Saveria G, **Leeuwenburgh C**, Calvani R. Integrated control of brown adipose tissue. *Heart Metab.* 2016 Mar;69:9-14.
- Flores-Toro JA, Go KL, **Leeuwenburgh C**, Kim JS. Autophagy in the liver: cell's cannibalism and beyond. *Arch Pharm Res.* 2016 Aug;39(8):1050-61. doi: 10.1007/s12272-016-0807-8. Review.
- Wawrzyniak NR, Joseph AM, Levin DG, Gundermann DM, **Leeuwenburgh C**, Sandesara B, Manini TM, Adhihetty PJ. Idiopathic chronic fatigue in older adults is linked to impaired mitochondrial content and biogenesis signaling in skeletal muscle. *Oncotarget.* 2016 Aug 16;7(33):52695-52709. doi: 10.18632/oncotarget.10685.
- Marsh AP, Applegate WB, Guralnik JM, Jack Rejeski W, Church TS, Fielding RA, Gill TM, King AC, Kritchevsky SB, Manini TM, McDermott MM, Newman AB, Stowe CL, Walkup MP, Pahor M, Miller ME; LIFE Study Investigators. Hospitalizations During a Physical Activity Intervention in Older Adults at Risk of Mobility Disability: Analyses from the Lifestyle Interventions and Independence for Elders Randomized Clinical Trial. *J Am Geriatr Soc.* 2016 May;64(5):933-43. doi: 10.1111/jgs.14114.
- Włodarczyk-Biegun MK, Farbod K, Werten MW, Slingerland CJ, de Wolf FA, van den Beucken JJ, **Leeuwenburgh SC**, Cohen Stuart MA, Kamperman M. Fibrous Hydrogels for Cell Encapsulation: A Modular and Supramolecular Approach. *PLoS One.* 2016 May 25;11(5):e0155625. doi: 10.1371/journal.pone.0155625. Erratum in: PLoS One. 2016;11(7):e0159893.
- Gill TM, Pahor M, Guralnik JM, McDermott MM, King AC, Buford TW, Strotmeyer ES, Nelson ME, Sink KM, Demons JL, Kashaf SS, Walkup MP, Miller ME; LIFE Study Investigators. Effect of structured physical activity on prevention of serious fall injuries in adults aged 70-89: randomized clinical trial (LIFE Study). *BMJ.* 2016 Feb 3;352:i245. doi: 10.1136/bmj.i245.
- Chmielewski TL, George SZ, Tillman SM, Moser MW, Lentz TA, Indelicato PA, Trumble TN, Shuster JJ, Cicuttini FM, **Leeuwenburgh C**. Low- Versus High-Intensity Plyometric Exercise During Rehabilitation After Anterior Cruciate Ligament Reconstruction. *Am J Sports Med.* 2016 Mar;44(3):609-17. doi: 10.1177/0363546515620583.
- Farbod K, Diba M, Zinkevich T, Schmidt S, Harrington MJ, Kentgens AP, **Leeuwenburgh SC**. Gelatin Nanoparticles with Enhanced Affinity for Calcium Phosphate. *Macromol Biosci.* 2016 May;16(5):717-29. doi: 10.1002/mabi.201500414.
- Joseph AM, Adhihetty PJ, **Leeuwenburgh C**. Beneficial effects of exercise on age-related mitochondrial dysfunction and oxidative stress in skeletal muscle. *J Physiol.* 2016 Sep 15;594(18):5105-23. doi: 10.1113/JP270659.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: MARSISKE, MICHAEL

eRA COMMONS USER NAME (agency login): mmarsiske

POSITION TITLE: Associate Professor and Associate Chair

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Toronto, Toronto, Ontario	BS	06/1987	Psychology
The Pennsylvania State University, University Park, PA	MS	12/1990	Human Development and Family Studies
The Pennsylvania State University, University Park, PA	PHD	12/1992	Human Development and Family Studies
Max Planck Institut fuer Bildungsforschung, Berlin	Postdoctoral Fellow	07/1995	Psychology and Human Development

A. Personal Statement

Michael Marsiske is an Associate Professor and Associate Chair for Research in the Department of Clinical and Health Psychology at the University of Florida (UF). Marsiske's major research emphases are: (a) the investigation of cognitive intervention strategies for older adults (cognitive training, video games, exercise, collaboration and prompting), and (b) development and evaluation of measures of everyday cognition. Marsiske has also been an instructor of advanced graduate coursework in statistics and methodology (particularly multivariate and longitudinal analyses) since 1996.

Currently an MPI of the multi-site NIA-funded "Augmenting Cognitive Training" study (with Adam Woods and Ronald Cohen), Marsiske was one of the PIs of the NIH-funded ACTIVE trial, a ten-year multi-site study of the long-term effects of cognitive interventions for older adults. Other support has come from the Robert Wood Johnson Foundation "Pioneer" program ("Games for Health") and the McKnight Brain Research Foundation. Marsiske is also the PI and training director of an National Institute on Aging (NIA) funded predoctoral T32 program in aging, funded since 2003. He also serves as the Core Leader for the Data Management and Data Analysis Core of the 1Florida Alzheimer's Disease Research Center at UF. Dr. Marsiske is a Fellow of the Gerontological Society of America, and is a past recipient of the Springer Award for Early Career Achievement in Research and Adult Development and Aging from Division 20 of the American Psychological Association. At UF, he has also received the university's UF Research Foundation Professorship and the UF Doctoral Mentoring Award. Marsiske is a past-Chair of the NIA-S (Behavioral and Social Sciences) Initial Review Group for the National Institute on Aging.

1. **Marsiske M**, Margrett JA. Handbook of the Psychology of Aging . 6th Edition ed. Birren JE, Schaie KW, editors. New York: Academic Press; 2006. *Everday problem solving and decision making*; p.315-342.
2. Cook SE, Sisco SM, **Marsiske M**. Dual-task effects of simulated lane navigation and story recall in older adults with and without memory impairment. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn*. 2013;20(4):383-404. **PubMed PMID: 23043546; PubMed Central PMCID: PMC3823673.**
3. Belchior P, **Marsiske M**, Sisco SM, Yam A, Bavelier D, Ball K, Mann WC. Video game training to improve selective visual attention in older adults. *Comput Human Behav*. 2013 Jul 1;29(4):1318-1324. **PubMed PMID: 24003265; PubMed Central PMCID: PMC3758751.**
4. Yam A, **Marsiske M**. Cognitive longitudinal predictors of older adults' self-reported IADL function. *J Aging Health*. 2013 Dec;25(8 Suppl):163S-85S. **PubMed PMID: 24385635; PubMed Central PMCID: PMC3882335.**

B. Positions and Honors

Positions and Employment

1992 - 1995 Postdoctoral Fellow, Max Planck Institut fuer Bildungsforschung, Berlin

1995 - 2000 Assistant Professor, Wayne State University, Detroit, MI

2000 - Associate Professor and Associate Chair, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

- 1987 - Member, American Psychological Association
- 1992 - Member, American Psychological Society
- 1992 - 2010 Member, International Society for the Study of Behavioral Development
- 1995 - 2014 Executive Member, Division on Adult Development and Aging, American Psychological Association
- 1998 - Fellow, Gerontological Society of America

- 2001 - 2003 Member, NIH Peer Review, NIA-S (Behavioral and Social Science of Aging)
- 2003 - 2003 Member, NIH Peer Review, Special Emphasis Panel (Roybal Centers)
- 2003 - 2005 Chair, NIH Peer Review, NIA-S (Behavioral and Social Science of Aging)
- 2003 - 2008 Member, Editorial Board, Journal of Gerontology: Psychological Sciences
- 2004 - Member, Member, Editorial Board, Aging, Neuropsychology and Cognition
- 2005 - 2005 Member, NIH Peer Review, Special Emphasis Panel (HRS/AHEAD)
- 2007 - 2007 Member, NIH Peer Review, Special Emphasis Panel (Program Project), NIA
- 2008 - 2008 Member, NIH Peer Review, Special Emphasis Panel (P50 Supplement), NIA
- 2009 - 2009 Member, NIH Peer Review, Special Emphasis Panel (Roybal Centers)
- 2009 - 2009 Member, NIH Peer Review, Special Emphasis Panel, Recovery Act Career Awards, RC2-SEP1 review
- 2009 - 2009 Member, Member, NIH Peer Review, Special Emphasis Panel, Research and Research Infrastructure "Grand Opportunities" (RC2).
- 2009 - 2009 Member, Member, NIH Peer Review, Special Emphasis Panel (Alzheimer's Disease Clinical Trials Special Emphasis Panel (BBBP-N[52]), National Institute on Aging, SBIR/STTR
- 2010 - 2010 Member, NIH Peer Review, Special Emphasis Panel (P50 Supplement), NIA
- 2012 - 2015 Chair-Elect/Chair, Membership Committee, Gerontological Society of America

Honors

- 1994 Fellowship for Summer Institute on Successful Midlife Development, MacArthur Foundation Research Network on Successful Midlife Development
- 1997 Springer Award for Early Career Achievement in Research on Adult Development and Aging, Division on Adult Development and Aging, American Psychological Association
- 2002 Fellowship status, Gerontological Society of America
- 2008 Research Mentorship Award, Graduate Student Organization, Univ. of Fla Dept. Clinical/Health Psychology
- 2009 Research Mentorship Award, Graduate Student Organization, Univ. of Fla Dept. Clinical/Health Psychology
- 2011 Audrey Schumacher Award for Teaching Excellence (2003, 2005, 2006, 2009, 2011), Graduate Student Organization, Univ. of Fla Dept. Clinical/Health Psychology
- 2011 University of Florida Research Foundation Professorship, University of Florida
- 2013 University of Florida Doctoral Mentoring Award, University of Florida

C. Contribution to Science

1. A key focus of my work has been on cognitive interventions with older adults. I had the privilege of training with Sherry Willis and Warner Schaie (PhD, Penn State) and Paul Baltes (Max Planck Institute) on some of the earliest NIH- and other funded research on cognitive training with older adults. This evolved to my role as a PI on the multi-site NIA-funded ACTIVE trial, where (in over 2,800 older adults) we showed that cognitive training in memory/reasoning/processing speed could yield improvements in trained domains that lasted at least ten years. Moreover, while transfer of training was narrow, self-reported everyday functioning showed attenuated age-related decline in adults who received training. A Robert Wood Johnson funded trial showed that off-the-shelf video games could also boost visual attention in older adults, and more recent work has focused on combinations of physical exercise, game play and cognitive training.
 - a. Margrett JA, Marsiske M. Gender differences in older adults' everyday cognitive collaboration. *Int J Behav Dev.* 2002 Jan;26(1):45-59. **PubMed PMID: 20657668; PubMed Central PMCID: PMC2909137.**
 - b. Willis SL, Tennstedt SL, Marsiske M, Ball K, Elias J, Koepke KM, Morris JN, Rebok GW, Unverzagt FW, Stoddard AM, Wright E. Long-term effects of cognitive training on everyday functional outcomes in older adults. *JAMA.* 2006 Dec 20;296(23):2805-14. **PubMed PMID: 17179457; PubMed Central PMCID: PMC2910591.**
 - c. Belchior P, Marsiske M, Sisco S, Yam A, Mann W. Older adults' engagement with a video game training program. *Act Adapt Aging.* 2012 Dec 19;36(4):269-279. **PubMed PMID: 23504652; PubMed Central PMCID: PMC3596832.**

- d. Thomas KR, **Marsiske M**. Verbal prompting to improve everyday cognition in MCI and unimpaired older adults. *Neuropsychology*. 2014 Jan;28(1):123-34. **PubMed PMID: 24219613; PubMed Central PMCID: PMC3935329.**
2. Much of my work has examined the measurement of “everyday cognition” or “everyday problem solving” in older adults. This work, which started with my dissertation, aims to answer the “so what” question in cognitive aging. How do we reconcile that apparent declines in the underlying cognitive process often seem to have little functional impact on elders’ maintenance of everyday independence. Theoretical speculation has argued that older adults may invoke compensatory domain-specific knowledge built up through years of experience. But our work (with Sherry Willis, Jason Allaire, Manfred Diehl and, recently, Kelsey Thomas) suggests that the rate of decline in everyday cognition may parallel that seen for traditional measures of cognition. As such, there may be a growing vulnerability in the ability to perform novel but important tasks (like medication use), even before clinically significant declines are observed.
- a. **Marsiske M**, Willis SL. Dimensionality of everyday problem solving in older adults. *Psychol Aging*. 1995 Jun;10(2):269-83. **PubMed PMID: 7662186; PubMed Central PMCID: PMC2923471.**
- b. Allaire JC, **Marsiske M**. Well- and ill-defined measures of everyday cognition: relationship to older adults’ intellectual ability and functional status. *Psychol Aging*. 2002 Mar;17(1):101-15. **PubMed PMID: 11931279; PubMed Central PMCID: PMC2909873.**
- c. Diehl M, **Marsiske M**, Horgas AL, Rosenberg A, Saczynski JS, Willis SL. The Revised Observed Tasks of Daily Living: A Performance-Based Assessment of Everyday Problem Solving in Older Adults. *J Appl Gerontol*. 2005;24(3):211-230. **PubMed PMID: 18160968; PubMed Central PMCID: PMC2153442.**
- d. Yam A, Gross AL, Prindle JJ, **Marsiske M**. Ten-year longitudinal trajectories of older adults’ basic and everyday cognitive abilities. *Neuropsychology*. 2014 Nov;28(6):819-28. **PubMed PMID: 24885451; PubMed Central PMCID: PMC4227959.**
3. Race-related disparities in cognition, the resulting differences in trajectories of cognitive aging as a function of disparities, and the underlying health antecedents of these disparities have been consistent through-lines in my research. The interest in race stemmed from opportunities (my site in the ACTIVE clinical trial included approximately 75% African American participants), collaborators (most notably Adrienne Aiken Morgan, now at Duke University, and Carolyn Tucker, a close colleague here at the University of Florida). Moreover, the lifetime opportunity structures associated with race-related disparities are a clear demonstration of developmental contextualism (as Paul Baltes called it), the role of environmental factors in shaping age-related change.
- a. Morgan AA, **Marsiske M**, Whitfield KE. Characterizing and explaining differences in cognitive test performance between african american and European American older adults. *Exp Aging Res*. 2008 Jan-Mar;34(1):80-100. **PubMed PMID: 18189169; PubMed Central PMCID: PMC2211729.**
- b. Aiken Morgan AT, **Marsiske M**, Dzierzewski JM, Jones RN, Whitfield KE, Johnson KE, Cresci MK. Race-related cognitive test bias in the active study: a mimic model approach. *Exp Aging Res*. 2010 Oct;36(4):426-52. **PubMed PMID: 20845121; PubMed Central PMCID: PMC2941916.**
- c. Tucker CM, **Marsiske M**, Rice KG, Nielson JJ, Herman K. Patient-centered culturally sensitive health care: model testing and refinement. *Health Psychol*. 2011 May;30(3):342-50. **PubMed PMID: 21553978; PubMed Central PMCID: PMC3092156.**
- d. **Marsiske M**, Dzierzewski JM, Thomas KR, Kasten L, Jones RN, Johnson KE, Willis SL, Whitfield KE, Ball KK, Rebok GW. Race-related disparities in 5-year cognitive level and change in untrained ACTIVE participants. *J Aging Health*. 2013 Dec;25(8 Suppl):103S-27S. **PubMed PMID: 24385632; PubMed Central PMCID: PMC3882334.**
4. Methodological and statistical consultation, especially in multivariate and longitudinal contexts, has been a through line in my research. Owing to early training with Warner Schaie, John Nesselroade and Paul Baltes at Penn State and Max Planck Berlin, I have leveraged my training to teach advanced graduate statistics (from regression and ANOVA through mixed effects, structural equation analysis and survival analysis) since 1996. I serve as methods/statistics co-investigator on several grants, and I direct the Data Analysis/Management Core for the NIA-funded Florida Alzheimer’s Consortium (alzfl.org).
- a. Jones RN, Rosenberg AL, Morris JN, Allaire JC, McCoy KJ, **Marsiske M**, Kleinman KP, Rebok GW, Malloy PF. A growth curve model of learning acquisition among cognitively normal older adults. *Exp Aging Res*. 2005 Jul-Sep;31(3):291-312. **PubMed PMID: 16036723; PubMed Central PMCID: PMC2908897.**
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- c. Zahodne LB, Marsiske M, Okun MS, Rodriguez RL, Malaty I, Bowers D. Mood and motor trajectories in Parkinson's disease: multivariate latent growth curve modeling. *Neuropsychology*. 2012 Jan;26(1):71-80. PubMed PMID: 22142359; PubMed Central PMCID: PMC3296901.
- d. Zahodne LB, Marsiske M, Bowers D. A latent class analysis of psychological disturbance in Parkinson's disease. *Int J Geriatr Psychiatry*. 2013 Oct;28(10):1054-60. PubMed PMID: 23307695; PubMed Central PMCID: PMC3656148.

Complete List of Published Work in My Bibliography:

<http://www.ncbi.nlm.nih.gov/myncbi/michael.marsiske.1/bibliography/40338977/public/?sort=date&direction=ascending>

D. Research Support

Ongoing Research Support (omits multiple awards for space)

2016/09/30-2021/09/29

R01 AG054077, National Institute on Aging (NIA)

COHEN, RONALD; MARSISKE, MICHAEL; WOODS, ADAM (MPI)

Augmenting cognitive training in older adults – the ACT grant

Role: PI

2015/05/31-2020/05/31

P50 AG047266, National Institute on Aging (NIA)

GOLDE, TODD (PI)

University of Florida – Mt. Sinai Medical Center AD Research Center

Role: Core Leader, Data Management and Data Analysis Core

2002/07/01-2018/04/30

T32 AG020499-14, National Institute on Aging (NIA)

MARSISKE, MICHAEL (PI)

Physical, Cognitive and Mental Health in Social Context

Role: PI

2013/05/01-2017/04/30

RX000339, Veterans Administration Rehabilitation Research and Development Service

Levy, Charles (PI)

Virtual Environments for Therapeutic Solutions (VETS) mTBI/PTSD Phase II

The goal of this study is to develop and test a virtual therapeutic environment (supermarket) in which cognitive and emotionally challenging situations can be presented; both assessment and intervention modules are being developed.

Role: Co-Investigator

2006/07/01-2021/03/31

P30 AG028740, National Institute on Aging

PAHOR, MARCO (PI)

Claude D. Pepper Older Americans Independence Center (OAIC) RC1

The goal of this center grant is to provide support to investigators conducting research on sarcopenia and the preservation of function in older adults. Marsiske directs the recruitment operation of the Clinical Core.

Role: PI

Completed Research Support

1996/09/30-2013/04/30

U01 AG014276-11, National Institute on Aging (NIA)

MARSISKE, MICHAEL (PI)

ACTIVE Phase III: UF/WSU Field Site

Role: PI

2014/09/15-2016/04/30

R21 AG044862, National Institute on Aging

DING, MINGZHOU (PI)

Measuring Cognitive Fatigability In Older Adults

The goal of this study is to investigate induction, covariates, and neural signatures, of cognitive fatigability in older adults.

Role: Co-Investigator

2011/09/01-2015/11/30

U01 AG022376, National Institute on Aging

PAHOR, MARCO (PI)

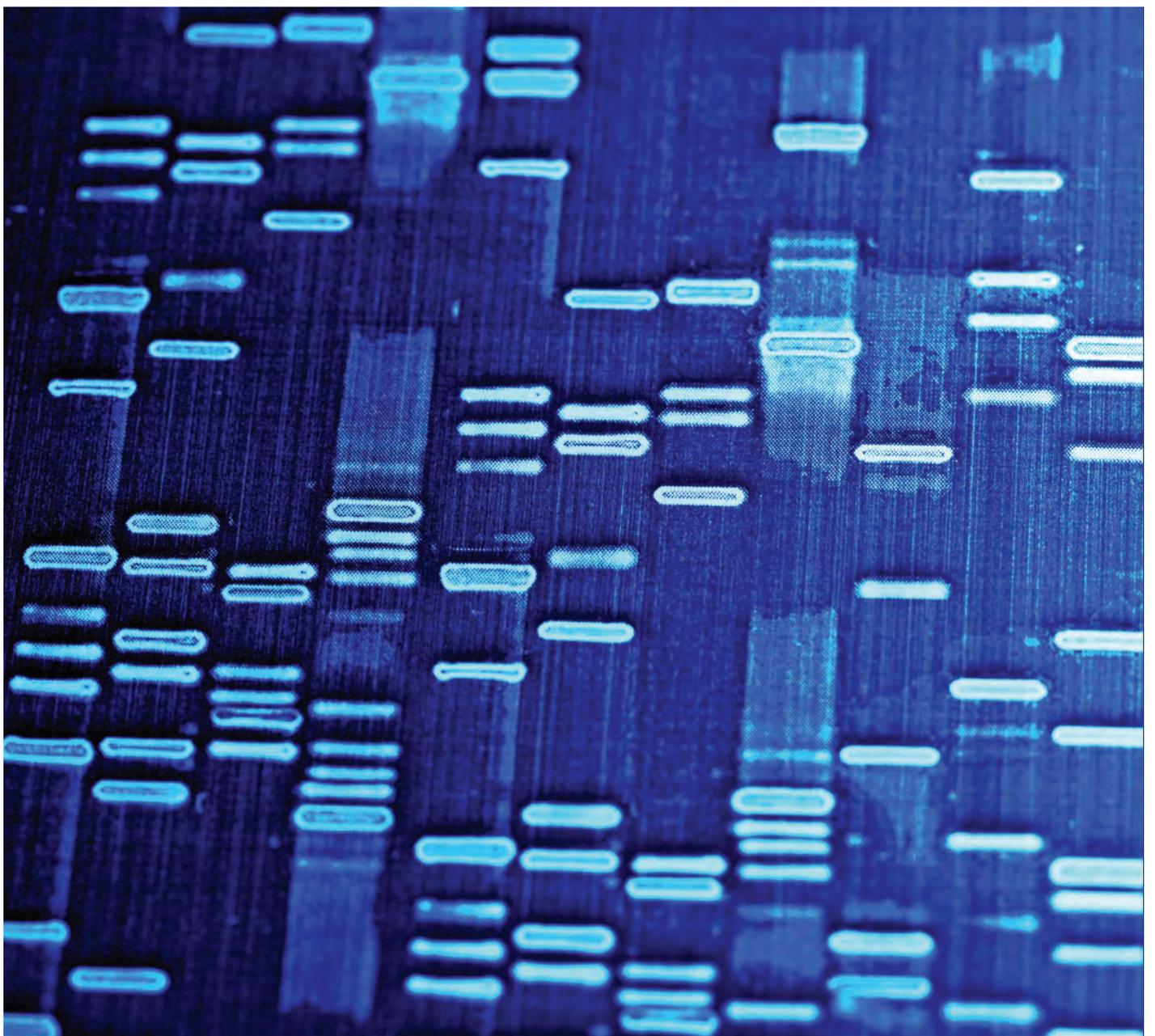
The LIFE Study

The goal of this study is to investigate the long-term effects of a physical exercise/walking intervention on the functional health of older adults.

Role: Co-Investigator

ARMIL

**Selected
Publications**



Effects of acute administration of the GABA(B) receptor agonist baclofen on behavioral flexibility in rats

B. Sofia Beas¹ · Barry Setlow^{1,2} · Jennifer L. Bizon^{1,2}

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Abstract

Rationale The ability to adjust response strategies when faced with changes in the environment is critical for normal adaptive behavior. Such behavioral flexibility is compromised by experimental disruption of cortical GABAergic signaling, as well as in conditions such as schizophrenia and normal aging that are characterized by cortical hyperexcitability. The current studies were designed to determine whether stimulation of GABAergic signaling using the GABA(B) receptor agonist baclofen can facilitate behavioral flexibility.

Methods Male Fischer 344 rats were trained in a set-shifting task in which they learned to discriminate between two response levers to obtain a food reward. Correct levers were signaled in accordance with two distinct response rules (rule 1: correct lever signaled by a cue light; rule 2: correct lever signaled by its left/right position). The order of rule presentation varied, but they were always presented sequentially, with the trials and errors to reach criterion performance on the second (set shift) rule providing the measure of behavioral flexibility. Experiments determined the effects of the GABA(B) receptor agonist baclofen (intraperitoneal, 0, 1.0, 2.5, and 4.0 mg/kg) administered acutely before the shift to the second rule.

Results Baclofen enhanced set-shifting performance. Control experiments demonstrated that this enhancement was not

simply due to improved discrimination learning, nor was it due to impaired recall of the initial discrimination rule.

Conclusions The results demonstrate that baclofen can facilitate behavioral flexibility, suggesting that GABA(B) receptor agonists may have utility for treating behavioral dysfunction in neuropsychiatric disorders.

Keywords Baclofen · GABA(B) receptor · Set shifting · Behavioral flexibility · Prefrontal cortex

Introduction

The ability to flexibly modify one's actions in response to changes in the environment is a critical aspect of normal adaptive behavior that is enabled by the prefrontal cortex (PFC). Deficits in behavioral flexibility are prevalent in psychiatric disorders such as schizophrenia and addiction and are also associated with normal aging (Beas et al. 2013; Buckner 2004; Cunha et al. 2013; D'Cruz et al. 2013; Everett et al. 2001). Impairments in behavioral flexibility can result in maladaptive perseveration on response strategies that no longer produce the desired outcome and can interfere with the ability to complete the normal activities of daily living. Despite the fact that interventions for improving behavioral flexibility could offer significant clinical benefit, no such pharmacological treatments currently exist.

Behavioral flexibility can be assessed in the laboratory using set-shifting tasks. Although the task details can vary, all involve shifting between response rules. Specifically, after acquisition of an initial response rule, that rule ceases to be reinforced and another response rule is introduced, the contingencies of which predict the correct response. Set shifting reflects the ability to inhibit responding to the initial rule and adapt responding according to the second rule. Damage to

✉ Jennifer L. Bizon
bizonj@ufl.edu

¹ Department of Neuroscience, University of Florida College of Medicine, McKnight Brain Institute, PO Box 100244, Gainesville, FL 32610-0244, USA

² Department of Psychiatry, University of Florida College of Medicine, Gainesville, FL, USA

primate dorsolateral PFC or the rodent homologue, medial PFC (mPFC), does not impede learning of the individual response rules but significantly impairs the ability to shift between rules (Birrell and Brown 2000; Bissonette and Powell 2012; Darrach et al. 2008; Demakis 2003; Dias et al. 1996; Floresco et al. 2008; Owen et al. 1991; Ragozzino 2007; Uylings et al. 2003). Beyond frank damage to PFC, behavioral flexibility is sensitive to perturbations in the balance of excitatory and inhibitory signaling within this brain region. Indeed, behavioral flexibility is impaired following a number of manipulations that disrupt GABAergic signaling, including neonatal ventral hippocampal lesions, intra-mPFC blockade of GABA(A) receptors, and genetically induced GABAergic interneuron dysfunction (Enomoto et al. 2011; Brady 2009; Cabungcal et al. 2014; Gruber et al. 2010; Lipska et al. 2003; Placek et al. 2013; Bissonette et al. 2014; Cho et al. 2015; Sparta et al. 2014). Together, these findings suggest that pharmacologically enhancing inhibition may facilitate set shifting.

Both ionotropic GABA(A) receptors and metabotropic GABA(B) receptors mediate inhibitory signaling in the PFC; however, GABA(B) receptors are of particular interest as a therapeutic target for improving PFC-supported cognition. Presynaptically, GABA(B) receptors are localized to both GABAergic and glutamatergic terminals, where they regulate neurotransmitter release. Postsynaptically, these receptors are localized to pyramidal neuron dendrites where they mediate slow inhibition and contribute to inhibitory tone (Bettler et al. 2004; McQuail et al. 2015; Wang et al. 2010). In transgenic mice with interneuron deficits, the selective GABA(B) receptor agonist baclofen normalizes pyramidal neuron hyperexcitability and restores gamma synchrony (Billingslea et al. 2014; Bortolato et al. 2007; Gandal et al. 2012; Henderson et al. 2012; Qin et al. 2015; Silverman et al. 2015). Moreover, drugs targeting GABA(B) receptors (including baclofen) enhance several aspects of cognitive function in preclinical animal models, including some that depend on the PFC (Banuelos et al. 2014; Beas et al. 2016; Lasarge et al. 2009; Qin et al. 2015; Zhang et al. 2015). Finally, baclofen has a strong safety profile, as it is used clinically as a treatment for spasticity, and has been explored as a treatment for addiction (Colombo et al. 2004; Franklin et al. 2009; Garbutt et al. 2010; Liu and Wang 2015; Margetis et al. 2014; Morley et al. 2014).

The goal of this study was to determine whether pharmacological stimulation of GABA(B) receptors with baclofen can enhance behavioral flexibility in a set-shifting task (Beas et al. 2013; Floresco et al. 2008). Rats initially learned to discriminate between response levers on the basis of one of two discrimination rules. After acquiring one of the rules, the rats were “shifted” to the other rule following administration of baclofen or vehicle. We hypothesized that baclofen would facilitate behavioral flexibility as evidenced by enhanced acquisition of the second rule.

Methods

Subjects

Male Fischer 344 rats ($N=145$ total, 5 months of age upon arrival) were obtained from Charles River and single-housed in the AAALAC-accredited vivarium facility in the McKnight Brain Institute Building at the University of Florida, in accordance with University of Florida IACUC and NIH guidelines. The facility was maintained at 25 °C with a 12-h light/dark cycle (lights on at 0800) with free access to food and water except as noted below. Prior to the start of experiments, rats were handled at least three times to minimize stress during testing. In addition, on the 2 days prior to drug injections, the rats were subjected to the handling procedures used during intraperitoneal (i.p.) injections (although no actual injections were given).

Apparatus

All testing was conducted in eight identical behavioral test chambers (30.5 × 25.4 × 30.5 cm, Coulbourn Instruments) composed of stainless steel front and back walls and transparent Plexiglas side walls. The floor was made of steel rods (0.4 cm in diameter) spaced 1.1 cm apart. A food pellet delivery trough was placed 2 cm above the floor in the center of the front wall. The food trough was equipped with a 1.12-W lamp for illumination and a photobeam for recording head entries. On each side of the trough, a retractable lever was located 11 cm above the floor, and a cue lamp (1.12 W) was placed 3.8 cm above each lever. Each chamber was located inside a sound-attenuating cubicle. An additional 1.12-W house light was mounted near the top of the rear wall of the cubicle. Food rewards consisted of individual 45-mg grain-based food pellets (PJAI, Test Diet) delivered into the food trough following a correct response. An infrared activity monitor was positioned above each test chamber to monitor locomotor activity. Test chambers were controlled by a computer equipped with Graphic State 3.01 software (Coulbourn Instruments).

Experimental procedures

Experiment 1: effects of systemic baclofen administration on set shifting from visual cue to left/right discrimination

Behavioral shaping The design of the set-shifting task was based on that used by Floresco et al. (2008) and Beas et al. (2013). Prior to the start of testing, rats ($n=45$) were reduced to 85 % of their free feeding weights over the course of 5 days and maintained at these weights for the duration of the experiment. Rats progressed through four stages of shaping prior to the start of the set-shifting task, with new stages beginning the day immediately after completion of the previous

stage. On the day prior to Shaping stage 1, each rat was given five 45 mg food pellets in its home cage to reduce neophobia to the food reward. Shaping stage 1 consisted of a 64-min session of magazine training, involving 38 deliveries of a single food pellet with an inter-trial interval (ITI) of 100 ± 40 s. Shaping stage 2 consisted of lever press training, in which a single lever (left or right, counterbalanced across groups) was extended and a press resulted in delivery of a single food pellet. After reaching a criterion of 50 lever presses in 30 min, rats were trained on the opposite lever using the same procedures.

Shaping stage 3 consisted of 90 trials designed to train rats to press the levers upon their insertion into the test chamber. Each 20-s trial began with illumination of the house light and insertion of a single lever (left or right, randomly selected within each pair of trials) into the test chamber where it remained for a maximum of 10 s. A response on the lever in this time window resulted in lever retraction, delivery of a food pellet, and continued illumination of the house light for an additional 4 s. If a rat failed to respond on the lever within 10 s, the lever was retracted and the house light turned off, and the trial was scored as an omission. Rats received a minimum of four daily sessions in this stage and were trained until reaching criterion performance of fewer than ten omissions out of the 90 trials.

Shaping stage 4 was designed to determine each rat's side bias (inherent preference for one lever over the other). Each trial consisted of multiple phases. In the first phase, the house light was illuminated and both levers were inserted into the test chamber. A response on either lever resulted in retraction of both levers and delivery of a single food pellet. In the second phase of a trial, both levers were again inserted, but only a response on the lever opposite to that chosen in the first phase was rewarded. A response on the same lever chosen in the first phase (i.e., "incorrect") resulted in the levers being retracted and the house light being extinguished. After a "correct" response in this second phase of a trial, a new trial was initiated, whereas after an incorrect response, the second phase was repeated until rats made a correct response. The session ended after a total of 45 completed trials. The side associated with the greatest number of total responses was considered a rat's "biased" side.

Visual cue discrimination Following shaping stage 4, rats were trained to press the lever associated with a visual cue (light). In this discrimination (Fig. 1a), illumination of a cue light over one of the two response levers signaled the correct response, irrespective of the left/right position of the cue. Each 20-s trial began with illumination of one of the cue lights (left or right, randomly selected in each pair of trials). After 3 s, the house light was illuminated and both levers were inserted into the chamber (the cue light remained illuminated while the levers were extended). A press on the lever corresponding to

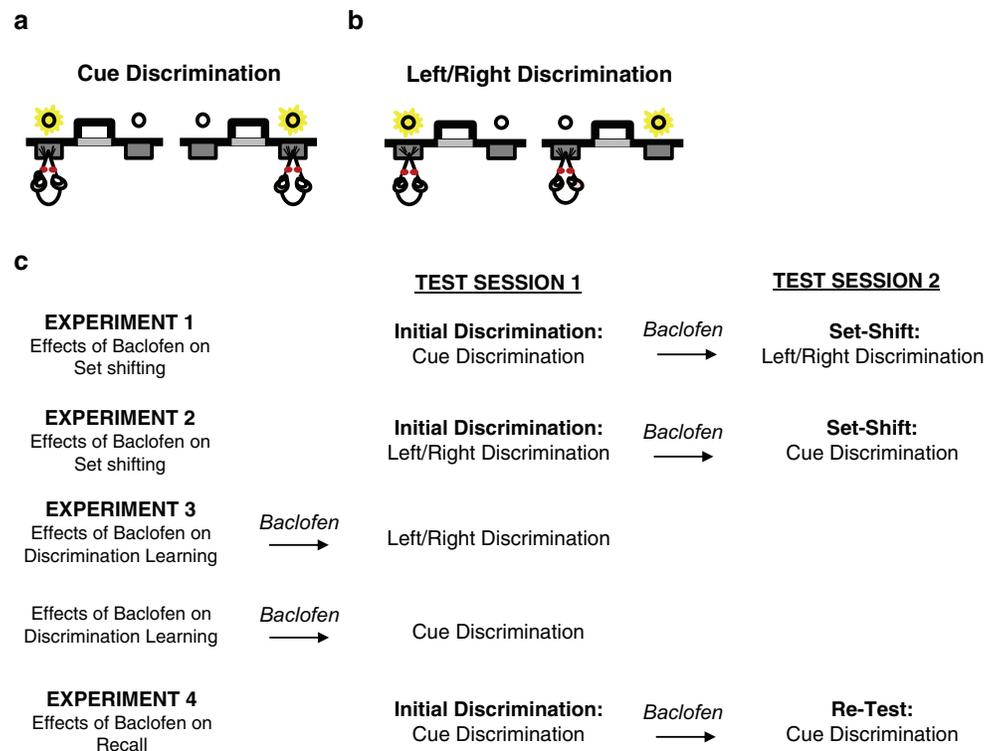
the cue light (a correct response) resulted in the house light remaining on for 4 s, during which time, the levers were retracted, the cue light was extinguished, and a single food pellet was delivered. A response on the opposite lever (an incorrect response) or failure to respond within 10 s (omission) resulted in retraction of both levers and all lights being extinguished. Rats were considered to have acquired the task upon reaching criterion performance of eight consecutive correct trials (and at least 30 total trials, excluding omissions), with the maximum number of trials per session set at 120. If rats failed to acquire the task in a single session, they received additional sessions on subsequent days.

Left/right discrimination (set shift) After reaching criterion performance on the visual discrimination, rats were tested the next day on the left/right discrimination (i.e., the set shift; Fig. 1b). In this condition, rats had to ignore the visual cue and instead choose the left or right lever (whichever was not their biased side as determined in shaping stage 4). Hence, accurate performance required rats to "shift" their responding away from the visual cue and toward the left/right position of the lever. Beyond the change in reward contingencies, trials were identical to those in the visual cue discrimination in all other respects (i.e., on each trial, both levers were presented, with the cue light illuminated over one lever, randomly selected in each pair of trials). Rats were considered to have acquired the task upon reaching criterion performance of eight consecutive correct trials, excluding omissions. The maximum number of trials per session was set at 120 and rats that failed to acquire the task in a single session received additional sessions on subsequent days.

Drug administration Experiment 1 (Fig. 1c) evaluated the effects of baclofen or vehicle on set shifting. Rats were assigned to one of the four drug conditions on the basis of their initial (visual cue) discrimination performance, such that the groups had approximately equivalent performance. Rats received an i.p. injection (1.0 ml/kg) of either the selective GABA(B) receptor agonist baclofen (1.0 ($n=11$), 2.5 ($n=9$), or 4.0 mg/kg ($n=6$); Tocris, Ballwin, MO) or 0.9 % saline vehicle ($n=19$) 20 min prior to set-shifting test sessions.

Data analyses Data files were exported from Graphic State software and compiled using a custom macro written for Microsoft Excel (Dr. Jonathan Lifshitz, University of Kentucky). Statistical analyses were conducted using SPSS 22.0. The total numbers of trials and errors required to achieve criterion on the initial discrimination and on the set shift (excluding trial omissions) were used as indices of performance. As the task design involved presentation of the same stimuli during both the initial discrimination and the set shift, the types of errors were also examined. Specifically, comparisons

Fig. 1 Schematic of the set-shifting task and experimental designs. The set-shifting task employed two types of discrimination: visual cue discrimination and left/right discrimination. **a** During the visual cue discrimination, rats were required to respond on the lever illuminated by a cue light, irrespective of its left/right location. **b** During the left/right discrimination, rats were required to respond on the lever in a particular location (e.g., as in the illustration, always press the left lever), irrespective of whether that lever was illuminated by the cue light. **c** Outline of each of the four experiments



between drug conditions were performed separately for errors that involved responses corresponding to previously reinforced choices (the cue light was incongruent with the correct lever location and the rat chose based on the previous visual discrimination rule) and for errors that were never reinforced (the cue light and spatial location were congruent, and the rat's choice was not correct according to either discrimination rule (Floresco et al. 2008; Ragozzino et al. 2002). In addition to these measures, numbers of omitted trials, response latencies (latencies to press one of the two levers after they were inserted into the chamber), and locomotor activity during ITIs were recorded. Comparisons between groups were conducted using one-way ANOVA and LSD post hoc tests when warranted. For all analyses, p values less than 0.05 were considered significant.

Experiment 2: effects of baclofen administration on set shifting from left/right to visual cue discrimination

Experiment 2 was designed to determine whether baclofen enhanced set-shifting performance when rats were required to shift from a left/right to a visual cue discrimination (Fig. 1c). A naïve cohort of rats ($n=36$) was initially shaped as described in experiment 1. Following shaping, rats were trained on a modified version of the left/right discrimination task, which was identical to that described in experiment 1 except that a minimum of 30 trials (in addition to performing eight consecutive correct trials) was required to achieve criterion performance. As in experiment 1, this first session ended

when rats reached criterion performance. These rats also received an additional session of 120 trials of left/right discrimination performance on the day after reaching criterion. This session was conducted to ensure that all rats developed an attentional “set” prior to the set shift, as pilot studies suggested that initial learning of the left/right discrimination was too rapid for rats to develop a robust bias for this rule. Rats were assigned to one of four drug conditions on the basis of their initial (left/right) discrimination performance, such that the groups had approximately equivalent performance. On the following day, rats received an injection of either 0.9 % saline vehicle ($n=10$) or one of three doses of baclofen (1.0 ($n=10$), 2.5 ($n=10$), or 4.0 ($n=6$) mg/kg), followed by testing in the visual cue discrimination.

Experiment 3: effects of baclofen administration on discrimination learning

This experiment was designed to test whether baclofen enhances left/right or visual cue discrimination learning in the absence of a rule shift (Fig. 1c). Two naïve cohorts of rats ($n=23$ and $n=18$) underwent shaping procedures as described in experiment 1. Following completion of shaping, rats were assigned to either the left/right or visual cue discrimination task and randomly assigned to drug conditions. Rats were given an i.p. injection of either 0.9 % saline vehicle or baclofen (1.0 or 2.5 mg/kg) prior to testing on acquisition of the left/right or visual cue discrimination task (i.e., in the absence of learning a prior discrimination rule). Group sizes

were $n=8$, 7, and 8 for groups in the left/right discrimination and $n=6$, 6, and 6 for groups in the visual cue discrimination, respectively.

Experiment 4: effects of baclofen on retrieval of a previously learned discrimination rule

This experiment was designed to test the possibility that baclofen impairs recall of a previously learned response rule (Fig. 1c). A naïve cohort of rats ($n=23$) was shaped and trained on the visual cue discrimination task as in experiment 1. After reaching criterion performance on the visual cue discrimination, rats were assigned to drug conditions such that the groups had approximately equivalent performance. The following day, rats received an i.p. injection of either 0.9 % saline vehicle ($n=8$) or baclofen (1.0 ($n=7$) or 2.5 mg/kg ($n=8$)) and re-tested in the same visual cue discrimination task.

Results

Experiment 1: effects of systemic baclofen administration on set shifting from visual cue to left/right discrimination

Comparisons of performance on the initial visual cue discrimination confirmed that there were no differences between groups in the number of trials needed to reach criterion (Fig. 2a). On the set shift (left/right discrimination), comparisons of performance following vehicle or baclofen administration revealed significant drug effects on both trials ($F(3, 44)=4.74$, $p<.05$; Fig. 2b) and errors ($F(3, 44)=5.73$, $p<.05$; Fig. 2c) to criterion. Post hoc comparisons showed that on both measures, the 2.5 and 4.0 mg/kg doses of baclofen significantly enhanced performance compared to vehicle ($ps<0.05$). Because the task design involved explicit presentation of the same set of stimuli during both the initial

discrimination and the set shift, the nature of the errors committed during acquisition of the set shift was further investigated. As shown in Fig. 2c, the analysis of error type revealed a main effect of drug on previously reinforced errors ($F(3, 44)=6.32$, $p<.05$), with post hoc comparisons showing that both the 2.5 and 4.0 mg/kg dose groups performed significantly better than the vehicle group. In contrast, there were no differences between drug groups in the number of never-reinforced errors. Considered together, these data suggest that systemic baclofen administration enhances behavioral flexibility.

Experiment 2: systemic baclofen administration enhances set shifting from left/right to visual cue discrimination

To determine whether the results of experiment 1 were independent of the type of discrimination learning involved, a new cohort of rats ($n=36$) was shifted in the opposite direction from the cohort in experiment 1. Specifically, rats were initially trained on the left/right discrimination task and, upon reaching criterion performance, received vehicle or baclofen followed by a shift to the visual cue discrimination task. Comparison of performance on the initial, left/right discrimination revealed no group differences in the number of trials required to reach criterion (Fig. 3a). As in experiment 1, comparison of performance on the set shift (visual cue discrimination) following vehicle or baclofen administration revealed significant drug effects on both trials ($F(3, 32)=4.22$, $p<0.05$; Fig. 3b) and errors ($F(3, 32)=4.91$, $p<0.05$; Fig. 3c) to criterion. Post hoc comparisons showed that on both measures, the 1.0 and 2.5 mg/kg baclofen groups performed significantly better than the vehicle group ($ps<0.05$). An error type analysis conducted as in experiment 1 revealed a main effect of drug on previously reinforced errors ($F(3, 32)=4.02$, $p<0.05$), but no effect on never-reinforced errors.

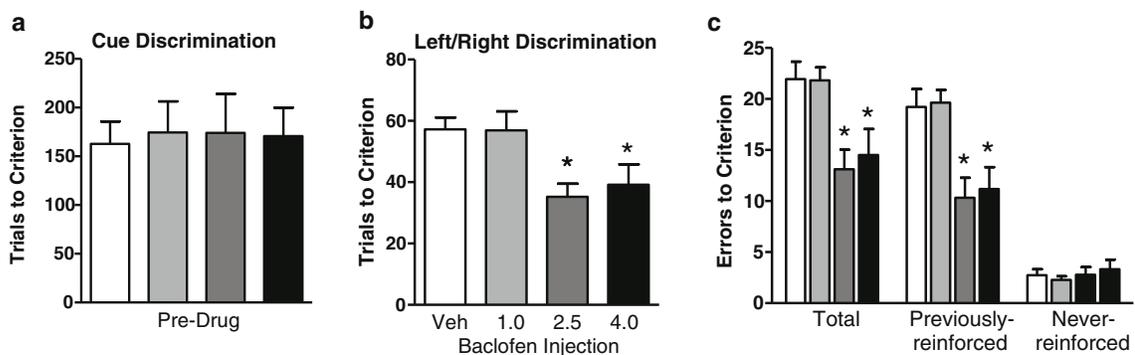


Fig. 2 Experiment 1: baclofen facilitated set shifting from visual cue to left/right discrimination learning. **a** Trials to criterion on the visual cue (initial) discrimination. **b** Trials to criterion on the left/right (set shift) discrimination following vehicle or baclofen (1, 2.5, or 4.0 mg/kg)

administration. **c** Errors to criterion on the left/right (set shift) discrimination shown for all errors total and broken out by previously- and never-reinforced error types. Data are expressed as mean + SEM. * $p<0.05$ compared to vehicle

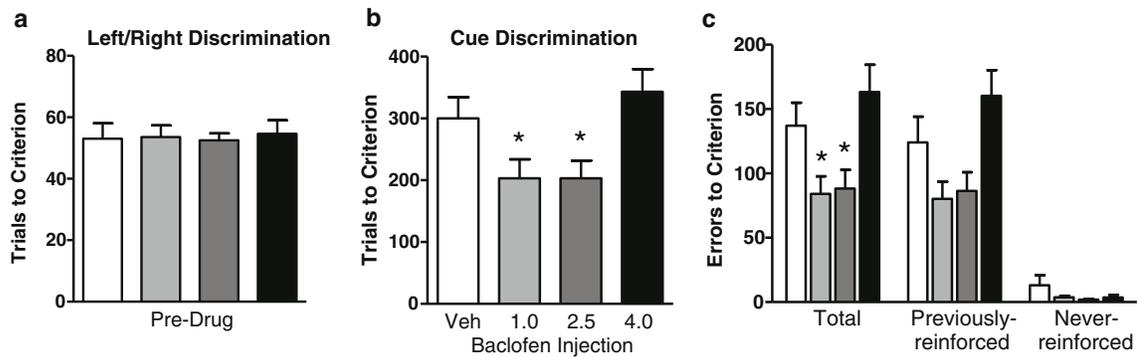


Fig. 3 Experiment 2: baclofen facilitated set shifting from left/right to visual cue discrimination learning. **a** Trials to criterion on the left/right (*initial*) discrimination following vehicle or baclofen (1.0, 2.5, or 4.0 mg/kg)

administration. **c** Errors to criterion on the visual cue (*set shift*) discrimination shown for all errors total and broken out by previously- and never-reinforced error types. Data are expressed as mean + SEM. * $p < 0.05$ compared to vehicle

Experiment 3: effects of baclofen administration on discrimination learning

Experiments 1 and 2 showed that baclofen enhanced acquisition of both a left/right and a visual cue discrimination rule in the context of a set shift (i.e., following learning of a previous rule). These data suggest that baclofen enhances behavioral flexibility; however, an alternative explanation is that baclofen more generally enhances discrimination learning. To assess this possibility, the effects of baclofen on acquisition of the left/right and visual discrimination rules were tested in the absence of prior rule learning. Comparisons of performance revealed no effects of drug condition on the number of trials to reach criterion on either the left/right (Fig. 4a) or visual cue (Fig. 4b) discrimination, suggesting that the effects of baclofen on set shifting observed in experiments 1 and 2 were not the result of non-specific enhancement of discrimination learning.

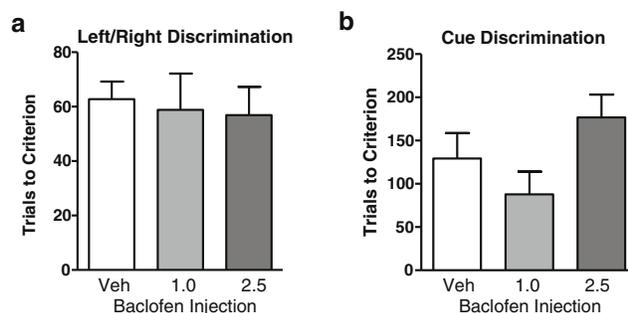


Fig. 4 Experiment 3: baclofen had no effect on acquisition of left/right or visual cue discriminations. **a** Trials to criterion on acquisition of the left/right discrimination following vehicle or baclofen (1.0 or 2.5 mg/kg) administration. **b** Trials to criterion on acquisition of the visual cue discrimination following vehicle or baclofen (1.0 or 2.5 mg/kg) administration. Data are expressed as mean + SEM

Experiment 4: effects of baclofen on retrieval of a previously learned discrimination rule

As baclofen can impair memory in certain contexts (Arolfo et al. 1998; Castellano et al. 1989; Stackman and Walsh 1994), it is possible that the enhanced set-shifting performance observed in experiments 1 and 2 resulted from effects of baclofen on recall of the initial discrimination rule. Specifically, impaired recall of the initial rule would be expected to minimize interference from the previously learned contingencies, thereby facilitating learning of the second rule. To test this possibility, rats were trained on the visual cue discrimination task. Upon reaching criterion, rats were assigned to drug groups as in experiment 1 and then re-tested on the visual discrimination task on the following day. Comparisons of performance revealed no differences between drug groups in the number of trials required to initially acquire the visual discrimination task (Fig. 5a). Following systemic administration of vehicle or

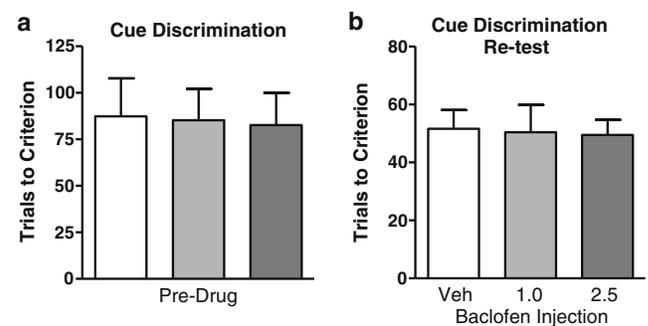


Fig. 5 Experiment 4: baclofen had no effect on recall of visual cue discrimination learning. **a** Trials to criterion on acquisition of the visual cue discrimination. **b** Trials required to re-establish criterion performance on re-test of the visual cue discrimination following vehicle or baclofen (1.0 or 2.5 mg/kg) administration. Data are expressed as mean + SEM

baclofen, comparisons of performance revealed no differences between drug groups in trials to reach criterion during the re-test of the visual discrimination task (Fig. 5b). These results show that baclofen does not impair recall of a previously learned response rule.

Effects of baclofen on trial omissions, locomotor activity, and response latency

In addition to choice accuracy described above, other measures of task performance were assessed with the intent of determining whether non-specific effects of baclofen could account for the drug's enhancement of set shifting. As reported below and in Table 1, baclofen did influence trial omissions, response latency, and locomotor activity in some experiments; however, these effects were inconsistent across experiments and could not account for baclofen's effects on set shifting. In experiment 1 (visual cue to left/right shift), baclofen produced an increase in the number of omitted trials ($F(3, 44)=4.53$, $p<0.05$) and mean latency to lever press ($F(3, 44)=5.26$, $p<0.05$), but no significant effects of baclofen were observed in experiment 2 (left/right to visual cue shift) on any of these measures. In experiment 3, baclofen produced a significant

increase in response latency ($F(2, 20)=11.92$, $p<0.05$) and a decrease in locomotor activity ($F(2, 20)=9.40$, $p<0.05$) on the left/right discrimination but had no significant effects on the number of trial omissions. In contrast, baclofen produced a significant increase in trial omissions ($F(2, 17)=10.02$, $p<0.05$) on the visual cue discrimination in experiment 3 but no changes in response latency or locomotor activity. Baclofen had no effect on any of the measures in experiment 4.

Discussion

Baclofen is used clinically for treatment of muscle spasticity associated with multiple sclerosis and cerebral palsy (Baker et al. 2014; Overgard et al. 2015; Rekan and Gronning 2011). More recently, it has been investigated for treatment of addiction and autistic disorders. (Kahn et al. 2009; Muzyk et al. 2012); (Berry-Kravis et al. 2012; Erickson et al. 2014). It is notable that these latter conditions are characterized by behavioral inflexibility, including impairments in laboratory set-shifting tasks (Casten et al. 2011; Maes et al. 2011; Van der Molen et al. 2012). Based on this prior clinical work, the goal

Table 1 Effects of baclofen on number of omitted trials, locomotor activity, and latency to respond at the lever

Experiment	Omitted trials	Locomotion (locomotor units/TTI)	Latency
Experiment 1			
Vehicle	3.05 (1.78)	6.49 (1.72)	1.71 (0.38)
1.0 mg/kg baclofen	3.36 (2.77)	4.22 (0.51)	1.11 (0.74)
2.5 mg/kg baclofen	20.55 (7.39)*	2.24 (0.79)	3.47 (0.62)*
4.0 mg/kg baclofen	31.17 (17.79)*	0.95 (0.14)	3.93 (1.29)*
Experiment 2			
Vehicle	9.90 (3.50)	4.18 (0.95)	1.72 (0.20)
1.0 mg/kg baclofen	3.40 (1.60)	5.32 (0.61)	1.18 (0.17)
2.5 mg/kg baclofen	11.00 (7.99)	3.58 (0.70)	1.56 (0.32)
4.0 mg/kg baclofen	11.67 (4.63)	3.41 (0.43)	1.64 (0.16)
Experiment 3 (left/right discrimination)			
Vehicle	6.12 (2.03)	7.00 (1.18)	2.08 (0.41)
1.0 mg/kg baclofen	1.18 (0.52)	6.48 (1.49)	1.76 (0.16)
2.5 mg/kg baclofen	26.62 (14.77)	1.03 (0.42)*	4.42 (0.55)*
Experiment 3 (visual cue discrimination)			
Vehicle	1.33 (0.61)	3.22 (0.53)	1.69 (0.25)
1.0 mg/kg baclofen	0.33 (0.21)	3.91 (0.67)	1.70 (0.10)
2.5 mg/kg baclofen	4.17 (0.87)*	2.03 (0.39)	1.90 (0.25)
Experiment 4			
Vehicle	8.50 (8.50)	8.25 (1.63)	1.24 (0.22)
1.0 mg/kg baclofen	0.28 (0.28)	7.05 (1.53)	1.20 (0.21)
2.5 mg/kg baclofen	0.12 (0.12)	5.25 (1.09)	1.23 (0.17)

Values represent means (SEMs)

*Significant difference from vehicle as indicated by post hoc comparisons

of the current studies was to test the utility of baclofen to specifically enhance behavioral flexibility. Indeed, impaired flexibility accompanies many neuropsychiatric diseases and can contribute to maladaptive perseverative behaviors and an inability to readily accomplish the activities of daily living (D’Cruz et al. 2013; Enomoto et al. 2011; Floresco et al. 2009; Gass et al. 2014; George et al. 2015; Gruber et al. 2010; Placek et al. 2013). The current experiments demonstrate that acute systemic baclofen administration facilitates behavioral flexibility in rats and suggest that this drug may be of utility for clinical conditions in which behavioral flexibility is impaired.

Experiments 1 and 2 used a set-shifting task to demonstrate that systemic baclofen administration enhances behavioral flexibility. This task required the effective inhibition of an initial discrimination rule and an adaptation to response contingencies associated with a second (set shift) rule. Rats given baclofen required fewer trials and errors to reach criterion performance on the second rule compared to rats given vehicle. These data are consistent with the interpretation that baclofen enhanced the ability to shift effectively from one response strategy to another. Notably, however, an alternative explanation for this pattern of performance is that baclofen directly enhanced learning of the second rule (left/right lever discrimination in experiment 1 and visual cue discrimination in experiment 2), rather than facilitating behavioral flexibility per se. Indeed, in rodents, baclofen is reported to enhance performance on delayed response and radial arm maze tasks and to reverse methamphetamine-induced deficits in object recognition (Arias et al. 2009; Escher and Mittleman 2004; Levin et al. 2004). Experiment 3 addressed this possibility by evaluating the effects of baclofen on acquisition of the left/right and visual cue discrimination in rats that had not already learned a competing response rule (i.e., when task acquisition did not require a rule shift). Under these conditions, baclofen had no effect on acquisition of either rule, demonstrating that this drug does not broadly enhance either type of discrimination learning. The distinct effects of baclofen in experiments 1 and 2 vs. experiment 3 are consistent with findings from prior behavioral pharmacology and lesion/inactivation studies demonstrating that different neural mechanisms mediate learning of an initial discrimination rule compared to learning to shift from one rule to another. Systemic administration of antagonists at muscarinic cholinergic and 5-HT₇ receptors (Chen et al. 2004; Nikiforuk 2012), as well as acute or chronic stress (Bondi et al. 2008; Butts et al. 2013), alter set-shifting performance without affecting initial discrimination learning. Similarly, lesions or inactivation of mPFC impair set shifting but not initial discrimination learning (Birrell and Brown 2000; Bissonette and Powell 2012; Bissonette et al. 2013; Floresco et al. 2008; Ragozzino 2007). These latter studies suggest that the mPFC is a potential site of action for the enhancing effects of baclofen on set

shifting; indeed, we recently showed that intra-mPFC baclofen administration enhances set-shifting performance in aged Fischer 344 rats in a manner similar to that in experiment 1 (Beas et al. 2016). Future experiments in which baclofen is administered directly into the young rat mPFC prior to set shifting would be useful for confirming the site of action for the behavioral enhancement reported here.

The fact that baclofen facilitated set shifting irrespective of the order of the presentation of discrimination rules (experiments 1 and 2) provides support for the conclusion that its enhancing effects are not unique to a particular set of discrimination contingencies but instead reflect improved behavioral flexibility (i.e., an enhanced ability to shift from one rule to another). It is notable, however, that while baclofen enhanced set shifting in both conditions, the most effective doses differed somewhat depending on the direction of the shift. Both 2.5 and 4.0 mg/kg baclofen improved performance of rats shifted from the visual cue to left/right discrimination, whereas 1.0 and 2.5 mg/kg baclofen improved performance of rats shifted from the left/right to visual cue discrimination. Importantly, pharmacological manipulations that enhance cognitive performance almost always, if not always, do so in an inverted U-shaped dose-response curve, such that doses that are too low have no effect and doses that are too high may also have no effect or even impair performance (Arnsten et al. 2015; Wood et al. 2014). The peaks of these curves (i.e., the most effective doses) are influenced by a variety of factors including the specific task demands, stress levels, and animal strain. In the current study, the differences in the effective doses of baclofen may relate to the relative difficulty of the two discriminations employed. A previous study showed that increasing the difficulty of the second (shift) discrimination rendered performance more sensitive to the effects of mPFC inactivation (Floresco et al. 2008). In a similar manner, the greater difficulty of the visual cue discrimination compared to the left/right discrimination in the present study (compare vehicle group performance in Figs. 2b and 3b) may have rendered performance more sensitive to the effects of a lower dose of baclofen.

Accurate performance on the set-shifting task requires not only acquisition of a new response rule but also effective inhibition of a previously learned response rule. Given that baclofen can impair memory in a variety of contexts (Arolfo et al. 1998; Castellano et al. 1989; Stackman and Walsh 1994), one explanation for its enhancing effects on set shifting is that it may interfere with recall of the initial discrimination rule. Impaired recall of the initial rule would be expected to facilitate set shifting, as there would be less interference from this prior learning during acquisition of the second rule. Experiment 4 addressed this possibility by evaluating the effects of baclofen on retention of an initial (visual cue) discrimination rule at the same time point at which the second rule was introduced in the set-shifting task in experiment 1.

Baclofen had no effect on performance in this context, suggesting that the enhancing effects of baclofen on set shifting cannot be attributed to impaired recall of the initial discrimination rule.

Baclofen can induce sedation and reduced locomotor activity in rodents (Beveridge et al. 2013; Li et al. 2013). Consistent with such findings, baclofen reduced locomotor activity and increased response latencies and trial omissions in some of the experiments (Table 1). For several reasons, however, it is unlikely that these effects account for baclofen's actions on set shifting. First, because the task employed a discrete-trial procedure, an increase in trial omissions or response latencies would not be expected to influence choice accuracy (which reflected whether trials were correct or incorrect rather than the number of correct trials, as omitted trials were excluded when calculating trials and errors to criterion). Second, the effects of baclofen on locomotion, response latencies, and trial omissions were inconsistent across experiments, suggesting that its non-specific actions on task performance were relatively weak. Most importantly, however, baclofen had no effect on locomotion, response latencies, or trial omissions in experiment 2, in which it robustly enhanced set shifting. This finding suggests that the effects of baclofen on these measures were unrelated to its enhancing effects on set shifting.

It is notable that some previous studies have reported that Fischer 344 rats are more anxious in comparison to other rat strains (Faraday 2002; van der Staay et al. 2009). As baclofen has been used clinically to reduce anxiety (Knapp et al. 2007; Li et al. 2013; Morley et al. 2014) and stress/anxiety can modulate behavioral flexibility (Butts et al. 2013; Hurtubise and Howland 2016), one interesting, albeit speculative, hypothesis is that the enhancement in behavioral flexibility produced by baclofen in the current study is via its anxiolytic properties. While this represents a fertile avenue of future investigation, it is notable that baseline (vehicle) Fischer 344 set-shifting performance in the current study was actually slightly better than in a previous study from our lab using identical task parameters in Long-Evans rats (Shimp et al. 2015). As the Long-Evans strain is reported to be less anxious than others (Turner and Burne 2014), it is unlikely that excessive anxiety in the Fischer 344 strain was the sole mediating factor for the enhancing effects of baclofen on behavioral flexibility observed here.

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PREFRONTAL CORTICAL GABAERGIC SIGNALING AND IMPAIRED BEHAVIORAL FLEXIBILITY IN AGED F344 RATS

B. S. BEAS,^a J. A. MCQUAIL,^a C. BAÑUELOS,^a
B. SETLOW^{a,b,c} AND J. L. BIZON^{a,b,*}

^a Department of Neuroscience, University of Florida College of Medicine, Gainesville, FL, United States

^b Department of Psychiatry, University of Florida College of Medicine, Gainesville, FL, United States

^c Department of Psychology, University of Florida, Gainesville, FL, United States

Abstract—The prefrontal cortex (PFC) is critical for the ability to flexibly adapt established patterns of behavior in response to a change in environmental contingencies. Impaired behavioral flexibility results in maladaptive strategies such as perseveration on response options that no longer produce a desired outcome. Pharmacological manipulations of prefrontal cortical GABAergic signaling modulate behavioral flexibility in animal models, and prefrontal cortical interneuron dysfunction is implicated in impaired behavioral flexibility that accompanies neuropsychiatric disease. As deficits in behavioral flexibility also emerge during the normal aging process, the goal of this study was to determine the role of GABAergic signaling, specifically via prefrontal cortical GABA(B) receptors, in such age-related deficits. Young and aged rats were trained in a set shifting task performed in operant chambers. First, rats learned to discriminate between two response levers to obtain a food reward on the basis of a cue light illuminated above the correct lever. Upon acquisition of this initial discrimination, the contingencies were shifted such that rats had to ignore the cue light and respond on the levers according to their left/right positions. Both young and aged rats acquired the initial discrimination similarly; however, aged rats were impaired relative to young following the set shift. Among aged rats, GABA(B) receptor expression in the medial prefrontal cortex (mPFC) was strongly correlated with set shifting, such that lower expression was associated with worse performance. Subsequent experiments showed that intramPFC administration of the GABA(B) receptor agonist

baclofen enhanced set shifting performance in aged rats. These data directly link GABAergic signaling via GABA(B) receptors to impaired behavioral flexibility associated with normal aging.

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Key words: aging, prefrontal cortex, behavioral flexibility, GABA(B) receptors, baclofen, rat.

INTRODUCTION

Aging results in a decline in prefrontal cortical-dependent cognitive capacities, which include behavioral flexibility or the ability to modify behavior in accord with changes in environmental contingencies. A loss of flexibility at advanced ages can result in perseverative behavioral strategies that can interfere with an individual's ability to complete activities of daily living and contribute to a loss of personal independence. Understanding the neurobiological mechanisms that underlie age-related behavioral flexibility deficits could provide insight into potential therapeutic targets to promote healthy cognitive aging.

Across species, behavioral flexibility can be assessed using “set shifting” tasks. Such tasks vary in design but typically involve subjects learning an initial response strategy or rule that is then followed by an unsignaled “shift” to a second response rule. The primary measure of behavioral flexibility is how adeptly the subject is able to inhibit responses to the initial rule and behave in accordance with the second rule. Extensive evidence demonstrates that set shifting performance is critically dependent on the dorsolateral prefrontal cortex (PFC) in primates, or the rodent homolog, medial prefrontal cortex (mPFC; Owen et al., 1991; Dias et al., 1996; Birrell and Brown, 2000; Demakis, 2003; Uylings et al., 2003; Ragozzino, 2007; Darrach et al., 2008; Floresco et al., 2008; Bissonette and Powell, 2012). In particular, behavioral flexibility appears sensitive to changes in prefrontal cortical GABAergic signaling and shifts in the normal balance of excitation and inhibition in this brain region. For example, intra-mPFC administration of the GABA(A) receptor antagonist bicuculline can impair set shifting performance in young rats (Enomoto et al., 2011). In addition, experimental disruption of prefrontal cortical GABAergic interneurons impairs set shifting as

*Corresponding author. Address: Department of Neuroscience, University of Florida College of Medicine, McKnight Brain Institute, P.O. Box 100244, Gainesville, FL 32610-0244, United States. Tel: +1-352-294-5149 (O); fax: +1-352-265-7983.

E-mail addresses: sofiabeas@ufl.edu (B. S. Beas), jmcquail@ufl.edu (J. A. McQuail), cristina.banuelos@nih.gov (C. Bañuelos), setlow@ufl.edu (B. Setlow), bizonj@ufl.edu (J. L. Bizon).

Abbreviations: ANOVA, analysis of variance; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid; GABA, gamma-aminobutyric acid; GABA(B)R, GABA(B) receptor; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic-acid, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid); ITI, inter-trial interval; mPFC, medial prefrontal cortex; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PFC, prefrontal cortex; TBS, Tris-buffered saline; TTC, trials to criteria.

well as other forms of behavioral flexibility in mice (Jacobson et al., 2006; Bissonette et al., 2012, 2014; Cho et al., 2015). These data are consistent with those from neuropsychiatric diseases such as schizophrenia, which are accompanied by both interneuron dysfunction and inflexible behavior (Everett et al., 2001; Hashimoto et al., 2008; Maldonado-Aviles et al., 2009; Gonzalez-Burgos et al., 2011; Lewis et al., 2012). Indeed, set shifting impairments are prominent in rodent models of schizophrenia that produce cortical hyperexcitability, including developmental exposure to NMDA receptor antagonists and neonatal ventral hippocampal lesions (Stefani and Moghaddam, 2005; Homayoun and Moghaddam, 2007; Brady, 2009; Gruber et al., 2010; O'Donnell, 2012; Placek et al., 2013; Ryan et al., 2013).

It is becoming increasingly clear that prefrontal cortical GABAergic signaling is dysregulated in advanced aging and that altered GABA signaling contributes to age-related cognitive decline (Bories et al., 2013; Baquelos et al., 2014; McQuail et al., 2015). GABAergic interneurons decline in number in the rat mPFC (Stranahan et al., 2012), and the density of inhibitory synapses (i.e., symmetric) is reduced in the aged cortex (Brunso-Bechtold et al., 2000; Poe et al., 2001; Peters et al., 2008). Despite these structural changes, electrophysiological studies conducted in both rodents and nonhuman primates indicate that some aged PFC pyramidal neurons are subject to increased inhibition (Luebke et al., 2004; Bories et al., 2013). Notably, age-related alterations in metabotropic GABA(B) receptors may mediate shifts in the tonic inhibition of PFC pyramidal neurons. GABA(B) receptors are localized presynaptically on GABAergic and glutamatergic terminals where they regulate neurotransmitter release, as well as postsynaptically on dendrites of pyramidal neurons where they have been implicated in tonic inhibition (Wang et al., 2010). GABA(B) receptor expression is markedly reduced in the aged PFC (McQuail et al., 2012); however it remains unclear how such reductions impact behavioral flexibility. The first goal of the current study was to determine how age-related changes in the expression of mPFC GABA(B) receptor subunits relate to performance on a mPFC-dependent set shifting task. The second goal of this study was to determine if pharmacological activation of GABA(B) receptors in the mPFC could improve set shifting performance in aged rats.

EXPERIMENTAL PROCEDURES

Subjects

Young ($n = 7$) and aged ($n = 40$) male Fischer 344 rats were used in Experiments 1 and 2. Rats were obtained from the National Institute on Aging colony (Charles River Laboratories, Raleigh, NC, USA) and housed in an AAALAC-accredited vivarium in the McKnight Brain Institute Building at University of Florida in accordance with the rules and regulations of the University of Florida Institutional Animal Care and Use Committee and NIH guidelines. The facility was maintained at a consistent 25 °C with a 12-h light/dark cycle (lights on at 0800 h) with free access to food and water except as noted below.

Experiment 1. Relationships between GABA(B) receptor expression and set shifting performance in aged rats

Prior to the start of behavioral testing, young and aged male Fischer 344 rats ($n = 7$ young, 6 months, and $n = 15$ aged, 22 months) were food restricted to 85% of their free feeding weights over the course of five days. Rats were maintained at this weight for the duration of behavioral testing.

Apparatus. The set shifting task was conducted in eight standard rat behavioral test chambers (30.5 × 25.4 × 30.5 cm, Coulbourn Instruments, Whitehall, PA, USA) with metal front and back walls, transparent Plexiglas side walls, and a floor made of steel rods (0.4 cm diameter) spaced 1.1 cm apart. Each test chamber was housed in a sound-attenuating cubicle, and contained a recessed food pellet delivery trough located 2 cm above the floor in the center of the front wall. The food trough contained a photobeam to detect head entries and a 1.12 W lamp for illumination. Food rewards in the task consisted of deliveries of a single 45-mg grain-based food pellet (PJAI, Test Diet, Richmond, IN, USA) into the trough for each correct response. Two retractable levers were located to the left and right of the food trough (11 cm above the floor), and a 1.12 W cue lamp was located 3.8 cm above each lever. An additional 1.12 W house light was mounted on the rear wall of the sound-attenuating cubicle. To monitor locomotor activity during task performance, an infrared activity monitor was positioned above each test chamber. The monitor contained an array of infrared (body heat) detectors focused on the chamber. Movement in the chamber was defined as a relative change in the infrared energy falling on the detectors. A computer interfaced with the test chambers and equipped with Graphic State 3.01 software (Coulbourn Instruments) was used for experiment control and data collection.

Shaping. The design of the set shifting task was based on that used by (Floresco et al., 2008; Beas et al., 2013). Prior to training, each rat was given five 45-mg food pellets in its home cage to reduce neophobia to the food reward. Training began with four stages of shaping, with each new stage beginning the day following completion of the prior stage. In Shaping Stage 1, rats received a 64-min session of magazine training, involving 38 deliveries of a single food pellet with an inter-trial interval (ITI) of 100 ± 40 s. In Shaping Stage 2, rats were trained to press each of the response levers. A single lever (left or right, counterbalanced across groups) was extended into the test chamber and a press resulted in delivery of a single food pellet. After reaching a criterion of 50 lever presses in 30 min, rats were then trained on the opposite lever using the same procedures.

In Shaping Stage 3, rats received 90 trials designed to train them to press the levers immediately after their insertion into the test chamber. Each 20-s trial began with the house light being illuminated and insertion of a single lever (either left or right, randomly selected within

each pair of trials) into the test chamber where it remained for a maximum of 10 s. A lever press in this time window caused the lever to retract, a single food pellet to be delivered, and the house light to remain on for an additional 4 s. If a rat failed to press the lever within 10 s, the lever was retracted and the house light turned off, and the trial was scored as an omission. Rats received at least four daily sessions in this stage, and training proceeded until rats reached a criterion of fewer than 10 omissions out of the 90 trials in a single session.

In Shaping Stage 4 each rat's side bias (i.e., preference for one lever over the other) was determined. Trials consisted of multiple phases. In the first phase of a trial, the house light was illuminated and both levers were inserted into the test chamber. A press on either lever caused both levers to retract and a single food pellet to be delivered. In the second phase of a trial, both levers were again inserted, but only a press on the lever opposite to that pressed in the first phase resulted in food delivery. A press on the same lever chosen in the first phase caused the levers to be retracted and the house light to be extinguished. After a "correct" response in this second phase of a trial, a new trial was initiated, whereas after an "incorrect" response, the second phase was repeated until rats made a "correct" response. The session ended after a total of 45 completed trials. The side associated with the greatest number of total responses across this phase of testing was considered a rat's biased side.

Initial discrimination (visual cue). On the day after the side bias determination session, rats were trained on the visual cue discrimination. As shown in Fig. 1A, illumination of a cue light over a lever signaled the correct response, irrespective of its spatial location (left or right). Each 20-s trial began with one of the cue lights being illuminated with the left or right side selected randomly for each pair of trials. The house light was illuminated 3 s later and remained on while both levers were inserted into the chamber. A press on the lever corresponding to the cue light (a correct response) caused the house light to remain on for 4 s, the levers to be retracted, the cue light to be extinguished, and a single food pellet to be delivered. A press on the lever that was not illuminated by the cue light (an incorrect response) or failure to respond within 10 s (omission) caused both levers to be retracted and all lights to be extinguished. The criterion for acquisition of the visual cue discrimination was eight consecutive correct trials

(and at least 30 total trials, excluding omissions). Rats performed a maximum of 120 trials per session. Rats not reaching criterion within a single session received additional sessions on subsequent days. The total number of completed trials required to reach criterion (combined across all sessions) was used as the measure of acquisition for the visual cue discrimination.

Set shift discrimination (left/right). The day after achieving criterion performance on the visual cue discrimination, the task contingencies were altered and rats were tested in the set shift condition. As shown in Fig. 1B, in this phase of the task, the trials were identical to those in the visual discrimination but the light illuminated over the levers no longer predicted the correct response. Instead, only responses on one side (left or right, whichever was not the rat's biased side as calculated in Shaping Stage 4) resulted in food delivery. As such, the rats were required to ignore the light illuminated over the lever (visual cue) and instead "shift" attention toward the spatial location of the lever in order to consistently receive the reward. Rats were considered to have acquired the set shift upon reaching criterion performance of 10 consecutive correct trials (excluding omissions). As with the visual cue discrimination, the maximum number of trials in each session was set at 120. If rats did not acquire the task within a single session, they received additional sessions on subsequent days.

Western blotting procedures

Sample preparation. Following completion of behavioral testing, rats were returned to free feeding for a minimum of one week before being sacrificed by decapitation. The brain was extracted from the skull and the mPFC was dissected from surrounding tissue on an ice cold plate and frozen on dry ice. Tissues were stored at -80°C until used for the preparation of membrane fractions as in (McQuail et al., 2012; Baqueños et al., 2014). Frozen tissue was weighed, thawed, and homogenized in ice cold buffer (50 mM HEPES, pH 7.4, 1 mM EDTA and 1 mM EGTA and protease inhibitors (Thermo Fisher Scientific, Waltham, Massachusetts, USA) using a glass-Teflon Dounce homogenizer. Homogenates were centrifuged at 14,000 rpm for 20 min at 4°C . The pellet was resuspended in 20 mL of the same buffer without protease inhibitors and incubated on ice for 30 min followed by centrifugation at 16,500 rpm for 15 min at 4°C . This pellet

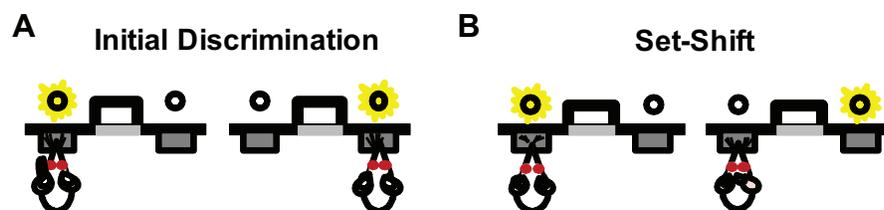


Fig. 1. Schematic of the set shifting task. (A) Rats were initially required to discriminate between two response levers on the basis of a cue light illuminated above the correct lever (visual cue discrimination). (B) Upon acquisition of the visual cue discrimination, the response strategy was "shifted", such that the rats had to ignore the cue light and respond on the basis of a particular lever location (e.g., always press the left lever).

was resuspended in seven volumes of 50 mM HEPES, pH 7.4, and aliquots were stored at -80°C until used for Western blotting assays. Protein concentration was determined using the Pierce BCA Kit according to the manufacturer's protocol (Rockford, IL, USA).

SDS-PAGE and immunoblotting. All reagents were purchased from Bio-Rad (Hercules, CA, USA), unless noted otherwise. Proteins were denatured and reduced in Laemmli sample buffer with 5% (vol/vol) β -mercaptoethanol (BioWorld, Dublin, OH, USA) and heated at 95°C for 5 min. In all Western blot experiments, $5\ \mu\text{g}$ of protein per lane were electrophoretically separated on a 4–15% Tris–HCl gel at 200 V for 35 min then transferred to nitrocellulose membranes using a wet transfer apparatus for 40 min at 200 V. Blots were washed three times with Tris-buffered saline (TBS; pH 7.4) then blocked for 1 h in blocking buffer (Rockland, Gilbertsville, PA, USA). Blots were then incubated overnight at 4°C with anti-GABA(B)R1 (made in rabbit; Cell Signaling Technology, Beverly, MA, USA), anti-vesicular GABA transporter (VGAT; made in rabbit; Millipore, Temecula, CA), and anti- β -tubulin (made in mouse; EnCor Biotechnology, Gainesville, FL, USA) diluted 1:1000 in blocking buffer with 0.1% Tween 20 (Bio-Rad, Hercules, CA, USA). Blots were then washed three times with TBS, incubated with either donkey-anti-rabbit IgG or donkey-anti-mouse IgG conjugated to IRDye 680RD or IRDye 800CW, (LI-COR Biosciences, Lincoln, NE, USA) and diluted 1:15,000 or 1:20,000 in blocking buffer (TBS (1:1) with 0.1% Tween 20) for 1 h. Following 3 additional TBS washes, blots were scanned on an Odyssey imaging system (LI-COR Biosciences). β -Tubulin was probed to verify equality of loading across conditions/experiments. Samples were assayed in triplicate; the loading position of the sample was varied between gels/experiments in a pseudorandom fashion to control for technical variation in the electroblotting procedure.

Statistical analyses

Set shifting statistical analysis. Data were exported from Graphic State 3.0 software as text files, and compiled using a custom macro written for Microsoft Excel (Dr. Jonathan Lifshitz, University of Kentucky). The numbers of trials and errors to reach criterion on the visual cue and left/right (set shift) discriminations were used as the measures of performance. Because the task involved presentation of the same set of stimuli (cue lights and levers) during both the initial discrimination and the set shift, the nature of the errors was also analyzed. Specifically, errors were divided into two subtypes. “Previously reinforced errors” were those in which the cue light was incongruent with the correct lever location and consistent with the previous cue discrimination rule. “Never-reinforced errors” were those in which the cue light and spatial location were congruent and the rat's choice failed to correspond with either the previous cue discrimination rule or the left/right discrimination rule (Ragozzino et al., 2002; Floresco et al., 2008; Beas et al., 2013). Additional performance measures were also col-

lected: number of omitted trials, response latency (latency to press one of the two levers after they were extended into the chamber), and locomotor activity during ITIs. For each measure, data are presented as mean + standard error of the mean and compared with independent *t*-tests. All statistical analyses were conducted using SPSS 22.0 (Cary, NC, USA) and GraphPadPrism (La Jolla, California). For all statistical comparisons, values of $p < 0.05$ were considered significant.

Protein expression statistical analysis. For Western blot analyses, integrated protein density was measured for each band using ImageStudio Software (LI-COR) and the individual values of both young and aged samples were normalized to the mean expression of young samples run on the same gel. Age comparisons of protein expression were conducted using independent *t*-tests. As a negative control, each blot was probed separately for tubulin expression to confirm that age effects were not a result of technical errors in protein quantification or loading ($t_{(20)} = .07$, $p = 0.94$). Effects of age on protein expression were analyzed using independent *t*-tests. Relationships between protein expression and behavioral flexibility performance (as measured by total trials to criterion (TTC) on the set shift) were assessed with bivariate correlations performed on data from aged rats.

Experiment 2: Effects of medial prefrontal cortex GABA(B) receptor activation on set shifting in aged rats

Subjects. Aged male Fischer 344 rats (20 months, $n = 25$) were obtained from the National Institute on Aging colony (Charles River Laboratories, Raleigh, NC) and housed as described above for two weeks prior to surgical procedures.

Surgery. Rats were initially anesthetized with isoflurane gas and then placed into a stereotaxic instrument fitted with atraumatic ear bars (Kopf Instruments, Tujunga, CA, USA). The incisor bar was set at -3.3 mm to provide a flat skull position. Bilateral guide cannulae, which consisted of a plastic body holding two 22-gauge stainless steel cannulae 1.4 mm apart (Plastics One, Roanoke, VA, USA), were implanted over the mPFC at the coordinates AP: $+2.7$ relative to the bregma, ML: ± 0.7 , DV: -3.8 relative to skull). The guide cannulae were affixed to the skull with dental acrylic and stainless steel screws, and wire stylets were used to occlude them to prevent infection.

Intracerebral microinjections of the GABA(B) receptor agonist baclofen. Two weeks post-surgery, rats were food restricted and trained in the set shifting task as described in Experiment 1. Just prior to testing on the visual cue discrimination, rats received a dummy injection during which the stylets were removed from the guide cannulae and injectors (28 ga. needles which extended 1.0 mm beyond the end of the guide cannulae; Plastics One) were lowered into the mPFC. This dummy injection (in which no fluid was injected)

served to acclimate the rats to the handling procedures necessary to administer the drug. Rats were then trained to criterion performance on the visual cue discrimination as in Experiment 1. Prior to the set shift session, rats received bilateral microinjections of the GABA(B)R agonist baclofen (0.5 or 1.5 nmol, Tocris, Ballwin, MO, USA) or vehicle (artificial cerebral spinal fluid, Harvard Apparatus, Holliston, MA, USA). Baclofen doses were chosen based on their efficacy in blocking cocaine-induced locomotor activity (Steketee and Beyer, 2005). Rats were assigned to drug conditions on the basis of their initial (visual cue) discrimination performance, such that all groups had approximately equal performance. Microinjections (0.5 μ l per hemisphere over 60 s) were administered 5 min prior to the start of the test sessions and were delivered via 10- μ l syringes connected to the injection needles (which extended 1.0 mm beyond the end of the guide cannulae) by a length of PE-20 tubing. The syringes were mounted on a syringe pump (Pump 11 Elite, Harvard Apparatus) and injection needles were left in place for 1 min after injections to allow for drug diffusion.

Histological assessment of cannulae placement. Following completion of testing, rats were euthanized with 100 mg/kg sodium pentobarbital, then perfused with 0.1 M phosphate-buffered solution (PBS) followed by 4% paraformaldehyde (PFA) in 0.1 M PBS. Brains were removed and postfixed in 4% PFA overnight and then cryoprotected in 20% sucrose in PBS. Brains were then flash frozen and sliced coronally at 40 μ m on a cryostat (Leica Jung Frigocut 2800E, Richmond, IL, USA). Every other tissue section was stained with thionin using methods described previously by Orsini et al. (2015) and visualized using a microscope under conventional bright-field illumination. Cannula tip placements were verified and mapped onto standardized coronal sections of the rat brain (Paxinos and Watson, 2007).

Statistical analyses. Analysis of performance in the set shifting task was calculated as described above in Experiment 1. Comparisons between drug groups were conducted using one-way analysis of variance (ANOVA) and LSD post-hoc tests when warranted.

RESULTS

Experiment 1: Relationships between GABA(B) receptor expression and set shifting performance in aged rats

Behavioral flexibility is impaired in aged F344 rats. In order to link age-related changes in GABA(B) receptor protein expression to mPFC-dependent behavioral flexibility, young adult and aged rats were characterized on a set shifting task (task schematic shown in Fig. 1). All rats in the young and aged groups reached criterion performance on both the initial (visual cue) and set shift (left/right) discrimination problems. Performance of young and aged rats is shown in Fig. 2. Consistent with previous findings from our lab and others (Barense

et al., 2002; Beas et al., 2013), independent samples *t*-tests indicated that young adult and aged rats required comparable numbers of trials to reach criterion performance on the initial (visual cue) discrimination ($t_{(20)} = 0.23$, $p = 0.81$, Fig. 2A). In contrast, aged rats were impaired relative to young on the set shift (left/right discrimination), requiring significantly more trials and errors to reach criterion performance ($t_{(20)} = 2.52$, $p < 0.05$, Fig. 2B; $t_{(20)} = 2.24$, $p < 0.05$; Fig. 2C). A two-factor repeated measures ANOVA (age X error type) revealed main effects of age (such that aged rats made more errors than young; $F_{(1,20)} = 5.05$, $p < 0.05$) and error type (such that rats made more previously reinforced than never-reinforced errors; $F_{(1,20)} = 38.29$, $p < 0.05$), but no interaction between the two variables (Fig. 2C). Planned *t*-test comparisons between error types and age groups showed that both young and aged rats made significantly more previously reinforced than never-reinforced errors (paired *t*-tests; young, $t_{(6)} = 4.95$, $p < 0.05$; aged, $t_{(14)} = 5.79$, $p < 0.05$), but that neither previous-reinforced nor never-reinforced errors differed significantly by age (previously-reinforced, $t_{(20)} = 1.67$, $p = 0.11$; never-reinforced, $t_{(20)} = 1.50$, $p = 0.14$). Fig. 2D shows individual performance (TTC) in the set shifting task. Some aged rats performed within the range of young adults whereas others fell well outside this range, demonstrating impairment. Additional analyses (Table 1) revealed no differences between young and aged rats in the number of omitted trials ($t_{(20)} = 0.14$, $p = 0.88$), mean response latencies ($t_{(20)} = 1.08$, $p = 0.29$), or baseline locomotor activity ($t_{(20)} = 0.91$, $p = 0.92$).

GABA(B) receptor protein expression in mPFC and set shifting performance. Previous work has shown that performance in the set shifting task critically depends on mPFC (Ragozzino, 2002; Floresco et al., 2008). Fig. 3A shows representative immunoreactive bands from young and aged mPFC samples when incubated with antibodies to the GABA(B)R1 subunit, VGAT, or β -tubulin. In the mPFC, two distinct GABA(B)R1-immunoreactive bands were detected (130 kDa and 95 kDa), corresponding to the two different isoforms of this subunit, GABA(B)R1a and GABA(B)R1b, respectively. The GABA(B)R1a isoform contains a pair of short consensus repeats at the N-terminal that traffic the GABA(B)R1a-containing receptor complex to presynaptic terminals where these receptors modulate neurotransmitter release. GABA(B)R1b lacks this N-terminal extension and is primarily localized to dendrites where it mediates postsynaptic inhibition (Kaupmann et al., 1998; Bettler et al., 2004; Biermann et al., 2010). Given the distinct localization and function of these R1 isoforms, R1a and R1b were analyzed separately. In agreement with previous work from our lab (McQuail et al., 2012; Baquelos et al., 2014), expression of both R1 isoforms was reduced in aged compared to young mPFC (Figs. 3B, D). Although the magnitude of the age-related reduction was similar for both R1a and R1b, the reduction in R1a did not quite reach statistical significance in this cohort (R1a: -43%, $t_{(20)} = 2.01$, $p = .05$; R1b: -46%, $t_{(20)} = 2.15$, $p < 0.05$). As shown

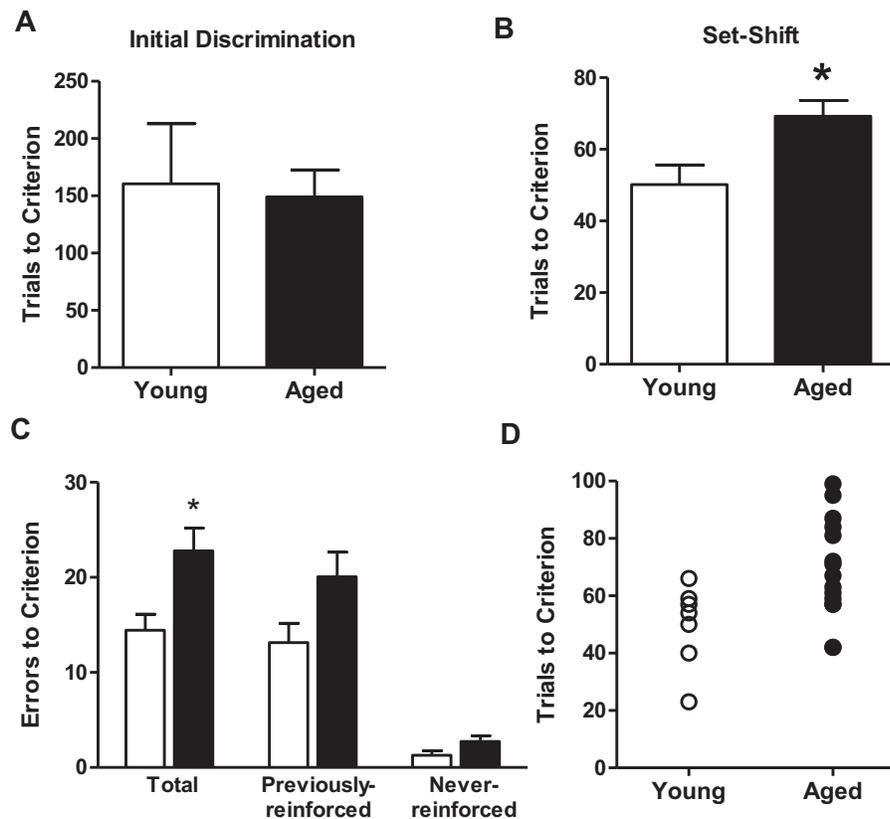


Fig. 2. Performance of young and aged rats on the set shifting task. (A) Bar graph shows that young and aged rats' performance was comparable on the initial (visual cue) discrimination, with young and aged rats requiring similar numbers of trials to reach criterion performance. (B) In contrast to initial discrimination learning, aged rats required significantly more trials than young to reach criterion performance on the set shift (left/right) discrimination. (C) Aged rats also made significantly more errors than young before reaching criterion performance on the set shift. In both young and aged rats, errors corresponded primarily to contingencies that had been reinforced previously (during the initial discrimination). Data are expressed as mean + SEM. $p < 0.05$.

Table 1. Trial omissions, locomotor activity, and response latencies in the set shifting task for Experiments 1 and 2.

	Trial omissions	Locomotor activity	Response latency
<i>Experiment 1</i>			
Young	1.57 (0.97)	6.09 (3.99)	1.36 (0.34)
Aged	1.73 (0.62)	3.12 (0.48)	1.39 (0.18)
<i>Experiment 2</i>			
Vehicle	0.50 (0.26)	4.33 (1.81)	1.02 (0.12)
Baclofen (0.5 nmol)	0.17 (0.17)	6.54 (2.54)	1.19 (0.17)
Baclofen (1.5 nmol)	1.43 (0.30)	4.60 (1.41)	1.29 (0.15)

Values represent means (SEMs).

in Fig. 3 and Table 2, lower expression of both GABA(B) R1a and GABA(B)R1b isoforms was significantly associated with a greater number of trials to reach criterion performance on the set shift phase of the task (R1a: $r = -0.55$, $p = 0.03$; Fig. 3C; R1b: $r = -0.70$, $p = 0.004$; Fig. 3E). Expression of R1b also predicted total ($r = -0.66$, $p = 0.008$) and previously reinforced ($r = -0.65$, $p = 0.009$) but not never-reinforced ($r = 0.24$, $p = 0.40$) errors to criterion on the set shift. One possibility is that these reductions in GABA(B)R subunits reflect an overt loss of inhibitory synapses in mPFC

with aging. To explore this possibility, expression of the vesicular transporter for GABA (VGAT) was evaluated in the same mPFC homogenates from young and aged rats. As shown in Fig. 3F, VGAT expression in mPFC did not differ between young and aged rats (VGAT: $t_{(20)} = 1.38$, $p = 0.18$), nor was there a significant relationship between mPFC VGAT expression and set shifting performance among aged rats (TTC: $r = -0.09$, $p = 0.74$; total errors: $r = 0.04$, $p = 0.90$; previously reinforced errors: $r = 0.10$, $p = 0.73$; never-reinforced errors: $r = -0.28$, $p = 0.32$; Table 2).

Experiment 2: Effects of medial prefrontal cortex GABA(B) receptor activation on set shifting in aged rats

Experiment 1 shows that GABA(B)R expression is reduced in aged mPFC, and that greater age-related reductions in both GABA(B)R1a and GABA(B)R1b expression are significantly associated with worse set shifting performance among aged rats. If this reduction in GABA(B)R expression is critical for set shifting performance deficits, it would be expected that direct stimulation of mPFC GABA(B) receptor activity should facilitate set shifting in aged rats. To test this hypothesis, aged rats were implanted with guide

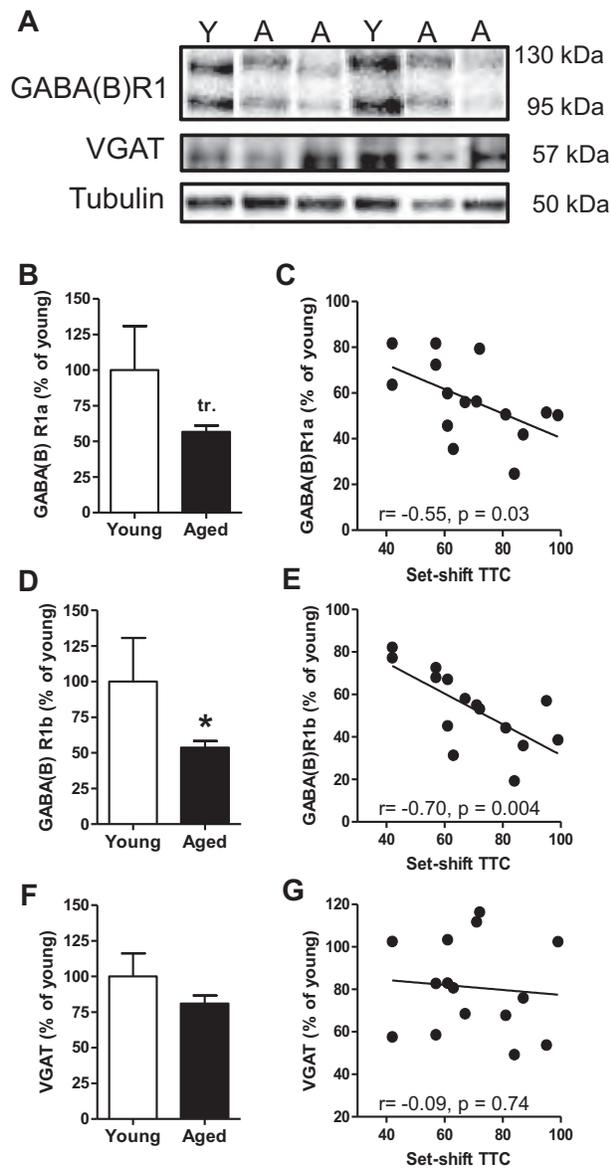


Fig. 3. Age-related changes in GABA(B) receptor protein expression and relationship to performance in the set shifting task. (A) Representative immunoreactive bands from young and aged mPFC homogenates following incubation with antibodies to GABA(B)R1, VGAT, and loading control β -tubulin. (B) Bar graph shows GABA(B) R1a expression in young and aged mPFC. While expression was numerically reduced in the aged mPFC, this reduction approached but did not reach significance ($p = 0.05$). (C) Among aged rats, expression of GABA(B)R1a was significantly associated with performance in the set shifting task such that that lower expression was associated with worse behavioral flexibility (more TTC). (D) Bar graph shows that GABA(B)R1b expression was significantly reduced in the aged compared to the young mPFC. (E) Among aged rats, expression of GABA(B)R1b was significantly associated with set shifting performance, such that lower expression robustly predicted worse behavioral flexibility. (F) Bar graph shows that VGAT expression in mPFC did not differ between young and aged rats. (G) Among aged rats, expression of VGAT was not associated with performance in the set shifting task. Data are expressed as mean + SEM. tr. $p = 0.05$, $p < 0.05$.

cannulae that permitted direct drug delivery to the mPFC. The cannulae and microinjection volume targeted both prelimbic and infralimbic subregions of the mPFC

(Fig. 4A) and $n = 4$ rats were excluded because cannulae were located outside these subregions, leaving a total of $n = 8$ rats in the vehicle group, $n = 6$ rats in the 0.5 nmol group, and $n = 7$ rats in the 1.5 nmol group. The number of trials needed to reach criterion on the visual cue discrimination (performed prior to drug administration) did not differ among these groups ($F_{(2,18)} = 0.14, p = 0.87$; Fig. 4B). In contrast, performance on the set shift (left/right discrimination), following vehicle or baclofen administration differed significantly among drug groups on both trials (Fig. 4C; $F_{(2,18)} = 7.60, p < 0.05$) and errors (Fig. 4D; $F_{(2,18)} = 4.49, p < .05$) to criterion measures. Post-hoc comparisons showed that the 0.5 nmol dose of baclofen significantly enhanced performance compared to both vehicle and the 1.5 nmol dose ($ps < 0.05$). Error analysis revealed a main effect of drug on previously reinforced errors ($F_{(2,18)} = 4.60, p < .05$), with the 0.5 nmol baclofen group having significantly fewer previously reinforced errors than the vehicle or 1.5 nmol groups ($ps < 0.05$). In contrast, there were no differences between drug groups in the number of never-reinforced errors ($F_{(2,18)} = 0.42, p = 0.65$). These results show that stimulation of GABAergic signaling through mPFC GABA(B) receptors can facilitate set shifting performance in aged rats. Analyses of additional task measures (Table 1) revealed that intra-mPFC baclofen did not significantly influence response latencies ($F_{(2,18)} = 0.89, p = 0.43$) or locomotor activity ($F_{(2,18)} = 0.37, p = 0.70$). There was a main effect of drug condition on trial omissions ($F_{(2,18)} = 6.04, p = 0.01$), with the 1.5 nmol group making significantly more omissions than the vehicle control group ($p < 0.05$). The magnitude of this effect was numerically very small, however, amounting to < 1 omitted trial difference between the 1.5 nmol and vehicle groups.

DISCUSSION

The ability to flexibly adapt behavior in response to changing environmental contingencies can be compromised in aged individuals. In the current study, a rat model was employed to demonstrate an important role for mPFC GABA(B) receptors in such age-related impairments. GABA(B) receptor expression was significantly reduced in the aged rat mPFC, with greater reductions associated with worse performance on a set shifting task. Consistent with these findings, set shifting performance in aged rats was enhanced by intra-mPFC administration of the GABA(B) receptor agonist baclofen.

Studies in rodents, non-human primates, and humans show that performance on set shifting tasks is impaired in aged compared to young subjects (Barense et al., 2002; Bizon et al., 2012; Nieves-Martinez et al., 2012; Beas et al., 2013). Uniformly, such age-associated deficits are evident only when subjects are expected to adapt behavior following a rule shift but not during simple discrimination learning that does not require such flexibility (Robbins et al., 1998; Volkow et al., 1998; Barense et al., 2002; Moore et al., 2003, 2006; Ashendorf and McCaffrey, 2008; Nieves-Martinez et al., 2012;

Table 2. Relationships between set shifting performance measures and protein expression in Experiment 1.

	GABA(B)R1a	GABA(B)R1b	VGAT
Trials to criterion	$r = -0.55, p = 0.03$	$r = -0.70, p = 0.004$	$r = -0.09, p = 0.74$
Total errors to criterion	$r = -0.49, p = 0.06$	$r = -0.66, p = 0.008$	$r = 0.04, p = 0.90$
Previously reinforced errors	$r = -0.42, p = 0.12$	$r = -0.65, p = 0.009$	$r = 0.10, p = 0.73$
Never-reinforced errors	$r = -0.08, p = 0.78$	$r = 0.24, p = 0.40$	$r = -0.28, p = 0.32$

Significant relationships ($p < 0.05$) are noted in **bold and italicized** text.

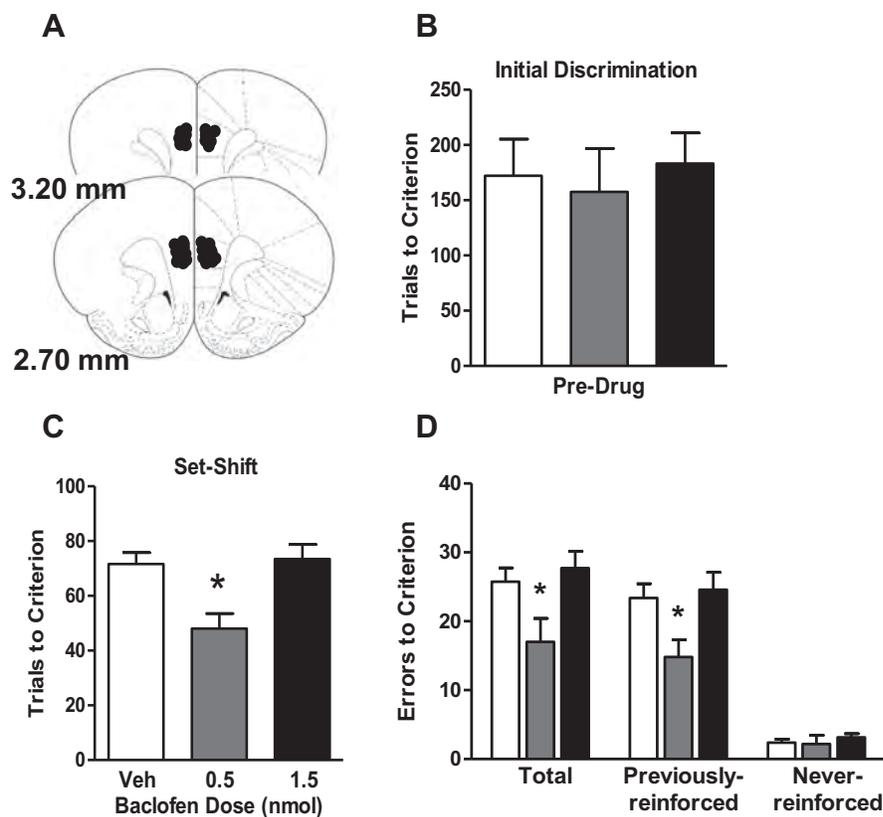


Fig. 4. Intra-mPFC baclofen administration enhanced set shifting performance in aged rats. (A) Schematic shows bilateral cannula placements in mPFC for each rat included in this experiment (illustrations adapted from Paxinos and Watson (2007)). (B) Bar graph shows that aged rats' performance on the initial (visual cue) discrimination prior to baclofen administration did not differ between drug conditions. (C) Intra-mPFC baclofen significantly enhanced set shifting performance in aged rats, with rats receiving intra-mPFC infusion of 0.5-nmol baclofen requiring significantly fewer trials to reach criterion performance on the set shift in comparison to the rats receiving vehicle. (D) Rats receiving intra-mPFC infusion of 0.5-nmol baclofen also made significantly fewer errors before reaching criterion performance on the set shift compared to those receiving vehicle. Error analysis revealed that rats in the 0-5 nmol condition made fewer previously reinforced errors than rats in the vehicle condition, but did not differ from the vehicle condition in the number of never reinforced errors. Data are expressed as mean + SEM. * $p < 0.05$ compared to vehicle.

Beas et al., 2013). The present findings are consistent with this prior body of work, in that aged rats performed no differently than young on an initial (visual cue) discrimination, but were impaired in their acquisition of a subsequent left/right discrimination that required a shift in behavioral strategy. A potential limitation of the design employed here is that all rats were shifted from visual cue to left/right discrimination. This design was necessary for several reasons, most notably because even in the absence of a strategy shift, young Fischer 344 rats differ significantly in their ability to acquire visual cue and left/right discriminations (Beas et al., under review). As such, a counterbalanced design results in a non-linear distribution of values for TTC that makes attempts to relate

neurobiological markers to behavioral performance challenging. For many reasons, it is likely that the age-related impairments reported in Experiment 1 are reflective of a response strategy "shift" and not simply an age-related impairment in left/right discrimination. First, a prior study employing a different set shifting task design showed that aged rats were selectively impaired on set shifting (but not initial discrimination learning), regardless of the direction of the shift (Barense et al., 2002). Second, numerous studies (including those using the same task design employed here) have shown that the rodent mPFC plays the same critical role in set shifting, regardless of the direction of the shift (Ragozzino et al., 1999; Birrell and Brown, 2000; Ragozzino, 2007; Floresco et al.,

2008; Bissonette and Powell, 2012; Bissonette et al., 2013), and that mPFC manipulations do not influence left/right discrimination performance if this discrimination is conducted in the absence of a shift (Floresco et al., 2008). Third, following the set shift, aged (as well as young) rats committed significantly more errors of the previously reinforced type compared to the never-reinforced type, indicating perseveration on the visual cue discrimination strategy. If the impaired performance in aged rats simply reflected a deficit in left/right discrimination abilities, equivalent numbers of previously- and never-reinforced errors would be expected. Finally, we recently showed that systemic administration of baclofen enhances performance in young rats on the same set shifting task used here, under conditions in which rats were shifted either from the visual cue to left/right discrimination or using the reversed design (from the left/right to the visual cue discrimination; Beas et al., under review). Additional control experiments in that study demonstrated that these enhancing effects of baclofen in young rats were not recapitulated when the left/right discrimination learning was not preceded by a visual cue discrimination (i.e., when no rule shift was required). Together, these findings strongly indicate that the age-related impairments described in the current study reflect an inability to shift behavior as adeptly as young rats, and that baclofen administered into the mPFC facilitates behavioral flexibility.

Findings from both neuropsychiatric disease and rodent models strongly implicate PFC GABAergic signaling in behavioral flexibility (Hashimoto et al., 2008; Brady, 2009; Maldonado-Aviles et al., 2009; Gruber et al., 2010; Enomoto et al., 2011; Bissonette et al., 2014; Cho et al., 2015). Changes in PFC GABAergic signaling in normal aging are evidenced by reductions in both GABAergic interneurons and inhibitory synapses (Grachev and Apkarian, 2001; Smith et al., 2004; Peters et al., 2008; Dumitriu et al., 2010; Soghomonian et al., 2010; Stranahan et al., 2012). Such structural changes suggest a shift in the normal balance of excitation and inhibition in this brain region that could be detrimental to cognitive capacities (Luebke et al., 2004; Stranahan et al., 2012; Bories et al., 2013; Baquelos et al., 2014; McQuail et al., 2015). Indeed, cortical hyperexcitability has been suggested to underlie cognitive dysfunction across a variety of psychiatric and neurological diseases in which perseverative behaviors are prominent, including schizophrenia, Alzheimer's disease, and autism (Volk and Lewis, 2002; Lewis et al., 2004; Hashimoto et al., 2008; Maldonado-Aviles et al., 2009; Enticott et al., 2010; Gonzalez-Burgos et al., 2011; Kellner et al., 2014; Silverman et al., 2015; Siwek et al., 2015). Consistent with reduced inhibition in the aged PFC, our laboratory has previously reported a marked decline in GABA(B) receptor expression in the aged mPFC (McQuail et al., 2012; Baquelos et al., 2014). This age-related decline was not evident in the hippocampus, which is a critical locus for spatial learning that can become compromised in aging (McQuail et al., 2012). The rats in this earlier study were evaluated for spatial learning in the Morris water maze prior to assessment of GABA(B) receptor expression. Consistent with evidence that the PFC is

not critical for this behavioral task, there was no relationship between GABA(B) receptor reductions and water maze performance among aged rats.

The current study extends this prior work by demonstrating that reduced mPFC GABA(B) receptor expression is correlated with set shifting performance among aged rats, such that lower GABA(B) receptor expression is associated with worse performance. It is notable that this relationship with set shifting was strongest with the GABA(B)R1b subunit, which is the isoform of the GABA(B) receptor that traffics the receptor to the dendrites of postsynaptic neurons. GABA(B) receptors on pyramidal neuron dendrites are largely localized extrasynaptically (Vigot et al., 2006; Biermann et al., 2010; Pinard et al., 2010) and signaling via these receptors contributes to tonic inhibition of PFC pyramidal neurons (Wang et al., 2010). Thus, the reductions in GABA(B)R1b likely confer increased pyramidal neuron excitability and highlight cortical hyperexcitability in aging as a potentially important mediator of impaired behavioral flexibility. The fact that set shifting performance in aged rats was enhanced by administration of baclofen directly into mPFC (Fig. 4) is consistent with this interpretation. Emerging evidence suggests, however, that the role of PFC GABAergic neurotransmission in behavioral flexibility is not limited to GABA(B) receptor signaling. Intra-mPFC administration of GABA(A) receptor antagonists in rats impairs set shifting, suggesting that signaling through this receptor is critical for flexible behavior (Enomoto et al., 2011; Tse et al., 2015). It will be of interest in future work to determine the extent to which GABA(A) receptors are altered in the aged PFC, and whether targeting these receptors is an effective strategy for reversing flexibility deficits in aged subjects.

The relationship between GABA(B)R1b and set shifting was not mirrored by a second marker of GABAergic synapses, VGAT, the transporter that packages GABA into vesicles in presynaptic inhibitory terminals. This latter finding suggests that the relationships between GABA(B) receptors and set shifting do not simply reflect a robust loss of inhibitory terminals. It should certainly be acknowledged that the biochemical methods employed here are likely insufficiently sensitive for detecting reductions in PFC inhibitory synapse number that have been documented using electron microscopy (Smith et al., 2004; Peters et al., 2008; Dumitriu et al., 2010; Soghomonian et al., 2010). In fact, it is possible that a loss of some inhibitory terminals may elicit compensatory changes to GABA synthesizing enzymes and signaling proteins that facilitate inhibitory neurotransmission at remaining inhibitory terminals in the aged PFC (Luebke et al., 2004; Bories et al., 2013; Baquelos et al., 2014; McQuail et al., 2015). The degree of compensation may vary among aged individuals, ranging from effective adaptation that preserves cognitive flexibility to insufficient or no compensation that produces cognitive rigidity. One interpretation from the current study is that GABA(B) receptors are a possible mediator of this compensatory process. Future work employing quantitative anatomical methodologies that enable immunolocalization of GABA(B) receptors to

young and aged synapses would offer additional insight into this possibility.

Behavioral flexibility in the context of set shifting is only one aspect of PFC function that is vulnerable to decline in aging (Glisky, 2007; Alexander et al., 2012; Bizon et al., 2012; Beas et al., 2013; Bañuelos et al., 2014). In addition, aged subjects across species (including Fischer 344 rats) show impaired working memory as assessed by delayed response tasks (Arnsten et al., 1994; Robbins et al., 1998; Holtzer et al., 2004; Dumitriu et al., 2010; Beas et al., 2013). In these tasks, subjects are required to remember the location of a response lever over a relatively short delay interval (<30 s) in order to earn a food reward. While performance on both delayed response and set shifting tasks critically depends upon the mPFC (Mishkin, 1957; Goldman and Rosvold, 1970; Freedman and Oscar-Berman, 1986; Floresco et al., 1997; Ragozzino et al., 1998), a previous study examining individual performance of aged rats cross-characterized on both tasks revealed a surprising inverse relationship between the two (Beas et al., 2013). As reproduced in Fig. 5A, this prior study showed that those aged rats with impaired set shifting performance demonstrated delayed response performance on par with young adult subjects. Conversely, aged rats with impaired delayed response performance, particularly at long delay intervals, showed intact behavioral flexibility as assessed on the set shifting task. The inverse relationship between these two aspects of executive function suggests that the neurobiological changes in the mPFC that adversely affect behavioral flexibility may enable preserved working memory, and vice versa.

Together with prior findings (Bañuelos et al., 2014), the data presented here support this hypothesis. In agreement with the current study, Bañuelos et al. (2014) showed that expression of GABA(B) receptor subunits is reduced in the aged rat mPFC relative to young. Notably, however, aged rats in the Bañuelos et al. study were characterized on the delayed response task, and expression of GABA(B)R1b in the aged mPFC was *inversely* related to performance such that lower GABA(B)R1b expression was associated with *better* delayed response performance (data reproduced in Fig. 5C). This relationship is opposite that shown in the current study, in which lower GABA(B)R1b in the aged mPFC was associated with *worse* set shifting performance (Fig. 5D). Consistent with the opposite directions of the relationships between GABA(B)R1b expression and performance on these two mPFC-dependent tasks in aged rats, intra-mPFC administration of a GABA(B) receptor antagonist enhances accuracy of aged rats on the delayed response task (Bañuelos et al., 2014), whereas intra-mPFC administration of the GABA(B) receptor agonist baclofen enhances aged rats' performance on the set shifting task. Fig. 5B shows a working model that is based on these collective findings and illustrates how age-associated shifts in the normal balance of excitation and inhibition within the PFC influences distinct executive functions. Young rats are proposed to have a balance between excitation and inhibition in the PFC that allows good performance on tests of both working memory and behavioral flexibility.

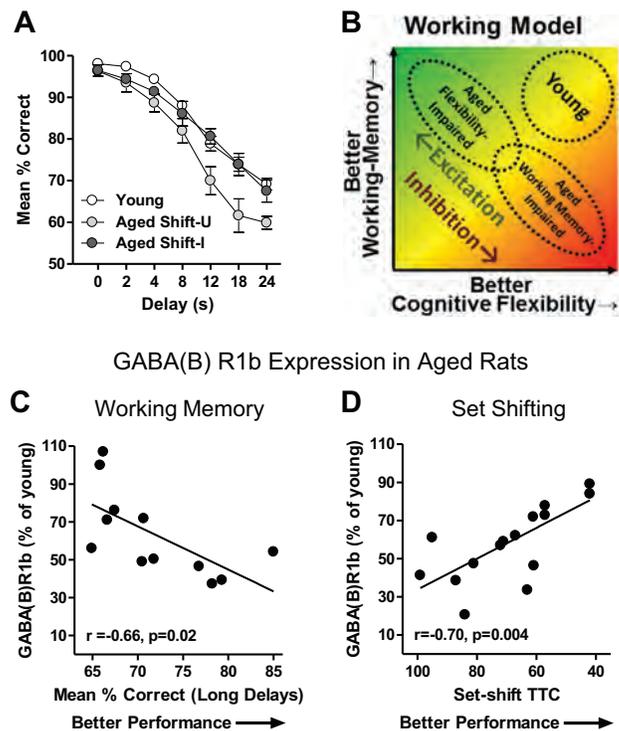


Fig. 5. GABAergic signaling may mediate an inverse relationship between behavioral flexibility and working memory in aged rats. (A) Line graph shows performance of young and aged rats cross-characterized on both the set shifting task used in the current study and a delayed response task that assesses working memory (Beas et al., 2013). In this prior study, aged rats that were unimpaired relative to young on the set shifting task (Shift-U) showed impaired performance relative to young on the delayed response task. In contrast, aged rats that were impaired on the set shifting task (Shift-I) showed intact performance on the delayed response task. (B) Working model of potential relationships between PFC excitatory/inhibitory signaling and PFC-supported executive functions that could account for the inverse relationship between set shifting and delayed response performance in aged rats. See text for detailed explanation. (C) Scatter plot reproduced from Bañuelos et al. (2014) showing the negative correlation between performance on a mPFC-dependent delayed response working memory task and mPFC GABA(B)R1b expression in aged rats. (D) Data from Fig. 3E plotted with the X-axis reversed to illustrate the positive correlation between set shifting performance and mPFC GABA(B)R1b expression.

In aging, GABAergic signaling in PFC becomes dysregulated such that net cortical inhibition is increased relative to young in some aged subjects and decreased in others. We posit that these divergent shifts in cortical inhibition have distinct consequences for executive function. Specifically, reduced cortical inhibition and a shift toward greater excitation (shown in green) contributes to impaired behavioral flexibility (“aged flexibility-impaired”) in aged rats but does not negatively impact working memory. In contrast, increased cortical inhibition (shown in red) contributes to impaired working memory in aged rats (“aged working memory-impaired”), but behavioral flexibility remains relatively preserved.

It is important to note that set shifting is not the only form of behavioral flexibility that can become compromised with age. Aged rats and primates are also impaired when the reward contingencies associated with

two stimuli are reversed (e.g., A+ ,B– is reversed to A–, B+; Bartus et al., 1979; Lai et al., 1995; Dias et al., 1996; Schoenbaum et al., 2002a,b). While reversal learning and set shifting depend upon distinct subregions of the PFC (orbitofrontal and medial PFC, respectively; Dias et al., 1996; Birrell and Brown, 2000; McAlonan and Brown, 2003), a prior study found a nearly significant relationship ($p = 0.06$) between age-associated impairments in the two types of behavioral flexibility (Barense et al., 2002). Moreover, similar to the set shifting impairments produced by GABAergic disruptions in mPFC (Enomoto et al., 2011), manipulations of GABAergic signaling within the orbitofrontal cortex can impair reversal learning (Bissonette et al., 2010). Together, these data suggest that age-associated alterations in GABAergic circuits across PFC subregions could contribute to general deficits in behavioral flexibility, encompassing both set shifting and reversal learning. It will be of significant interest in future studies to determine the status of GABA(B) receptor signaling in aged orbitofrontal cortex and, in particular, whether baclofen can exert broadly enhancing effects across different forms of impaired behavioral flexibility.

The present study replicates previous findings demonstrating reduced GABA(B) receptor expression in mPFC during normal aging. This reduction is strongly related to set shifting abilities such that greater expression is associated with better preservation of behavioral flexibility in aged rats. Moreover, mPFC GABA(B) receptor stimulation enhances set shifting in aged rats. These findings further our understanding of behavioral consequences of altered excitatory–inhibitory dynamics within the aged PFC and highlight the role of cortical inhibition in modulating behavioral flexibility. Lastly, these findings suggest GABA(B) receptors as a potential therapeutic target for improving cognitive functions supported by the PFC.

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Preclinical Magnetic Resonance Imaging and Spectroscopy Studies of Memory, Aging, and Cognitive Decline

Marcelo Febo^{1*} and Thomas C. Foster²

¹ Department of Psychiatry, William L. and Evelyn F. McKnight Brain Institute, University of Florida, Gainesville, FL, USA,

² Department of Neuroscience, William L. and Evelyn F. McKnight Brain Institute, University of Florida, Gainesville, FL, USA

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*Correspondence:

Marcelo Febo
febo@ufl.edu

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Neuroimaging provides for non-invasive evaluation of brain structure and activity and has been employed to suggest possible mechanisms for cognitive aging in humans. However, these imaging procedures have limits in terms of defining cellular and molecular mechanisms. In contrast, investigations of cognitive aging in animal models have mostly utilized techniques that have offered insight on synaptic, cellular, genetic, and epigenetic mechanisms affecting memory. Studies employing magnetic resonance imaging and spectroscopy (MRI and MRS, respectively) in animal models have emerged as an integrative set of techniques bridging localized cellular/molecular phenomenon and broader *in vivo* neural network alterations. MRI methods are remarkably suited to longitudinal tracking of cognitive function over extended periods permitting examination of the trajectory of structural or activity related changes. Combined with molecular and electrophysiological tools to selectively drive activity within specific brain regions, recent studies have begun to unlock the meaning of fMRI signals in terms of the role of neural plasticity and types of neural activity that generate the signals. The techniques provide a unique opportunity to causally determine how memory-relevant synaptic activity is processed and how memories may be distributed or reconsolidated over time. The present review summarizes research employing animal MRI and MRS in the study of brain function, structure, and biochemistry, with a particular focus on age-related cognitive decline.

Keywords: fMRI, DTI, magnetic resonance spectroscopy, hippocampus, memory, preclinical MRI, aging neuroscience

INTRODUCTION

Among the various techniques in neuroscience, magnetic resonance imaging and spectroscopy are uniquely suited for longitudinal measurements; providing in-depth assessments of neural activity, tissue microstructural organization, and chemistry in the aging brain. Functional and diffusion magnetic resonance imaging (fMRI and dMRI, respectively) are among the most promising MRI modalities that may be used to investigate the relationship between regional changes in neural activity and structural connectivity. These neuroimaging methods have been employed in aging humans to suggest that variability in the decline of several cognitive processes

results from changes in defined neural circuits (O'Sullivan et al., 2001; Gunning-Dixon and Raz, 2003; Salat et al., 2005; Dennis et al., 2008; Kaufmann et al., 2008; Duverne et al., 2009; Wang et al., 2009; Lighthall et al., 2014). However, cellular and synaptic mechanisms underlying regional differences in vulnerability to aging are difficult to assess in human subjects. Thus, the utility of fMRI and dMRI in studying functional and neuroanatomical correlates of the human aging brain is strengthened by animal studies that combine imaging with invasive assessments. Animal imaging approaches combining fMRI with electrophysiological recordings, direct electrical stimulation and/or optogenetic modulation of neuronal activity, may bring us closer to characterizing links between neural activity and memory formation, both in healthy aging and with cognitive impairment. Other animal imaging methods not widely used in human subjects, such as pharmacological MRI (Box 1), may be used to discern specific drug effects on BOLD activity in memory networks. Functional and anatomical imaging techniques find strong complementation with *in vivo* magnetic resonance spectroscopy (MRS), which describes biochemical correlates in memory regions.

The present article provides an overview of animal studies that use fMRI, dMRI, and MRS to assess functional, structural, and chemical characteristics in brain areas involved in learning and memory. Rather than providing an extensive overview of the full breadth of the animal imaging literature, the review focuses on studies that are particularly relevant to normal aging animal models, and on imaging and spectroscopy studies of temporal lobe, prefrontal cortical and striatal circuits. The medial temporal lobe episodic memory system and a prefrontal cortex and striatal executive function system are highly vulnerable to changes in structure and activity associated with cognitive decline in humans, monkeys, rats, and mice. Furthermore, there is a rich repertoire of behavioral paradigms that can be applied to study of age-related decline in memory and executive function across species (Moss et al., 2007; Nagahara et al., 2010; Zeamer et al., 2011; Alexander et al., 2012; Bizon et al., 2012; Foster et al., 2012; Holden and Gilbert, 2012; Roberson et al., 2012; Crystal and Wilson, 2015). Monkeys may have an advantage for behaviors that depend on the higher complexity of the cortex. In this regard, the anatomy of the prefrontal cortex in Old world primates is more analogous to that of humans. However, aged non-human primates may exhibit extensive neuronal loss in the prefrontal cortex, which is not evident in aging humans or other animal models (Smith et al., 2004; Burke and Barnes, 2006). Furthermore, while comparative studies have identified similarities in connectivity for auditory and visual system pathways, some connections involving the prefrontal cortex may be absent in non-human primates (Rilling, 2014), which may underlie differences in behaviors between humans and non-human primates (Stoet and Snyder, 2003). In contrast, rodents are a common model of "normal" cognitive aging, particularly for studies seeking to understand cellular and molecular mechanisms underlying age-related changes in brain structure and function. We will attempt to offer interpretations on the summarized literature and discuss how the imaging findings might be reconciled with what is

BOX 1 | Pharmacological MRI is a term used to describe the use of functional magnetic resonance imaging modalities to measure the BOLD response to neuropharmacologically active compounds (Chen et al., 1999; Salmeron and Stein, 2002). Studies typically employ BOLD imaging, however, the term is inclusive of arterial spin labeling, iron contrast-based cerebral blood volume measurements, and manganese enhanced MRI studies designed to screen brain activity in response to CNS drugs.

known on the synaptic circuitry and mechanisms of learning and memory.

EMERGING APPROACHES TO DRIVE AND RECORD FROM MEMORY CIRCUITS DURING fMRI

Findings from fMRI experiments are perhaps the most intriguing among preclinical imaging studies because of the potential of resolving functional changes involving hippocampal and prefrontal circuits during specific stages of memory formation and reconsolidation. A major question concerns the underlying neuronal activity that generates the BOLD signal. Is the signal related to region specific neuronal discharge activity or does it reflect synaptic activity associated with afferent input and local circuits? Several studies have examined neuronal discharge activity in behaving animals. Based on visual stimulation induced changes in BOLD signal in humans and neuronal discharge activity in monkeys it was suggested that the BOLD signal is representative of neuronal firing rate (Heeger et al., 2000; Rees et al., 2000). However, in both rats and non-human primates BOLD fMRI signals correlate more closely with local field potentials (LFPs) than with multi-unit activity (MUA; Logothetis et al., 2001), although recent work in rats indicates that cerebral blood flow (CBF) correlates better with LFPs than do BOLD signals or cerebral blood volume (CBV; Herman et al., 2013). The LFPs represent relatively slow changes in membrane depolarization and hyperpolarization due to afferent input and local circuit synaptic activity. Thus, the BOLD response is largely associated with local neuronal processing of synaptic inputs, as well as excitatory and inhibitory synaptic activity of the local circuit, rather than the consequent neuronal discharge activity which represents the output computation (Logothetis and Wandell, 2004). Thus, it is possible that neuromodulatory influences that inhibit the discharge activity of principle cells can increase the BOLD response, while increased discharge activity due to GABA antagonist may not alter the BOLD response (Thomsen et al., 2004). In this regard, the BOLD signal will differ across regions due to the local excitatory/inhibitory configuration of the circuit.

Functional Imaging of Hippocampal Networks

Taking advantage of the well-defined synaptic circuitry of the hippocampal formation, Angenstein et al. (2007, 2009) have utilized direct current stimulation and neural recordings across a

series of studies to determine the relationship between local field activity and the BOLD signal, particularly in relation to its evoked spatial and temporal properties in hippocampus (Tiede et al., 2012; Angenstein, 2014; Scherf and Angenstein, 2015). Electrical stimulation of afferents and recording in specific hippocampal regions allowed this group to control input activity to the dorsal CA1/dentate region, where BOLD signals were measured. Using this technique they determined important properties of hippocampal BOLD responses in relation to the neuronal activity driving this signal. For BOLD response originating in the dentate gyrus, it seems that afferent synaptic activity of the perforant path correlates better with BOLD responses rather than the discharge response of the population of granule cells (Angenstein et al., 2007). Furthermore, the propagation of BOLD activity across interconnected hippocampal subregions is influenced by the internal processing dynamics and synaptic plasticity in this region (Angenstein et al., 2009). The induction of long-term potentiation (LTP) requires activation of *N*-methyl-D-aspartate (NMDA) receptors (Foster, 2012), thus treatment with an NMDA receptor blocker (MK801) prior to afferent stimulation blocks hippocampal network activity (Tiede et al., 2012). Hence, increased BOLD signal changes associated with the induction of LTP suggests that memory-related changes in neural activity are measurable with fMRI (Canals et al., 2008; Angenstein et al., 2013).

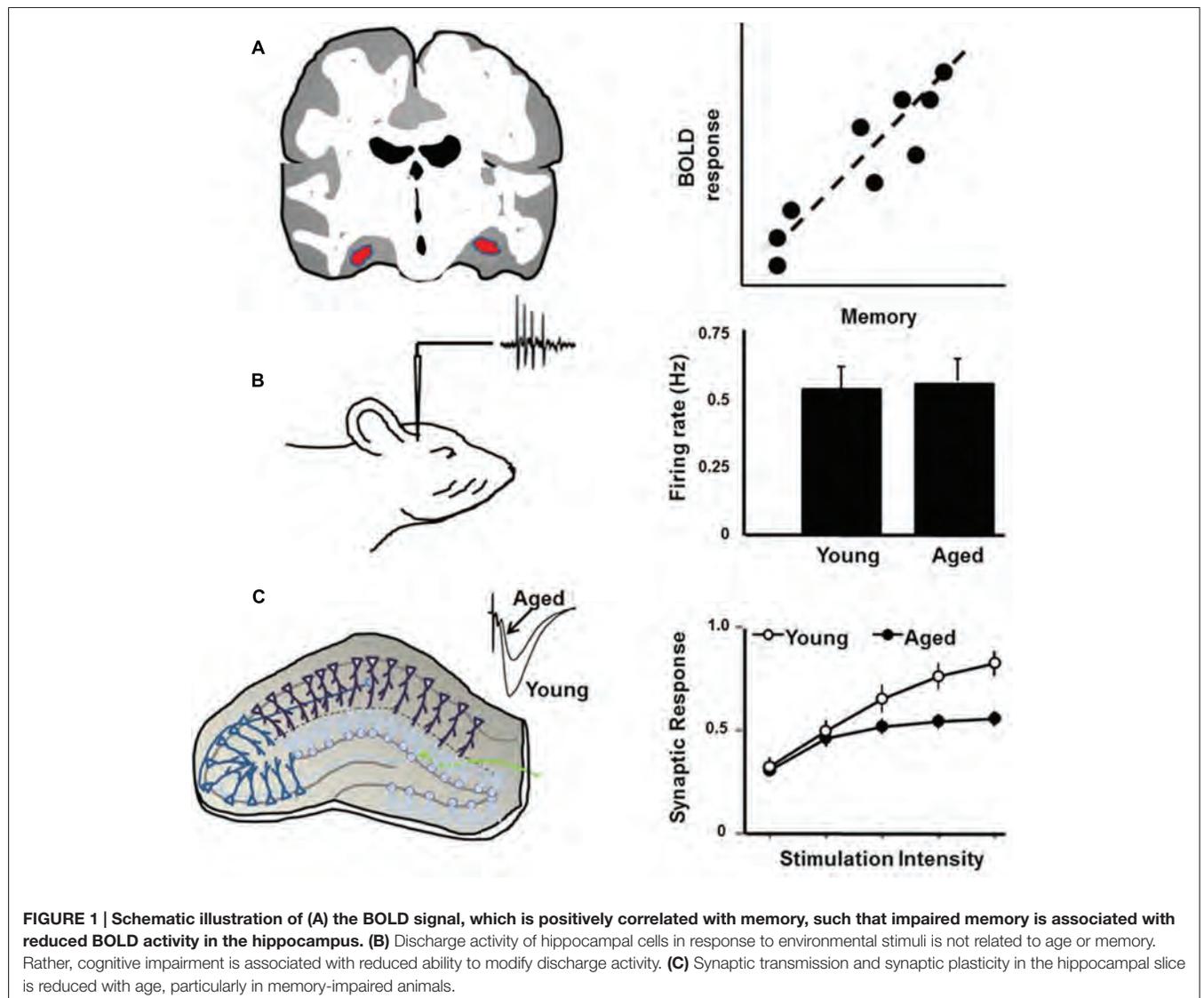
Here is where animal-imaging studies may provide key insights in the regulation of neural activity during memory formation and recalling specific events, as reported in human subjects (Ranganath et al., 2004; Flegal et al., 2014). Parahippocampal areas of normal healthy human subjects show greater BOLD responses during correct recall of events than with incorrect recall (Eldridge et al., 2000). In awake Rhesus macaques, correct recall of events on a serial probe recognition task was associated with increased BOLD in caudal entorhinal cortex, perirhinal cortex and hippocampus (Miyamoto et al., 2014). While this temporal lobe network in primates reflects early consolidation phases, the intraparietal sulcus plays a role in long-term retrieval processes (Miyamoto et al., 2013). The idea that synaptic activity mediates the BOLD response has important implications for interpreting the response as it relates to mechanisms for cognitive function during aging (Figure 1). Impaired memory encoding and retrieval is associated with decreased BOLD activity in the hippocampus and medial temporal lobe of humans (Daselaar et al., 2003; Morcom et al., 2003; Dennis et al., 2008; Salami et al., 2012; Pudas et al., 2013; Sherman et al., 2015). Conversely, an increase in frontal cortex neural activity is observed in older humans and may relate to performance of executive function tasks (Rosano et al., 2005; Turner and Spreng, 2012; Maillet and Rajah, 2014). Studies in animals suggest that the level of cell discharge activity is not dramatically altered in the hippocampus or prefrontal cortex of older cognitively impaired animals (Oler and Markus, 2000; Burke and Barnes, 2006; Wang et al., 2011; Caetano et al., 2012). Rather, cognitive decline is associated with an inability to modify cell discharge activity. In turn, modifiability of cell discharge activity depends on synaptic plasticity, and thus age-related cognitive decline is associated with a decrease in the strength

of excitatory synapses and impaired synaptic plasticity (Luebke et al., 2004; Foster, 2012; Guidi et al., 2015b).

In spite of the aforementioned insights, direct electrical current stimulation presents technical challenges preventing straightforward interpretations that can link these results to human imaging work. Among these is the non-specificity of neuronal groups targeted for stimulation, a lack of control over excitatory versus inhibitory activity, and off-target antidromic activation of afferent inputs to the stimulation site that could hinder clear interpretations of fMRI data. Some of these limitations may be resolved through the use of optogenetics in conjunction with fMRI. Initial studies applying this strategy have focused on hippocampal and prefrontal cortical regions. Therefore, these types of experiments are highly relevant to characterizations of circuit adaptations in memory and normal aging. Following a seminal study using the light sensitive cation channel rhodopsin 2 (ChR2) to drive motor thalamocortical BOLD responses (Lee et al., 2010), Lee and colleagues conducted a similar opto-fMRI study centered on eliciting hippocampal activation (Duffy et al., 2015). Light stimulated excitation of dorsal CA1 pyramidal neurons increased BOLD activation in the hippocampus and its output regions in the medial septum (Duffy et al., 2015). Increasing stimulation frequencies to levels capable of triggering seizure-like after discharges elicited a greater distribution of BOLD activation to contralateral hippocampus, neocortex, and mediodorsal thalamus, an effect closely resembling optogenetically evoked BOLD activation in mouse CA1 circuitry (Takata et al., 2015). Among the implied conceptual benefits of the opto-fMRI studies of hippocampal networks is the potential for measuring how neural activity moves through subregions of hippocampal memory networks. Importantly, future studies are likely to target specific cell groups to characterize hippocampal network activity and how memory and recall mechanisms modify activity through this structure. Studies directed at understanding specific roles for neurotransmitter systems in modulating BOLD activation through their effects on LFPs and MUA are coming to fruition in non-human primates (Rauch et al., 2008; Arseneault et al., 2013; Zaldivar et al., 2014). Applying such targeted cell- and receptor-specific approaches in imaging hippocampal networks is likely to provide powerful insight into effects of aging on hippocampal activity, memory, and cognitive behaviors.

Functional Imaging of Prefrontal Networks

The prefrontal cortex plays a role in working memory and its role in normal aging is functionally distinct from that of hippocampus and amygdala. Due to the complexity of the prefrontal cortex, in terms of afferents, efferents, and local circuits, optogenetics is essential in order to specify neuronal activation patterns with memory and in aging. Optically exciting output neurons from the prelimbic area of the prefrontal cortex of awake rats has been shown to increase BOLD activation in ventral striatum, other neocortical areas, and the mediodorsal thalamus (Liang et al., 2015). It should be noted that BOLD activity in mediodorsal thalamic nucleus



occurs with optogenetic stimulation of the hippocampus and prefrontal cortex. This region is known to be an integral part of anterior thalamic limbic circuitry involved in memory and learning (Aggleton and Brown, 1999; Aggleton et al., 2010). Interestingly, mediodorsal thalamic BOLD activation observed when driving prefrontal neurons of awake rats was not observed in rats under anesthesia. Conversely, hippocampally driven mediodorsal thalamus activation occurred under anesthetized conditions (Duffy et al., 2015). The distinct responsiveness of the mediodorsal thalamus to stimulation of these two brain areas thus appears to vary according to the state of consciousness of the animals. It is possible that prefrontal cortex-to-mediodorsal thalamus activation requires an awake state whereas it does not appear to be necessary in hippocampal networks. This brings up interesting possibilities regarding the potential properties of temporal and prefrontal lobe interactions in memory networks. Mediodorsal thalamic neurons project to limbic frontal areas such as the prelimbic, orbital, insular and

anterior cingulate regions (Gabbott et al., 2005). Here, they form asymmetric synaptic contacts with layer III pyramidal neurons projecting back to mediodorsal thalamus (Kuroda et al., 2004). Mediodorsal thalamic neurons also synapse onto two types of GABAergic interneurons that modulate both pyramidal cells and GABAergic interneurons, thus offering a potential network controlling and modifying thalamocortical and corticocortical activity (Kuroda et al., 2004). This medial thalamic circuitry also modulates hippocampal-to-prefrontal activity (Floresco and Grace, 2003). Driving hippocampal activity to the prefrontal cortex is modulated by stimulation of mediodorsal thalamus (Floresco and Grace, 2003). Tetanic stimulation of mediodorsal thalamus-to-prefrontal neurons potentiates ventral hippocampal-to-prefrontal activity (Floresco and Grace, 2003). Thus, the hippocampal-prefrontal circuitry shows synaptic plasticity that is under partial control by mediodorsal thalamic neurons. These, and other results, strongly suggest that mediodorsal thalamic neurons regulate the transit

of limbic activity to and from frontal cortical and hippocampal networks, and it also offers a pathway that can be targeted for further opto-fMRI studies.

The functional role of the dorsolateral area of the prefrontal cortex in working memory has been characterized using fMRI in humans and neurophysiological recordings in non-human primates (Funahashi et al., 1989; Curtis and D'Esposito, 2003; Nee and D'Esposito, 2016). Working memory tasks that engage this region elicit a BOLD activation pattern that reflects its temporary storage buffer and processing capacity (D'Esposito et al., 1999a; Rypma and D'Esposito, 1999), with a temporal neural activity profile similar to that measured electrophysiologically in non-human primates (Funahashi et al., 1989; Compte et al., 2000). It appears that with aging there is compensatory increased activation in the dorsolateral prefrontal cortex, with reduced BOLD activation in caudal sensory processing structures, such as the temporal and occipital cortices (Gigi et al., 2010; Fakhri et al., 2012). Older adults show greater BOLD activation in this prefrontal region that expands to the contralateral site when compared to young individuals (Cabeza et al., 2004). The greater BOLD activation in older versus young individuals may be related to a compensatory activation of neurons in this region during a working memory task. Interestingly, it was previously shown that the BOLD response to a high load working memory task is higher and more lateralized (to the right hemisphere of the laterodorsal prefrontal cortex) than in a low load working memory condition (Rypma and D'Esposito, 1999). More recent work in older individuals has shown that this pattern may vary, with high cognitive load eliciting weaker activation (failure to meet demands) and low load activating the region more strongly (compensation for the functional loss; Cappell et al., 2010; Toepper et al., 2014). It is unclear if the expansion is in fact compensation in order to facilitate behavior, or is a sign of decreased specificity, and/or a sign of the impairment. Comparable studies have not been carried out in rats in order to address this matter more directly by manipulation of frontal cortical brain areas (Liang et al., 2015). Therefore, this is an area that would greatly benefit from opto-fMRI studies in rodents. During working memory tasks, the discharge activity of some cells does not increase to the same extent in aged rats (Caetano et al., 2012) and monkeys (Wang et al., 2011). In turn, the shift in discharge activity is thought to result from a shift in the balance of excitatory/inhibitory synaptic activity (Luebke et al., 2004; Guidi et al., 2015b), including the loss of dendritic spines (Dumitriu et al., 2010). If the BOLD expands in older humans, then the decline in discharge activity would not directly explain this. An alternative would be that decreased activity in the region may result in decreased lateral inhibition, permitting increased activity of other regions (thus the expansion of BOLD to other areas). Such a shift in the balance of excitatory/inhibitory synaptic activity could explain the expansion, and is an intriguing target for optogenetic manipulations in aged rats.

In summary, fMRI studies designed to activate the hippocampus of rats reveal that causally driving afferent inputs to the dentate gyrus via the perforant path increases BOLD in this region, and in downstream areas, and this appears to involve dynamic processing of synaptic activity. Variations in

BOLD associated with high frequency pattern stimulation are likely due to synaptic plasticity in local circuits. These plasticity mechanisms, which can be engaged during learning or due to pathology such as epilepsy, influence the spread of neural activity to connected regions. Finally, a decrease in synaptic strength or plasticity, or a shift in the balance of excitatory/inhibitory synaptic activity may underlie changes in the BOLD response in association with cognitive aging.

IMAGING RESTING STATE NETWORKS INVOLVED IN MEMORY

Resting state fMRI is becoming a highly valuable strategy for characterizing neural circuits involved in learning and memory, especially when measures of behavioral performance on cognitive tasks are also assessed. Resting state connectivity provides information on intrinsic functional brain organization, which under baseline conditions involves correlated BOLD signals between specific subsets of brain areas (e.g., default, executive, salience networks can be assessed). Similarity in resting state functional connectivity of the hippocampus is observed between humans and animals models, including rodents, rabbits and monkeys (Becerra et al., 2011; Hutchison et al., 2011; Jonckers et al., 2011; Schroeder et al., 2016). Resting state connectivity has been examined in awake marmoset and rhesus monkeys and in anesthetized macaques. Effective connectivity (which estimates directionality of connectivity) between hippocampus and parietal cortex increases during memory retrieval in awake macaques (Miyamoto et al., 2013). Areas of the default mode network (e.g., medial and lateral parietal areas, anterior and posterior cingulate, and medial prefrontal cortex) exhibit decreases in activity during performance of goal-directed and attention-demanding tasks, and show increase functional coupling when the brain is in an "idle" mode (Raichle et al., 2001). This network appears to be active in anesthetized monkeys (Hutchison et al., 2011; Mantini et al., 2011, 2013) and rats (Upadhyay et al., 2011; Lu et al., 2012), and activity is decreased as monkeys attend to external stimuli (Mantini et al., 2011). Macaques and humans have a homologous temporal-parietal resting state network that involves parahippocampal areas, retrosplenial, posterior cingulate, superior temporal gyrus, and posterior parietal cortex, which may be involved in mnemonic processes (Vincent et al., 2010). Some of these areas are also part of the default network and this further strengthens the notion that this system is preserved across species of primates and rodents (Kojima et al., 2009; Lu et al., 2012), although a difference in the role of the striatum within the default system has been reported between human and non-human primates (Kojima et al., 2009).

Functional connectivity networks are increasingly used to assess network-level alterations associated with learning and memory and conditions of impaired cognition including aging. In rats, it has been shown that training-induced improvement in performance on a Morris water maze (MWM) task is associated with increased connectivity within hippocampal regions and between the hippocampus and other memory associated regions such as the septum, retrosplenial cortex, entorhinal cortex, and

task associate regions such as the visual and motor cortices and thalamus (Nasrallah et al., 2016). The increased connectivity between these regions was again reduced 7 days after the last MWM session, suggesting a waning of memory associated network BOLD activity (Nasrallah et al., 2016). In humans, resting state connectivity is reduced with age (Achard and Bullmore, 2007; Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Wu et al., 2011; Dennis and Thompson, 2014; Song et al., 2014; Ferreira et al., 2015; Huang et al., 2015; Li et al., 2015; Scheinost et al., 2015; La et al., 2016). Thus, one possibility is that a decrease in resting state connectivity is an indication of impaired memory formation or consolidation during aging. In a study that examined changes in resting state connectivity, older rats exhibited a postoperative impairment in cognition associated with decreased resting state connectivity, which recovered over time (Xie et al., 2013). In contrast, results obtained in middle age non-human primates showed increased connectivity strength between hippocampus and neocortical areas in animals with low memory performance scores (Koo et al., 2013). These animals showed reduced white matter integrity, suggesting that loss of memory performance with aging is associated with increased functional connectivity to compensate for structural white matter losses (Koo et al., 2013). Similarly, it should be noted that patients diagnosed with mild cognitive impairment exhibited increased connectivity between hippocampal and prefrontal regions, which the authors suggest is a result of a maladaptive reorganization of the brain (Gardini et al., 2015). It is thus possible that increased hippocampal functional connectivity reflects compensatory increases in neuronal activity in temporal lobe and neocortical networks of middle aged individuals, or individuals with milder forms of cognitive impairment. Although not yet determined, such compensatory neuronal activity might fail at later ages, or with the progression of senescent synaptic function with more advanced age and/or dementia.

In sum, experimental paradigms recruiting memory systems in normal aging may modify patterns of resting state functional connectivity across specific functional networks involving default mode and temporal lobe areas that are preserved across species (hippocampal areas in case of rats). While there is an extensive literature linking aspects of Alzheimer's disease and other forms of neurodegenerative dementia's to alterations in these networks, normal aging connectivity patterns need further investigation. Of note is the fact that functional connectivity analysis is based on statistical correlation methods and, as a result, limits the establishment of causal links to cellular and synaptic mechanisms. In spite of this limitation, future animal imaging studies should further define links between neuronal aging mechanisms and distinct functional connectivity patterns associated with impaired cognitive function.

FUNCTIONAL IMAGING AND NEUROVASCULAR COUPLING DEFICITS IN COGNITIVE AGING

Neurovascular coupling is a critical aspect of BOLD fMRI that can be impacted by cellular and molecular events altered in

aging, especially as it relates to vascular mechanisms (D'Esposito et al., 1999b). This in turn could directly affect cognitive performance, even in the absence of data indicating impairments in synaptic plasticity. Cerebral metabolic rates for oxygen, arterial perfusion and blood volume changes contribute to the BOLD effect and may all be independently influenced by an aging cerebrovasculature (Mehagnoul-Schipper et al., 2002). Also, supporting cells, such as astrocytes, and the expression of vasoactive molecules, which play an important role in neurovascular coupling (Takano et al., 2006; Drake and Iadecola, 2007) may also be affected by aging mechanisms and in turn affect functional MRI results. Functional MRI alterations in the aging brain may, therefore, be influenced not only by changes in synaptic activity and strength, but may also occur as a result of changes in neurovascular coupling.

Aged rats show lower oxyhemoglobin, CBF and percent increases in BOLD signal in cortex in response to hypercapnia than young rats (Desjardins et al., 2014). These perfusion deficits worsen with age, and even more so with hypertension (Lee et al., 2011, 2014). Interestingly, deficits in response to hypercapnic challenge show a linear relationship with mild cognitive impairments in aged rats and are thought to be predictive of reduced performance on cognitive tasks (Mitschelen et al., 2009). Direct effects of aging on neurovascular uncoupling may contribute to reductions in cognitive performance, even in the absence of a change in synaptic function. Inhibiting the vasoactive signaling molecules cyclooxygenase-2, epoxygenase, and nitric oxide synthase (NOS) reduced CBF in response to whisker barrel stimulation. Reduced CBF was in turn associated with reduced performance in Y- and T-maze tasks and object recognition in the absence of altered synaptic strength (Tarantini et al., 2015). Cerebrovascular insufficiency has been shown to be associated with reduced performance on a MWM task, increased CA1 neuron damage, increased glial acidic fibrillary protein (GFAP) expression, reduced hippocampal blood flow, and increased ³¹P-phosphomonoester, which may be an indicator of altered membrane phospholipid turnover rates (de la Torre et al., 1992). The results suggest that vascular impairments with age might lead to blunted BOLD signal responses compared to young adults and contribute to impaired cognition.

In aged animals the BOLD response is linked to several biological markers that are thought to contribute to cognitive deficits. Transcriptional profiling in vulnerable brain regions has revealed a relationship between age, cognitive function and gene expression (Prolla, 2002; Blalock et al., 2003; Loerch et al., 2008; Zeier et al., 2011). In general, aging is associated with increased expression of genes associated with the immune response and a decrease in expression of genes linked to synaptic connectivity and neural activity. Gene changes are relatively region-specific and suggest regional vulnerability to aging (Wang and Michaelis, 2010; Zeier et al., 2011). The regional specificity for an altered BOLD response suggests that the blunted BOLD signal may be due to local changes in synaptic function, metabolism, and neuroinflammation associated with these gene expression changes (Blau et al., 2012; Moreno et al., 2012; Sanganahalli et al., 2013). In support of this notion, several recent studies have combined gene manipulations with neuroimaging to understand

the relationship between transcriptional markers of aging or neurodegenerative disease and the progression brain changes (Song et al., 2004; Maheswaran et al., 2009; Moreno et al., 2012; Lewandowski et al., 2013; Pavlopoulos et al., 2013; Zerbi et al., 2014; Micotti et al., 2015; Sevgi et al., 2015). Mutation or absence of the cholesterol transporter protein apolipoprotein- ϵ (ApoE) is associated with deficits in functional connectivity and in CBF in the mouse hippocampus (Zerbi et al., 2014). The functional impairments are associated with increased mean diffusivity, which in turn are linked to synaptic loss and presence of pro-inflammatory cells in the region. In one study, a decline in the expression of histone binding protein RbAp48 was observed specific to the dentate gyrus of humans and mice over the course of aging (Pavlopoulos et al., 2013). Expression of a dominant-negative inhibitor of RbAp48 resulted in impaired memory and a decrease in BOLD activity within the dentate gyrus suggesting that altered histone regulation underlies cognitive impairment and/or decreased BOLD activity. The idea that age-related cognitive impairments are associated with a decrease in activation of the dentate gyrus is supported by work in a primate model of aging showing reduced CBV, which was associated with reduced expression of the neural activity marker *Arc* (Small et al., 2004). Thus, the above-cited studies appear to arrive at a consensus that age related reductions in CBF are particularly robust in the dentate gyrus. This represents a promising direction for preclinical imaging research.

IN VIVO HIPPOCAMPAL AND CORTICAL VOLUMETRIC CHANGES ASSOCIATED WITH AGING

A reduction in synaptic connectivity in the hippocampus with aging may consequently produce atrophy of this structure, impair memory functions, and this may explain not only impaired BOLD fMRI and vascular changes, but also volumetric changes in this region. There is currently a debate as to whether cognitive aging is associated with a decline in hippocampal volume in humans (Jernigan et al., 1991; Golomb et al., 1993; Sullivan et al., 1995; Raz et al., 2000), non-human primates (Shamy et al., 2006; Willette et al., 2013), and dogs (Su et al., 1998; Tapp et al., 2004; Kimotsuki et al., 2005). In aged Rhesus macaques, spatial memory was not associated with the size of the hippocampus; although, expression of the synaptic marker synaptophysin was reduced in animals with impaired memory (Haley et al., 2012). In contrast, another study indicated that gray matter areas of the prefrontal and temporal cortices of macaques show age related reductions in volume accompanied by reduced performance on delayed non-match to sample task (Alexander et al., 2008; Wisco et al., 2008). Synaptic proteins and mRNA levels, and hippocampal volumes, decline in aged-memory impaired rats, with volumes lower in aged and middle age rats compared to young rats (Smith et al., 2000; Blalock et al., 2003; Driscoll et al., 2006). The decline in hippocampal volume correlated with reduced performance on a MWM task. Similarly, transgenic mice expressing ApoE4 show greater aged related reductions in hippocampal and cortical volumes, and

also suffer greater cognitive deficits than wild-type mice (Yin et al., 2011). The reduced hippocampal volume is associated with increased microglial marker *iba1* and tumor necrosis factor α , suggesting a role for neuroinflammation. Finally, aged lemurs that performed poorly on shifting and discrimination tasks also show significant volumetric reductions in caudate-putamen, hippocampus, septum, and temporal, occipital and cingulate cortices (Picq et al., 2012). Volumetric reductions may be influenced by co-occurring conditions affecting the aging population. For instance, using a heart failure model, Suzuki et al. (2015) showed a significant reduction in gray matter volume in rats with coronary ligation. There is also promising evidence suggesting that physical activity may ameliorate reductions in cortical and hippocampal volumes (Fuss et al., 2014; Mariotti et al., 2014; Sumiyoshi et al., 2014).

Volumetric changes can be linked to functional changes through the use of manganese enhanced MRI (MEMRI) in animal studies (Koretsky and Silva, 2004). The paramagnetic manganese ion (Mn^{2+}) enters voltage dependent Ca^{2+} channels (VDCC), which are pervasively present on synapses across the brain. It is therefore used as a surrogate marker of synaptic activity during baseline conditions and following chronic disease states, memory tasks, or drug stimulation (Pautler et al., 2003; Pautler, 2004). Intra-synaptic sequestering and transsynaptic transport allows for the measurement of neural circuit activity-associated increases in signal intensity in high resolution T_1 weighted images. A popular application of MEMRI is to quantify rates of signal intensity change in major fiber pathways in order to indirectly estimate *in vivo* brain axonal transport rates (Bearer et al., 2007; Smith et al., 2007, 2010, 2011; Kim et al., 2011). Using this methodology, Cross et al. (2008) showed a significant decline in olfactory pathway transport rates in aged vs. young and middle aged rats. This has been demonstrated as well in mouse models of amyloidosis, tauopathy, and neurodegeneration (Serrano et al., 2008; Majid et al., 2014).

Interestingly, given its mechanism involving VDCC uptake, this imaging strategy can provide an indication of Ca^{2+} regulation (Lu et al., 2007; Berkowitz et al., 2014; Groschel et al., 2014). Dysregulation of Ca^{2+} during aging is thought to underlie changes in cell excitability (Landfield, 1988; Disterhoft et al., 1993; Foster, 2007; Thibault et al., 2007; Kumar et al., 2009; Oh and Disterhoft, 2010) and the senescence of synaptic function (Foster and Norris, 1997; Kumar et al., 2009; Foster, 2012). The MEMRI technique has been employed to demonstrate increased Ca^{2+} of sensory systems associated with an age-related impairment of sensory processing (Bissig et al., 2013; Groschel et al., 2014). For example, 13- to 18-month-old mice with significant hearing loss show greater accumulation of Mn^{2+} signal in auditory networks and the hippocampus relative to 3-month-old mice (Groschel et al., 2014). An increase in MEMRI signal intensity in the pyramidal cell layer of the CA1 is also observed in 6- to 18-month-old rats (Bissig and Berkowitz, 2014). However, it is unclear if the changes represent accumulation in active neurons, active synapses, or glial cells (Nairismagi et al., 2006; Hsu et al., 2007; Immonen et al., 2008; Eschenko et al., 2010; Perez et al., 2013; Zhang et al., 2015). For example, an increase in Mn^{2+} signal intensity in dorsal CA1 and dentate

gyrus of mice showing neurodegeneration and forebrain atrophy might be associated with increased presence of glial cells in the region (Perez et al., 2013) and an increase in the area of Mn^{2+} intensity was observed at the mossy fiber to CA3 synaptic terminal region following a learning (Zhang et al., 2015). In spite of these interesting results, a significant limitation of MEMRI is the neurotoxic effects of Mn^{2+} on dopamine neurons (Aschner et al., 2007), and its actions as a glutamatergic NMDA receptor blocker, both of which may interfere with its intended use of measuring neuronal activity and aging related changes in neuronal activity (Guilarte and Chen, 2007; Liu et al., 2013). Furthermore, because Mn^{2+} competes with Ca^{2+} , it will have effects on Ca^{2+} -dependent processes including the release of transmitter and possible synaptic plasticity (Eschenko et al., 2010). Systemic administration of Mn^{2+} may also affect overall health and chronically affect weight gain in rats (Jackson et al., 2011), thus further reducing the utility of this method for longitudinal MRI studies.

PHARMACOLOGICAL MRI OF POTENTIAL COGNITIVE MODULATORS

BOLD fMRI, arterial spin labeling, and superparamagnetic iron oxide nanoparticle based functional imaging of blood volume are also used for *in vivo* measurement of drug-induced brain activation. The use of these modalities initially started with administration of psychoactive substances in studies of drug abuse and addiction (Mandeville et al., 1998; Marota et al., 2000; Schwarz et al., 2003; Febo et al., 2004, 2005a,b), but over the last decade other applications, particularly the testing of modulators of cognitive function and mood has emerged. For instance, one of the key mechanisms involved in LTP is an increase in AMPA receptor-mediated synaptic currents through the insertion of AMPA receptors into the post-synaptic terminal (Luscher et al., 2000). Drugs that enhance AMPA mediated effects can thus be considered to be potential targets for modulating memory through a well-defined synaptic mechanism. Administration of an AMPA receptor agonist (LY404187) increased BOLD activation largely in the dorsal hippocampus and septum and this was blocked by pretreatment with the AMPA/kainite antagonist LY293558 (Jones et al., 2005). This is consistent with previous work with the same AMPA agonist compound showing increases in cerebral metabolic rates for glucose and *c-fos* expression in the same regions (Fowler et al., 2004). Cholinergic modulation in the brain has also been assessed using drugs that activate muscarinic and nicotinic receptors (Hoff et al., 2010, 2011; Haley et al., 2011; Bruijnzeel et al., 2014). Experiments focusing on the cholinergic system show pronounced thalamocortical activation, which is very low in studies examining AMPA receptor activation. This shows the capacity of combining pharmacology and fMRI to distinguish among these two drug classes acting through different receptor systems. Bruijnzeel et al. (2014) showed dose-dependent nicotine-induced BOLD activation of anesthetized rat brain, which was blocked by the general nicotine receptor antagonist mecamylamine (Bruijnzeel et al., 2014). Administration of the non-specific muscarinic receptor antagonist scopolamine to aged

monkeys resulted in greater increases in hippocampal BOLD signal, but only in animals performing well on spatial maze task (Haley et al., 2011). This correlated with greater levels M1 but not M2 receptor density in greater performing than poorly performing animals.

In humans, an age-related decline in activity within the posterior brain regions including the hippocampus is associated with increased activity in the prefrontal cortex (Sperling, 2007; Kaufmann et al., 2008; Park and Reuter-Lorenz, 2009; Turner and Spreng, 2012; Lighthall et al., 2014; Tromp et al., 2015). Interestingly, inhibition of NMDA receptors in the hippocampus or prefrontal cortex drives activity in the prefrontal cortex (Jodo et al., 2005; Homayoun and Moghaddam, 2007). Furthermore, low level NMDA receptor blockade impairs hippocampal function and improves executive processes that depend on the prefrontal cortex (Guidi et al., 2015b). Chin et al. (2011) showed that ketamine, an amnesic/dissociative agent that blocks NMDA receptors produced robust activation of cortical regions and the hippocampus of awake rats. This effect was modulated by the glutamate metabotropic agonist LY379268 (Chin et al., 2011). More recent work has confirmed that ketamine increases functional interactions between brain regions involved in memory (hippocampus, areas of the limbic prefrontal cortex, and retrosplenium; Gass et al., 2014; Grimm et al., 2015). These studies illustrate the potential for pharmacological MRI to investigate brain wide activation in response to cognitive modulators.

DIFFUSION BRAIN IMAGING IN NORMAL AGING RATS

Compared with fMRI, diffusion MRI has been applied more extensively to the study of animal models of neurodegenerative diseases. However, we will mostly focus here on dMRI studies relevant to normal cognitive aging. In diffusion MRI, directionally applied diffusion-sensitizing magnetic field gradients tag protons in slowly moving (diffusing) water molecules (in the order of $10^{-3} \mu m^2/s$), and thus omit water moving at faster rates (e.g., inside blood vessels, as measured in the above-cited fMRI modalities; Le Bihan et al., 2001). The fractional anisotropy (FA) index is one of the main scalars estimated from a series of diffusion-sensitized MRI images. FA has been used extensively as an indicator of underlying tissue microstructural integrity (Mori and Zhang, 2006). This value is most reliable when assessed in major white matter (WM) tracts in rodent brain at high fields, although a growing number of studies are also reporting FA for gray matter regions. FA values range from 0 to 1, with 1 indicating *high directionality* of water diffusion (anisotropic diffusion) and 0 *low directionality* (isotropic diffusion). Thus, lowest FA values are measured from cerebroventricles (because of the high mobility of unbound water molecules have in this compartment), whereas highest values are measured in WM fiber bundles, such as the corpus callosum, fimbria, internal capsule, where water molecules show a high net directionality due to the presence of highly organized barriers to diffusion formed by myelinated axonal fibers. Reductions in

myelination, increases in fluid filled inter-axonal spaces, altered cellular density, local cellular inflammatory responses, and edema can all reduce FA (Peled, 2007). Reduced FA is thought to represent WM alterations in aging and pathologies of Alzheimer's disease in humans (Bozzali et al., 2001; Teipel et al., 2010; Douaud et al., 2011, 2013). Recent evidence for a link between reduced FA and pathology was provided by studies in a mouse overexpressing microtubule associated protein tau (Sahara et al., 2014). Similar to human studies, the results demonstrate a progressive reduction of FA in WM structures of the tau-expressing mice. Changes in FA were associated with an increase in tau pathology and disorganization of unmyelinated processes. Indeed, changes in dMRI were detectable as early as 2.5 months, before the emergence of obvious overt pathology. Similar age-associated reductions in FA (and concomitant increases in diffusivity scalars, axial, radial, and mean diffusivities) have been reported in a transgenic rat model of Huntington disease (Antonsen et al., 2013), and in corpus callosum of normal aged rats (Guo et al., 2015). In spite of these correlations, the mechanistic basis for changes in FA still remains unclear.

In humans, it has been known for many years that WM content in brain shows an inverted U maturational change that peaks at middle age (45 years of age). This was first demonstrated in postmortem tissue and subsequently supported by Bartzokis et al. (2004) using MRI. The loss of WM begins early in middle-age in humans and rhesus monkeys, with a prolonged decline during aging (Makris et al., 2007; Westlye et al., 2010; Yeatman et al., 2014). In contrast, WM loss is initiated much later for chimpanzees, suggesting that older chimpanzees exhibit decreased atrophy relative to humans (Chen et al., 2013). Rodents show a age progressive change in WM similar to humans, occurring at earlier stages in the rodents lifespan, with a steep rise in FA from 0–40 days (ending at mid adolescence), and a gradual but progressive decline thereafter (Calabrese et al., 2013). Interestingly, mean diffusivity peaks earlier between 10–20 days of age and then remains stable, or shows a steady decline (Mengler et al., 2014), at least until day 80 (Calabrese et al., 2013). FA values in outer cortical layers of developing rat brain is reduced during the first 10 postnatal days (Huang et al., 2008). Therefore, diffusion MRI can distinguish between non-WM maturational changes. Thus, these represent early life maturational changes, perhaps associated with early brain development (Mengler et al., 2014). Synaptic and axonal pruning and increases in myelination of major fiber tracts account in part for these early life changes in FA and mean diffusivity (Huang et al., 2008). Compared to 3-month-old rats, 12-month-old rats show lower apparent diffusion coefficient (ADC) values in cortex (Heiland et al., 2002). ADC mapping is typically used in preclinical stroke research, with reduced gray matter ADC values indicative of early hemorrhagic events and high values indicative of progressive edematous tissue damage. Aged rats sustaining transient global ischemia also show greater reductions in ADC than young animals imaged under the same conditions (Canese et al., 1998, 2004). Hypotension-associated with a single hemorrhage event causes a greater reduction in hippocampal ADC in 18-month-old compared to 12-month-old rats (Plaschke et al., 2009). Thus, vascular events that increase in risk with age

are observed to alter diffusion MRI indices of tissue integrity. While this is an area that needs further investigation, it points to the possibility of developing the diagnostic capabilities of diffusion MRI as a technique that offers tissue quantitative measures that could assess risk or vulnerability in aging brain under specific challenges. For example, age-related reduction in FA in corpus callosum is prevented in aged rats subjected to a caloric restriction (Guo et al., 2015).

PROTON MAGNETIC RESONANCE SPECTROSCOPY

A major advantage of ultra-high field imaging (7 T and above), apart from the greater signal-to-noise, is the improved capacity to resolve or separate the chemical shift peaks of various biomolecules involved in neurotransmitter metabolism in cells (Di Costanzo et al., 2003; Moser et al., 2012). MRS, particularly involving hydrogen (^1H) nuclei, has been used for years to assess various chemical species in brain diseases, both neuropsychiatric and neurologic conditions (Maddock and Buonocore, 2012; Rossi and Biancheri, 2013). Imaging techniques have better spatial resolution than MRS techniques, but ^1H -MRS offers strong complementary data because of its specificity and quantitative capabilities, which permit the assessment of tissue concentrations of distinct biologically relevant molecules and metabolic intermediates. ^1H -MRS can detect molecular markers for neurons and glia, transmitters, and antioxidant capacity (see **Table 1**).

For example, *N*-acetylaspartate (NAA) and myo-inositol are measured as neuronal and glial markers, respectively (Demougeot et al., 2004; Harris et al., 2015). Following experimental ischemia, a decline in NAA is observed in the most vulnerable brain regions and correlates with cell loss (Higuchi et al., 1997; Sager et al., 2001). However, there is also evidence for a decline in NAA not linked to neuronal loss (Jenkins et al., 2000). NAA is synthesized in the mitochondria and a rapid decline in NAA following ischemia may represent impaired neuronal function, which can recover during reperfusion (Demougeot et al., 2004). Indeed, minor levels of hippocampal cell loss following mild ischemia was not associated with a change in hippocampal NAA (Galisova et al., 2014).

In Alzheimer's disease, a decline in NAA is observed in hippocampus and posterior cingulate in association with a loss of synaptic markers and increased hyperphosphorylated tau, consistent with a neuronal loss. Interestingly, increased myo-inositol levels were more prominent in the cortex in association with amyloid-beta levels, suggesting activation of glial markers may precede neuronal loss (Murray et al., 2014; Wang et al., 2015). In this case, the increase in glial markers may be an early sign, possibly representing neuroinflammation. Indeed, APP/PS1 transgenic mice, an increase in myo-inositol precedes the decline in NAA and changes in both markers precede cognitive impairment (Chen et al., 2012).

In the case of normal aging in humans and in animal models of brain aging, an increase in glial markers, possibly as a sign of neuroinflammation, is observed in the absence of

TABLE 1 | Proton MRS markers relevant to aging, inflammation, neurodegeneration, and excitatory neurotransmission.

Molecule or metabolic intermediate	Marker for	Age-related change
NAA	Neuronal health	No change with normal aging, decreased in neurodegenerative disease
myo-inositol	Glia	Increased in normal aging, possibly as a sign of neuroinflammation
Acorbate, GSH	Oxidative stress	Generally decreased with age, indicating brain regions that are vulnerable to oxidative stress
Glutamate, GABA	Neurotransmitters	Region specific changes may reflect a shift in the balance of excitatory/inhibitory transmission

profound neuronal loss. Studies of ^1H -MRS profiles in aging humans indicates no change or a small decrease in NAA and an increase myo-inositol, consistent with an increase in glial markers (Boumezbeur et al., 2010). Similar changes are observed in ^1H -MRS profiles of aging non-human primates (Herndon et al., 1998; Ronen et al., 2011) and rodents (Driscoll et al., 2006; Duarte et al., 2014; Harris et al., 2014), consistent with an increase in astrocytic markers associated with chronological age. A consistent observation of aging in humans and animal models, is a persistent low level increase in serum markers of inflammation and this pro-inflammatory phenotype is thought to influence neuroinflammation, the activation glial cells, astrocytes and microglia, and may contribute to age-related cognitive decline (Rafnsson et al., 2007; Gimeno et al., 2008; Bettcher et al., 2012; Scheinert et al., 2015). Studies that employ ^1H -MRS and correlate brain levels of myo-inositol with measures of systemic inflammation indicate a positive relationship (Eagan et al., 2012; Schneider et al., 2012). Interestingly, just as with neurodegenerative disease and aging, not all brain regions are equally vulnerable to the effects of systemic inflammation (Semmler et al., 2005; Silverman et al., 2014; Scheinert et al., 2015) and regional markers of inflammation, including levels of myo-inositol correlate with behavioral changes (Schneider et al., 2012; Scheinert et al., 2015).

One important goal for the neurobiology of aging is to understand regional differences in neuronal vulnerability to aging (Jackson et al., 2009; Wang and Michaelis, 2010; Zeier et al., 2011). Total choline levels may reflect membrane turn over including demyelination and inflammation and brain levels increase with age in humans and animal models (Duarte et al., 2014; Harris et al., 2014). However, it is unclear whether the age-related increase in total choline is due to membrane turnover or linked to functional changes including cognitive decline (Charlton et al., 2007). Thus, animal studies could provide a rich source for hypotheses concerning the cellular and molecular constituents of total choline measures, as well as a test of regional vulnerability. Oxidative stress increases with advancing age and oxidative damage may contribute to neuronal death associated with neurodegenerative disease. Recent work indicates that the oxidation–reduction (redox) status of neurons underlies senescent physiology including impaired synaptic plasticity and the emergence of cognitive impairment (Bodhinathan et al., 2010a,b; Kumar and Foster, 2013; Lee et al., 2014; Guidi et al., 2015a). ^1H -MRS can be employed to examine redox status by measuring the level of antioxidant molecules, ascorbate and glutathione (GSH). GSH is mainly observed in astrocytes (Slivka et al., 1987; Keelan et al., 2001) and an increase in GSH

could indicate activation of astrocytes. Alternatively, a decline in GSH could signal an increase in oxidative stress. In rats, maturation is associated with an increase in the level of GSH in the prefrontal cortex and prenatal immune activation interferes with an inability to increase GSH levels (Vernon et al., 2015). In general, aging is associated with a decline in antioxidant molecules in the brain. Moreover, the decline is regionally selective and can vary by gender (Terpstra et al., 2006; Emir et al., 2011; Duarte et al., 2014; Harris et al., 2014), which provides grist for hypotheses concerning vulnerability to aging and neurodegenerative disease.

Finally, ^1H -MRS can detect the level of certain transmitters. The most common measures are for glutamate, aspartate, and gamma-aminobutyric acid (GABA). Changes in transmitter levels are observed in several animal models of neurological diseases (Biedermann et al., 2012; Bagga et al., 2013; Berthet et al., 2014; Santin et al., 2014). In some cases, the animal models exhibit a good correspondence with the human condition. For example, altered levels of glutamate are observed in the prefrontal cortex of schizophrenia patients and animal models of schizophrenia (Iltis et al., 2009; Maddock and Buonocore, 2012; Puhl et al., 2015). Similarly, a decrease in prefrontal cortex glutamate is observed in depressed patients and in animal models of stress (Knox et al., 2010; Hemanth Kumar et al., 2012; Drouet et al., 2015). Examination of transmitters over time may give clues to mechanisms and how processes change over time. A decline in both glutamate and GABA show a progressive decline in animal models of Alzheimer's disease (Nilsen et al., 2012) and a differential decline in glutamate and GABA is observed during the development of temporal lobe epilepsy (van der Hel et al., 2013). An increase in glutamatergic transmission may contribute to the development of Parkinson's disease. MRS studies examining glutamate levels in the striatum of humans with Parkinson's disease do not agree possibly due to the etiology, stage of the disease, or pharmacological intervention (Kickler et al., 2007; Griffith et al., 2008; Modrego et al., 2011). Similarly, in animal models differences in glutamate levels may depend on the etiology or the animal model (Delli Pizzi et al., 2013). For example, treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine decreased glutamate levels in felines (Podell et al., 2003), no change in canines (Choi et al., 2011), and increased glutamate levels in mice (Chassain et al., 2008, 2013). Even when examined in the same animal model differences may arise associated with the power of the magnet (Kickler et al., 2009; Coune et al., 2013).

Regional differences in transmitter levels are associated with aging in humans (Grachev et al., 2001; Gao et al., 2013;

Zahr et al., 2013; Riese et al., 2015). In male rats, aspartate and glutamate exhibited a decline with in specific brain regions, which may reflect a shift in the balance of excitatory and inhibitory transmission (Harris et al., 2014). In contrast, measures of both GABA and glutamate or aspartate declined in an aging male and female mice (Duarte et al., 2014). Future studies will need to investigate these discrepancies which may include regions examined and sex differences in glutamate over the course of aging (Zahr et al., 2013), as well as variability associated with variability in cognitive decline.

CONCLUDING REMARKS

One of the key aspects of aging brain is a gradual loss of memory to the point where this affects the individuals' normal daily activities (Gauthier et al., 2006). In normal aging cognitive impairment can be progressive, and while not necessarily entailing a severe loss of neurons, as in neurodegenerative diseases, the animal literature supports alterations in neuronal activity (Kumar and Foster, 2007; Watson et al., 2012; Foster et al., 2016). Indeed, these changes may involve alterations in excitatory/inhibitory balance, changes in synaptic proteins and intracellular signaling mechanisms, and spine density and morphology (Foster et al., 1996, 2000, 2012, 2016; Foster and Norris, 1997; Foster and Dumas, 2001; Blalock et al., 2003; Foster, 2007, 2012; Kumar and Foster, 2007, 2013; Kumar et al., 2009; Dumitriu et al., 2010; Guidi et al., 2015a,b). Translating

these cellular mechanisms of animal aging models to human aging is a difficult challenge and it may be possible that preclinical imaging and spectroscopy studies could serve a role in this task. We have reviewed different MRI modalities used in primate and rodent models to characterize functional activation in hippocampal and prefrontal memory networks, anatomical changes and their correlations with cognitive decline, changes in neurovascular coupling with aging, and biochemical alterations relevant to aging. Results from the different MR modalities presented here can be enhanced by combining these with invasive *in vivo* and *ex vivo* approaches to determine their relationship to changes at the synaptic, proteomic, and genetic levels, for an integrative assessment of brain aging and reduced cognitive capacity.

AUTHOR CONTRIBUTIONS

MF and TF contributed equally to the outline, drafting and editing of the present manuscript.

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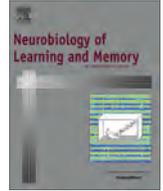
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Medial prefrontal-perirhinal cortical communication is necessary for flexible response selection

Abbi R. Hernandez^a, Jordan E. Reasor^a, Leah M. Truckenbrod^a, Katelyn N. Lubke^{a,b}, Sarah A. Johnson^a, Jennifer L. Bizon^a, Andrew P. Maurer^{a,b}, Sara N. Burke^{a,c,*}

^aMcKnight Brain Institute, Department of Neuroscience, University of Florida, United States

^bDepartment of Biomedical Engineering, University of Florida, United States

^cInstitute on Aging, University of Florida, United States

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ABSTRACT

The ability to use information from the physical world to update behavioral strategies is critical for survival across species. The prefrontal cortex (PFC) supports behavioral flexibility; however, exactly how this brain structure interacts with sensory association cortical areas to facilitate the adaptation of response selection remains unknown. Given the role of the perirhinal cortex (PER) in higher-order perception and associative memory, the current study evaluated whether PFC-PER circuits are critical for the ability to perform biconditional object discriminations when the rule for selecting the rewarded object shifted depending on the animal's spatial location in a 2-arm maze. Following acquisition to criterion performance on an object-place paired association task, pharmacological blockade of communication between the PFC and PER significantly disrupted performance. Specifically, the PFC-PER disconnection caused rats to regress to a response bias of selecting an object on a particular side regardless of its identity. Importantly, the PFC-PER disconnection did not interfere with the capacity to perform object-only or location-only discriminations, which do not require the animal to update a response rule across trials. These findings are consistent with a critical role for PFC-PER circuits in rule shifting and the effective updating of a response rule across spatial locations.

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1. Introduction

The capacity to update one's actions based on environmental contingencies is critical for adaptive behaviors. Dysfunction in this type of cognitive flexibility is associated with schizophrenia (Enomoto, Tse, & Floresco, 2011), aging (Barense, Fox, & Baxter, 2002; Beas, Setlow, & Bizon, 2013; Moore, Killiany, Herndon, Rosene, & Moss, 2003), and other disease states (Buckner, 2004; Cunha et al., 2013; Lavoie & Everett, 2001). Cognitive flexibility is supported by the dorsolateral prefrontal cortex in humans (Demakis, 2003; Owen, Roberts, Polkey, Sahakian, & Robbins, 1991) and other primates (Dias, Robbins, & Roberts, 1996a, 1996b; Moore, Schettler, Killiany, Rosene, & Moss, 2009), and the homologous medial prefrontal cortex (mPFC) in rodents (Birrell & Brown, 2000; Bissonette & Powell, 2012; Floresco, Block, & Tse, 2008; Uylings, Groenewegen, & Kolb, 2003). Specifically, the ability to extinguish a cue-driven behavior, one measure of cognitive flexibility, is mediated by increased neuronal activity within the

infralimbic region of the rodent mPFC (Burgos-Robles, Vidal-Gonzalez, Santini, & Quirk, 2007; Milad & Quirk, 2002; Quirk & Mueller, 2008), and age-related decreases in the excitability of these neurons have been linked to flexibility impairments in old animals (Kaczorowski, Davis, & Moyer, 2012).

While the prefrontal cortex, and mPFC in particular, is unequivocally involved in behavioral flexibility (Logue & Gould, 2014), this brain region does not act in isolation to update response selection. In support of this idea, damage to the hippocampus has been shown to impair performance on attentional set-shifting tasks of behavioral flexibility (Cholvin et al., 2013; Malá et al., 2015). Furthermore, both mPFC and hippocampal activity are associated with the inhibition of an incorrect response (Lee & Byeon, 2014), and functional connectivity between the frontal cortices and medial temporal lobe is involved in dynamic task switching (Clapp, Rubens, Sabharwal, & Gazzaley, 2011; Wais & Gazzaley, 2014). While these data support that communication between the mPFC and hippocampus is important for behavioral flexibility, the anatomical projections between these areas are relatively sparse (Beckstead, 1979; Sesack, Deutch, Roth, & Bunney, 1989; Vertes, 2002), suggesting that other cortical regions may be important

* Corresponding author at: University of Florida, P.O. Box 100244, 1149 Newell Dr, Gainesville, FL 32610, United States.

for updating associations between sensory information and desirable outcomes.

A candidate brain region that could be critical for facilitating flexible response selection is the perirhinal cortex (PER), an area within the medial temporal lobe that receives direct input from all sensory modalities (Burwell & Amaral, 1998a; Suzuki & Amaral, 1994). The PER is involved in both memory (Buffalo et al., 1999; Suzuki, Zola-Morgan, Squire, & Amaral, 1993) and higher-order sensory perception (Barense, Gaffan, & Graham, 2007; Barense, Ngo, Hung, & Peterson, 2012; Bartko, Winters, Cowell, Saksida, & Bussey, 2007a, 2007b). Moreover, the PER shares reciprocal connections with the mPFC (Agster & Burwell, 2009; Burwell & Amaral, 1998a; Delatour & Witter, 2002; McIntyre, Kelly, & Staines, 1996; Sesack et al., 1989) and the hippocampus (Naber, Witter, & Lopez da Silva, 1999; Witter, Wouterlood, Naber, & Van Haeften, 2000; Witter et al., 2000). Furthermore, communication within the mPFC-PER-hippocampal circuit is necessary for an animal's ability to detect when the relationship between an object and its spatial location has changed (Barker, Bird, Alexander, & Warburton, 2007; Barker & Warburton, 2008, 2015). Thus, the PER is positioned to contribute stimulus-specific information as well as link activity patterns in the hippocampus to the mPFC in support of flexible behavior. Consistent with this idea, mPFC activity enhances interactions between the PER and entorhinal cortex (Paz, Bauer, & Pare, 2007). As PER-entorhinal cortical interactions are believed to gate the flow of information into the hippocampus (de Curtis & Pare, 2004), mPFC modulation of rhinal cortical activity is likely critical for higher cognitive function.

Although the mPFC is necessary for an animal's ability to inhibit an incorrect response (Lee & Byeon, 2014; Lee & Solivan, 2008), and mPFC-PER communication is involved in an animal's ability to detect novel object-place associations (Barker & Warburton, 2008, 2015; Barker et al., 2007; Jo & Lee, 2010a, 2010b), it is not known if communication between these brain areas is critical for flexible behavior. The objective of the current experiments was to examine whether mPFC-PER communication is necessary for performance on the object-place paired association (OPPA) task (Jo & Lee, 2010b), which tests an animal's ability to flexibly update which of two objects is rewarded based on an incrementally learned object-in-place rule that requires knowledge of both object identity and current spatial location. After rats acquired the biconditional association, the necessity of mPFC-PER communication was investigated by infusing the GABA_A receptor agonist muscimol (MUS) into one hemisphere of the mPFC and the contralateral PER to reversibly disconnect these areas. This approach capitalizes on the fact that the mPFC and PER are densely connected within the same hemisphere, but not across hemispheres (Bedwell, Billett, Crofts, MacDonald, & Tinsley, 2015). Thus, unilateral mPFC and contralateral PER inactivation blocks communication between these areas. Importantly, because only one hemisphere of each region is inactivated, the PER and mPFC remain functional as independent entities.

2. Materials and methods

2.1. Subjects and handling

Twelve male Fischer 344 rats (NIA colony at Taconic; 6–13 mo. old) were single housed and kept on a reverse 12-h light/dark cycle, with all testing occurring during the dark phase. After histological verification of cannula placement (see below), 9 rats were included in the current analyses. Upon arrival to the facility, rats acclimated to the colony room for 7 days. After acclimation, the rats were handled by the experimenters for several days before being placed on food restriction. Rats were restricted to 85% of

their initial free-feeding body weight, with *ad libitum* access to water for the duration of the experiment. For all surgical and behavioral procedures, adequate measures were taken to minimize pain or discomfort to all animals. All protocols were in accordance with the *Guide for the Care and Use of Laboratory Animals* and the University of Florida Institutional Animal Care and Use Committee.

2.2. Habituation and training

Rats were habituated to the OPPA arena (Fig. 1A) for 10 min a day for 2 days prior to training. For all experiments, a two-arm maze constructed from wood and sealed with waterproof black paint was used (Fig. 1A; Hernandez et al., 2015). The two arms of the maze were separated by black poster board with distinctive markings on each side to prevent the rat from seeing the opposite arm while providing environmental cues to differentiate the two arms. The arms radiated from a starting platform that was 48.3 cm in diameter. Each arm was 84.0 cm long and had a rectangular choice platform (31.8 cm × 24.1 cm) attached at the end. Each choice platform contained two food wells (2.5 cm in diameter) that were recessed into the maze floor by 1.0 cm and were separated by 12.8 cm. The arms and choice platforms had 5.5 cm high walls. During habituation, food rewards (Froot Loops; Kellogg's, Battle Creek, Michigan) were scattered throughout the maze to encourage exploration. Once comfortable with foraging, rats were shaped to alternate arms by placing a single reward on the choice platform of the unoccupied arm. After the rat retrieved the reward, the opposite arm was baited. Once a rat consistently alternated 30 times in less than 20 min, they began training on the OPPA task.

2.3. Object-place paired-association task and pre-training

Rats were trained to use an object-in-place rule, in which the rewarded object of a pair was contingent on the spatial location (see, Hernandez et al., 2015; Lee & Solivan, 2008) prior to surgery for cannula placement. Rats began the trial in either the left or right arm of the maze, randomly chosen. During pre-training, rats were required to traverse between the left and right arms for a total of 32 trials, regardless of how many correct choices were made. Arms were not blocked so rats had to correctly remember to alternate. A failure to alternate was recorded as a working memory error. The incidence of this type of error was low (≤ 1 /testing session), and did not significantly vary across infusion conditions ($p > 0.05$ for all comparisons). Because rats freely alternated between the 2 arms to initiate a new trial, the inter-trial interval was based on the amount of time it took a rat to ambulate from one arm to the next and variable across trials.

The same two objects were presented in both arms of the maze, with a food reward hidden in the well beneath the correct object. For example, in the left arm, the “chicks” object was always the correct choice, regardless of whether it was presented on the left or right well (Fig. 1B). In the right arm, the “chicks” object was the incorrect choice, and instead the “frog” object was always rewarded. The locations of objects within the arms pseudorandomly varied across trials in order to ensure that the left and right wells were equally rewarded. The rat was required to push the object off of the well it was covering to retrieve the food reward beneath the correct object. If the incorrect object was pushed or touched by the rat's nose, the objects and the food reward were removed from the arm and the rat was not allowed to make another choice in that arm.

During testing, as well as during all training and control sessions, the experimenter stood behind the choice platforms so that the next trial could be set up before the rat exited the current arm it was in. The barrier located between the arms ensured that the rat could not observe which well was baited. All rats completed 32 tri-

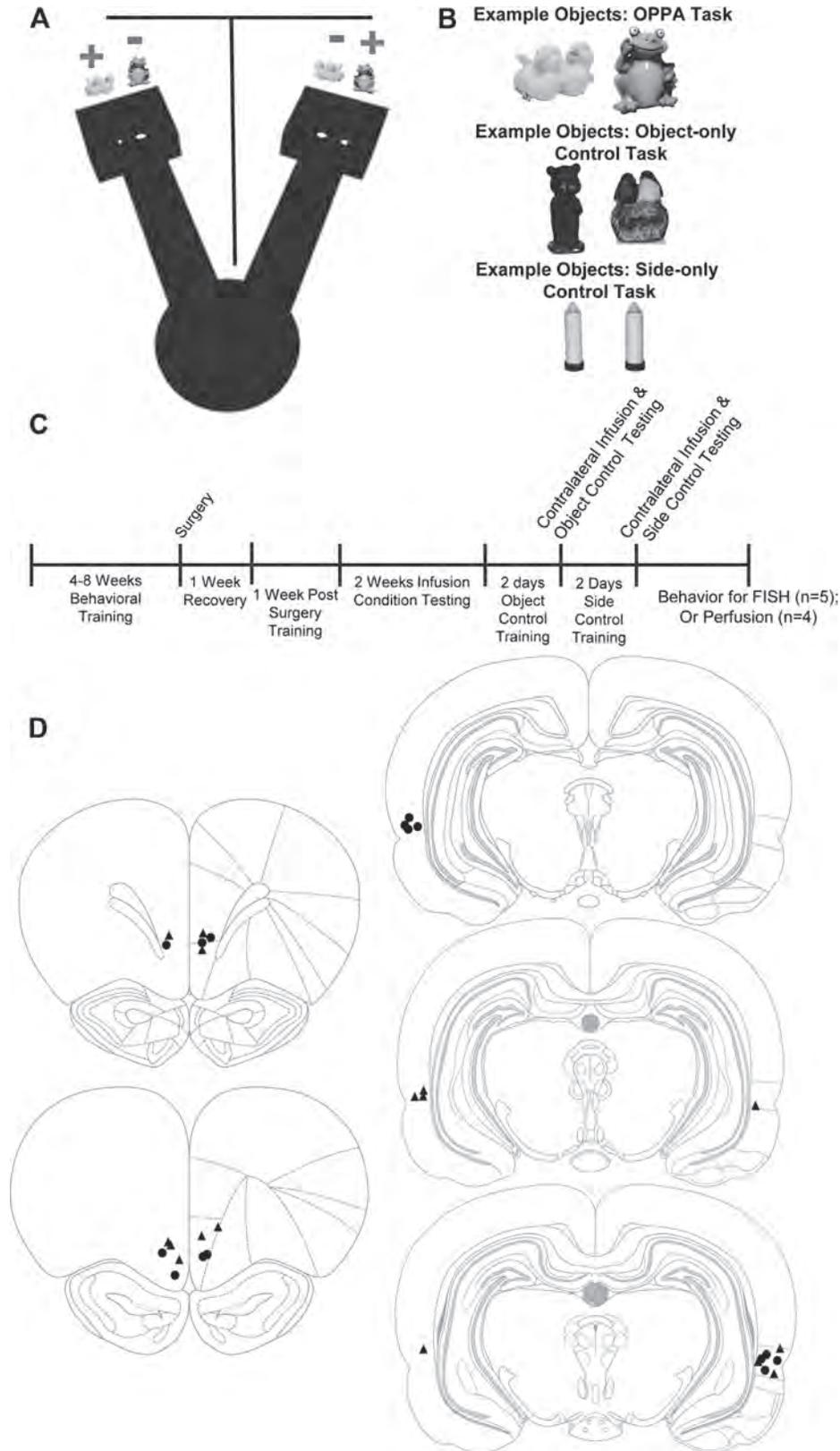


Fig. 1. Experimental design and cannulae placement. (A) The Object-Place Paired Association (OPPA) Task apparatus. Two objects were placed at the end of each arm, covering a food well with a hidden food reward underneath the correct object. The rat had to choose the correct choice in each arm while alternating back and forth. Arms were separated by black poster board with different markings on each side to prevent the rat from viewing the opposite arm and to differentiate between the two arm locations. Each object was correct in only one arm of the maze and the same objects were used within a test condition. The same apparatus was used for object-only and side-only control tasks with one arm blocked off during each task. (B) Representative objects used during the OPPA task (top), object only control task (middle), and the left versus right side discrimination control task (bottom). (C) Timeline of experimental procedures. (D) Bilateral cannulae placement in the mPFC and PER shown for each rat included in analyses. Triangles indicate the placement from animals in which tracts were verified with histology and circles represent placements from animals in which *Arc in situ* hybridization was used to verify selectivity of inactivation.

als/day for 6 days a week until they achieved criterion performance of 26/32 correct trials 2 days in a row. In cases in which there was a delay between OPPA training and cannulation surgery, rats were tested 3 days per week to maintain OPPA performance.

2.4. Surgery

Each rat was stereotaxically implanted with cannulae bilaterally targeting the mPFC (infralimbic cortex) and PER under isoflurane anesthesia (1–3%). An incision was made to expose Bregma. Small holes were drilled for the placement of 22-gauge guide cannulae (Plastics One, Roanoke, VA; C313G-L20/SPC) at +3.2 mm AP and ± 0.9 mm ML from Bregma and 3.8 mm ventral to the skull surface for mPFC, and -5.5 mm AP, ± 6.6 mm ML from Bregma and 6.5 mm ventral to the skull surface for PER. The mPFC coordinates were determined based on a previous study showing a role for the infralimbic cortex in the conceptual set-shifting task of behavioral flexibility (Beas, McQuail, Bañuelos, Setlow, & Bizon, 2016). Anchoring screws (1/8" length; 00-120) were placed in the skull and all hardware was secured with dental cement. Dust caps with dummy stylets were screwed into each cannula to prevent tubing from becoming obstructed. During surgery and post-operatively, the non-steroidal anti-inflammatory Meloxicam (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO; 1.0 mg/kg S.C.) was administered as an analgesic. All animals were given 7 days to recover before resuming behavioral testing.

2.5. Infusions

The GABA_A receptor agonist muscimol (MUS) (0.5 μ g/0.5 μ l; Sigma-Aldrich, St. Louis, MO) was injected intracerebrally with a microinfusion pump (Harvard Apparatus; Holliston, MA) for the temporary inactivation of the PER and/or mPFC. Polyethylene tubing (Plastics One) was attached to a 10 μ l syringe (Hamilton, Franklin, MA), backfilled with sterile water, and then loaded with 2 μ l muscimol. An air bubble of 1–2 μ l volume was maintained between the backfill and drug to prevent mixing, as well as ensure that the pump was infusing the correct volume of drug. Dust caps were removed and 28-gauge needles (Plastics One, 81C313ISPCXC) were placed into the proper guide cannulae. Each injector needle protruded 1 mm below the guide cannula into the brain, such that the depth of injection was 7.5 mm from the surface of the skull for the PER and 4.8 mm from the surface of the skull for the mPFC infusions. MUS was infused at a rate of 0.1 μ l/min over 5 min. Upon infusion completion, needles were left in place for a minimum of 2 min to allow diffusion of the drug. Dust caps were reinserted and the animal was returned to their home cage for a 30-min period before testing began.

2.6. Behavioral task post surgery

Fig. 1C shows the order and timeline for all experimental procedures. Once rats recovered from surgery, they were retrained on the OPPA task until performance was at or above criterion (26/32 trials, $\geq 81.25\%$ correct) for at least 2 consecutive days. A pseudo-randomized infusion schedule was used for each rat, with a baseline testing session (no infusion) between each infusion day for a total of 15 test days. The infusion conditions were: (1) bilateral mPFC MUS, (2) bilateral PER MUS, (3) left ipsilateral mPFC-PER MUS, (4) right ipsilateral mPFC-PER MUS, (5) contralateral left mPFC-right PER MUS, (6) contralateral right mPFC-left PER MUS, (7) contralateral left mPFC-right PER vehicle control, (8) contralateral right mPFC-left PER vehicle control.

Upon completion of these 8 infusion conditions, rats were trained on 2 additional control tasks. First, rats were trained on an object-only control task (Fig. 1B middle panel), during which

only one choice platform of the maze was used. In this task, rats traversed back and forth between a single choice platform and the other arm, choosing a single target rewarded object regardless of its position over the left or right well. Thus, in this task only object information was required to make a correct choice. Once the rat reached criterion performance of 26/32 trials on the object-only control task, a contralateral MUS infusion was administered 30 min prior to testing. After completion of this object discrimination control task, rats were then trained on a side-only task (Fig. 1B bottom panel) in the opposite choice platform. In this control task, the correct choice of two identical objects was determined by their placement over a single well on a specific side (left versus right). For example, a rat had to learn to always select the object over the right well, and the left well never contained the reward. The rewarded well was counterbalanced across rats. Rats performed this task until a criterion of 26/32 trials was reached and a final contralateral MUS mPFC-PER infusion was administered 30 min prior to testing.

2.7. Histology and fluorescence *in situ* hybridization

At the conclusion of behavioral testing, rats were sacrificed and tissue collected to evaluate accurate placement of the guide cannula in the PFC and PER with either standard histological techniques ($n = 7$), or by labeling the expression of the activity-dependent immediate-early gene *Arc* ($n = 5$). For the 7 rats that underwent standard histology, a lethal dose of sodium pentobarbital (Vortech Pharmaceuticals, Dearborn, MI) was administered prior to transcardial perfusion with 4% paraformaldehyde. Brains were stored in 4% paraformaldehyde with 30% sucrose at 4 °C for 72 h and then sectioned at 40 μ m on a cryostat (Microm HM550; Thermo Scientific, Waltham, MA), thaw-mounted on Superfrost Plus slides (Fisher Scientific, Waltham, MA) and nuclei were stained prior to cannulae placement confirmation with microscopy. Fig. 1D shows the cannulae placement for all rats included in the current experiments. Based on the histology 3 rats were excluded from the analyses because the guide cannula could not be localized to the mPFC and PER.

In a second subset of rats ($n = 5$), fluorescence *in situ* hybridization for the activity-dependent immediate-early gene *Arc* was used to provide a functional assay of MUS infusion specificity to the target regions. In these animals, MUS was infused unilaterally into the PFC and PER 30 min prior to sacrifice (left PFC-PER $n = 2$, right PFC-PER $n = 2$, control $n = 1$). Just prior to sacrifice, the 4 MUS infused rats performed the OPPA task for 10 min, completing an average of 46 trials ($SD = 1.22$). At the conclusion of the behavioral session, rats were deeply anesthetized with isoflurane (Abbott Laboratories, Chicago, IL) and euthanized by rapid decapitation. Tissue was extracted and flash frozen in chilled 2-methyl butane (Acros Organics, NJ). One additional rat was sacrificed directly from the home cage as a caged control. Tissue was stored at -80 °C until it processing for fluorescence *in situ* hybridization.

Fluorescence *in situ* hybridization (FISH) for the immediate-early gene *Arc* was performed as previously described (e.g. Burke, Hartzell, Lister, Hoang, & Barnes, 2012; Guzowski, McNaughton, Barnes, & Worley, 1999). Tissue was sliced at 20 μ m thickness on a cryostat (Microm HM550) and thaw-mounted on Superfrost Plus slides (Fisher Scientific). *In situ* hybridization for *Arc* mRNA was performed and z-stacks were collected by fluorescence microscopy (Keyence; Osaka, Osaka Prefecture, Japan) to confirm that target regions were inactivated and adjacent structures did not have a significant blockage of activity-dependent *Arc* induction. Briefly, a commercial transcription kit and RNA labeling mix (Ambion REF #: 11277073910, Lot #: 10030660; Austin, TX) was used to generate a digoxigenin-labeled riboprobe using a plasmid template containing a 3.0 kb *Arc* cDNA. Tissue was incubated with the probe

overnight and *Arc* positive cells were detected with anti-digoxigenin-HRP conjugate (Roche Applied Science Ref #: 11207733910, Lot #: 10520200; Penzberg, Germany). Cyanine-3 (Cy3 Direct FISH; PerkinElmer Life Sciences, Waltham, MA) was used to visualize labeled cells and nuclei were counterstained with DAPI (Thermo Scientific). Two images were taken per region from each hemisphere of all infused rats and the caged control for the PER, mPFC, lateral entorhinal cortex (LEC), area TE and anterior cingulate cortex (AC). This process was repeated on a second section of tissue from each rat. Two rats did not have tissue analyzed for the mPFC and AC, as the *Arc* signal was degraded. For these rats, cannula placement was confirmed with fluorescence microscopy.

Following FISH, z-stacks were taken at increments of 1 μm and the percentage of *Arc* positive cells was determined by experimenters blind to infusion condition using ImageJ software. In order to exclude nuclei that were cut off by the edges of the tissue, only those cells that were visible within the median 20% of the optical planes were included for counting. All nuclei were counted with the *Arc* channel off, so as to not bias the counter. When the total number of cells in the z-stack were identified, the *Arc* channel was turned on to classify cells as positive or negative for *Arc*. A cell was counted as *Arc* positive if the fluorescent label could be detected above threshold anywhere within or around the nucleus on at least 3 adjacent planes.

2.8. Statistical analysis

To examine the effect of bilateral inactivation of the PER and mPFC on the percent of correct responses, bilateral infusions of MUS were compared to the vehicle control condition with a repeated measures analysis of variance (ANOVA) with 3 different drug conditions of: (1) vehicle control ($n = 9$), (2) bilateral MUS in PER ($n = 9$), and (3) bilateral MUS in mPFC ($n = 7$). Because the primary comparisons of interest were the rats' performances following inactivation of the PER or mPFC relative to vehicle controls, a planned simple contrast was used to compare each bilateral MUS condition to the control.

In order to examine the effects of contralateral mPFC and PER inactivation on OPPA task performance, a second repeated measures ANOVA was used to test the effects of 3 different infusion conditions on behavior: (1) vehicle control ($n = 9$), (2) contralateral mPFC-PER inactivation ($n = 9$), and (3) ipsilateral mPFC-PER inactivation ($n = 9$). For the conditions in which there were two infusions in different brain hemispheres, the mean was determined for each rat such that sample size (n) was the number of rats for each infusion type rather than the total number of infusions. This was done as to not arbitrarily inflate statistical power. In order to quantify the effect of blocking mPFC-PER communication on behavior, the comparisons of interest were contralateral mPFC-PER inactivation relative to the vehicle control and to the ipsilateral mPFC-PER inactivation. Therefore, a planned simple contrast was used to test whether there was a statistical difference between the contralateral mPFC-PER MUS infusion relative to the vehicle control, and relative to the ipsilateral mPFC-PER MUS infusion. Table 1 summarizes the statistical tests used for the primary comparisons of infusion condition described above.

The same statistical model described above was also used to test the effect of inactivation condition on the side and object bias indices. Rats often display response biases during the acquisition of the OPPA task prior to learning the object-in-place rule (Hernandez et al., 2015; Jo & Lee, 2010a, 2010b; Lee & Byeon, 2014). Thus, indices of a side bias (left vs. right well) and object bias (e.g. "chicks" vs "frog") were calculated during initial training and for each infusion condition. Side bias was calculated as the absolute value of (total number left choices-total number right choices)/total number of trials. The object bias was calculated as the absolute

value of (total number of object 1 choices-total number of object 2 choices)/total number of choices.

Finally, for two-way comparisons of the effects of hemisphere on performance, and *Arc* expression in infused versus non-infused hemispheres, significance was tested with paired-samples *T* tests. All analyses were performed with the Statistical Package for the Social Sciences v23 (IBM, Armonk, NY), and statistical significance was considered at *p* values less than 0.05.

3. Results

3.1. Muscimol selectively blocked activity-dependent *Arc* expression in perirhinal and medial prefrontal cortices

To confirm the MUS infusion in the current study, the expression of the neural activity-dependent gene *Arc* was used to determine if MUS infusion blocked neuronal activity selectively in the PER and mPFC. Specifically, if MUS blocked PER/mPFC activity then the expression of *Arc* should be reduced in these brain areas compared to adjacent areas not targeted for inactivation: LEC, area TE and AC. To test this idea, 4 rats received ipsilateral infusions of MUS 30 min prior to performing the OPPA task, while the other hemisphere served as the non-infused control. These rats were tested on OPPA for 10 min and were then immediately sacrificed. In 2 of these rats, tissue was processed for the PER, LEC, area TE, mPFC, and AC. In the other 2 animals, *Arc* labeled tissue was not available for the mPFC and AC. Fig. 2 shows representative images from the hemisphere that received MUS infusion and the control hemisphere for the mPFC (Fig. 2A) and the PER (Fig. 2B). Note the reduced *Arc* signal (red¹) in the MUS infused regions. Fig. 2C shows the mean proportion of *Arc* positive cells in the PER, mPFC, area TE, LEC, and AC for the hemisphere infused with MUS, the control hemisphere, and the caged controls. Repeated-measures ANOVA with the within subjects factor of infusion hemisphere (muscimol versus control) and the between subjects factor of brain region (targeted for muscimol versus adjacent, non-targeted area) revealed a significant main effect of brain region ($F_{[1,14]} = 8.13$, $p < 0.02$), such that the targeted regions (PER and mPFC) had fewer *Arc* positive neurons compared to the non-targeted regions. Moreover, there was a trend towards a significant interaction effect of infusion hemisphere and target region ($F_{[1,14]} = 3.20$, $p = 0.09$). Post hoc analysis indicated that the percent of *Arc* positive cells in the mPFC and PER was significantly reduced in the hemisphere that received MUS infusions relative to the non-infused hemisphere ($p < 0.04$; Tukey), which was not the case for adjacent regions not targeted for infusions ($p > 0.73$; Tukey).

The percent of *Arc* positive cells in the caged control rat was low (4.0%; $SD = 3.53$). For the mPFC and PER the levels of *Arc* expression after MUS infusion were not significantly different than those observed in the caged controls ($T_{[5]} = 0.51$, $p = 0.63$; one sample). In contrast, in the hemisphere that was not infused, *Arc* expression was significantly greater relative to the caged control ($T_{[5]} = 3.37$, $p < 0.02$; one sample). Moreover, in the adjacent brain regions that were not targeted for inactivation, *Arc* expression in the MUS infused hemisphere was significantly greater than the caged control ($T_{[9]} = 4.55$, $p < 0.01$; one sample). The observation that MUS blocked *Arc* expression is consistent with previous data (Kubik, Miyashita, Kubik-Zahorodna, & Guzowski, 2012). Moreover, the reduction in activity-dependent *Arc* expression observed in the mPFC and PER, but not LEC, area TE or AC, of MUS infused hemispheres indicates that neural activity in the target regions of the current experiments was selectively blocked. Although this was

¹ For interpretation of color in Fig. 2, the reader is referred to the web version of this article.

Table 1

Summary of infusion condition statistical model. The effect of infusion condition was tested with repeated measures ANOVAs and planned contrasts.

Main effect	Statistical test	Comparison	n	Statistic	p value
Bilateral infusion (3 levels: PER, mPFC, control)	Repeated measures ANOVA F[2,12] = 10.25, p < 0.01	Vehicle control vs bilateral MUS in PER	9	Simple contrast	p < 0.01
		Vehicle control vs bilateral MUS in mPFC	7	Simple contrast	p < 0.01
Disconnection infusion (3 levels: contralateral mPFC-PER, ipsilateral mPFC-PER, control)	Repeated measures ANOVA F[2,12] = 23.62, p < 0.001	Contralateral mPFC-PER MUS vs Vehicle control	9	Simple contrast	p < 0.01
		Contralateral mPFC-PER MUS vs Ipsilateral mPFC-PER MUS	9	Simple contrast	p < 0.01

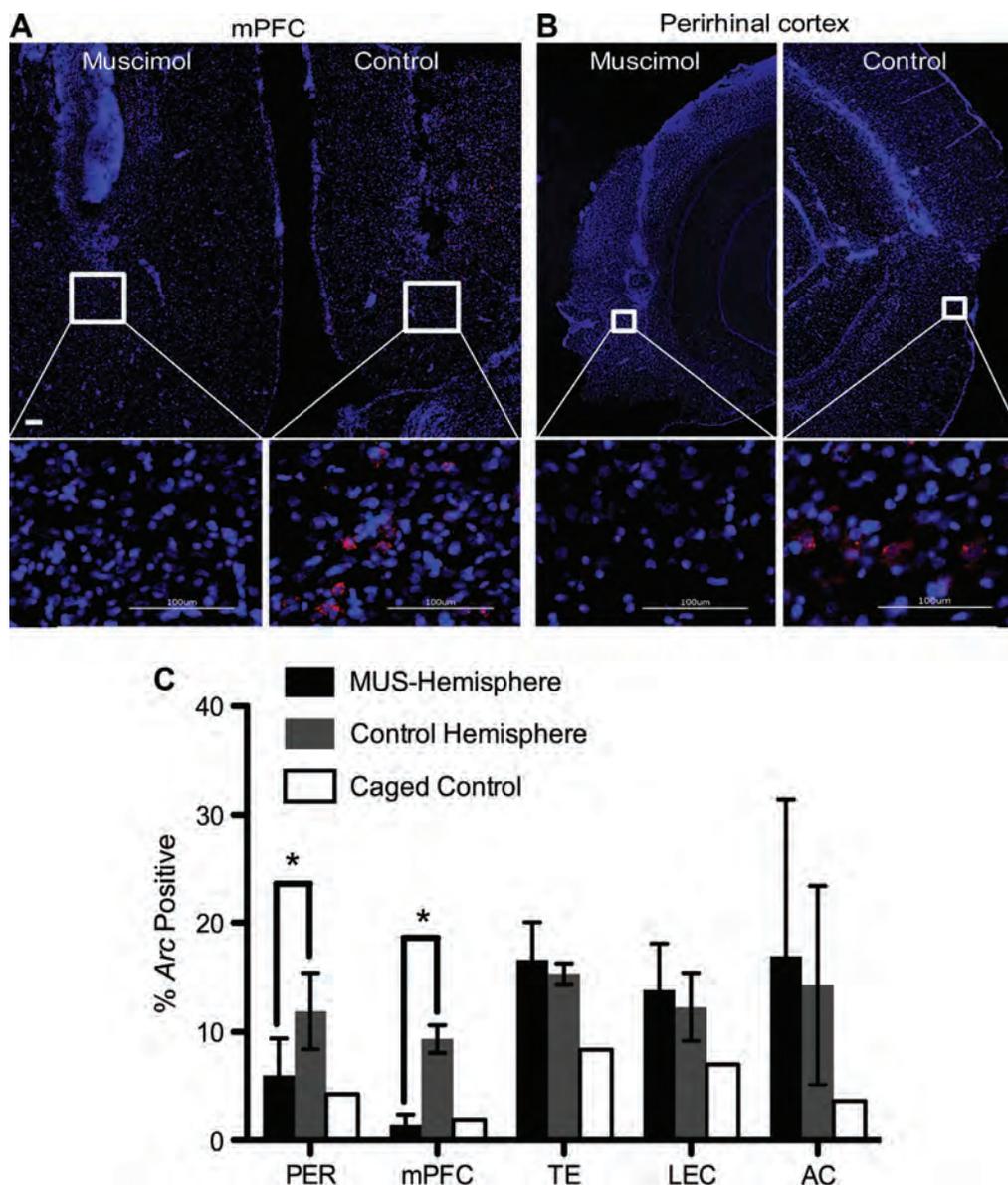


Fig. 2. *Arc* expression following muscimol (MUS) infusion. (A) A representative image of *Arc* expression in the mPFC in which the left hemisphere was inactivated with MUS and the right was intact (top). 40 \times images of the regions of interest outlined in top panel from MUS infused (left panel) and non-infused tissue (right panel) (bottom). (B) A representative image of *Arc* expression the PER in which the right hemisphere was infused with MUS and the left as intact (top). 40 \times images of the regions of interest outlined in top panel from non-infused (left panel) and MUS infused tissue (right panel) (bottom). (C) Percent of *Arc* positive cells in the perirhinal cortex (PER), medial prefrontal cortex (mPFC), area TE, lateral entorhinal cortex (LEC), and anterior cingulate cortex (AC) in the MUS infused (black) and non-infused control (grey) hemispheres relative to caged controls (white). For the PER and mPFC, there was a significantly greater percent of *Arc* positive cells in the hemisphere that was not infused with MUS ($p < 0.05$), indicating that the MUS inhibited activity-dependent gene expression in the mPFC and PER. This was not the case for the adjacent brain regions (TE, LEC and AC) that were not targeted for infusions ($p > 0.05$ for all comparisons). Error bars are ± 1 Standard error of the mean (SEM).

qualitatively the case for both the PER and mPFC, it was not possible to compare *Arc* expression across hemispheres for the individual regions due to lack of statistical power from the small sample sizes.

3.2. Pre-training and initial response bias

During the initial training sessions on the OPPA task, rats exhibit a "side bias" for selecting the object over a well on a particular side,

regardless of the object or the arm of the maze (Hernandez et al., 2015; Jo & Lee, 2010a; Lee & Byeon, 2014; Lee & Kim, 2010). This bias has to be suppressed, presumably through mPFC activity projecting back to sensorimotor areas (Lee & Byeon, 2014) before OPPA task performance shows an improvement. The mean side bias and percent correct responses as a function of days before reaching criterion performance are shown in Fig. 3A and B, respectively. The rats in the current study began with a side bias and over the course of 20 days of testing, this bias decreased as indicated by a significant main effect of test day ($F_{[19,76]} = 12.80$, $p < 0.001$; repeated measures). Planned orthogonal contrasts comparing the side bias on each day to the mean of the preceding days indicated that the side bias did not significantly change across testing days until one day prior to reaching criterion performance ($p > 0.1$ for all comparisons; difference contrast). The day before criterion performance was achieved, however, the mean side bias significantly decreased from the preceding day ($p < 0.005$; difference contrast; Fig. 3A). Importantly, the significant shift away from a side bias corresponded with rats' improved performances on the OPPA task. In fact, although rats showed a significant main effect of testing day on performance ($F_{[19,76]} = 14.22$, $p < 0.001$), planned orthogonal contrasts comparing performance on each test day to the mean of the preceding test days did not detect a significant performance improvement across testing days ($p > 0.1$ for all comparisons; difference contrast) until one day prior to reaching criterion performance ($p < 0.001$; difference contrast; Fig. 3A). Together these data suggest that the rats perseverated, using a maladaptive response strategy that prevented them from making incremental progress on the task over weeks of training, and that reductions in this side bias were associated with rats reaching criterion performance. Consistent with this idea, when the response bias was plotted against percent correct for all testing days, there was a significant negative correlation over all days of testing between the response bias and percent correct on the OPPA task ($R^2_{[181]} = 0.71$, $p < 0.001$; Fig. 3C). When a correlation value was calculated separately for each rat so that an animal only contributed 1 data point, the mean correlation was also significant ($R^2_{[8]} = 0.87$, $p < 0.01$). Together, these data indicate that rats must move away from using a non-adaptive side bias in order to acquire the biconditional response of flexibly choosing the correct object associated a given maze arm.

3.3. Bilateral inactivation of the mPFC or PER impaired behavioral flexibility

Before examining the effect of disconnecting the mPFC and PER, whether these regions are independently critical for normal performance on the OPPA task was tested. Rats were bilaterally infused with MUS into the mPFC or PER during separate testing sessions. Infusions involving the left mPFC cannula were excluded for 2 of the 9 rats due to this cannula being misplaced, but the PER infusion

conditions were included for these animals. Mean performance was 90.23% correct (SD = 9.10) for vehicle control infusions. Bilateral MUS infusion into the mPFC decreased the percent of correct trials to 69.64% (SD = 16.43) and bilateral PER MUS infusions resulted in 63.67% correct (SD = 15.76; Fig. 4). The overall main effect of bilateral infusion condition was statistically significant ($F_{[2,12]} = 10.25$, $p < 0.01$). Planned comparisons of performance across the different drug conditions indicated that the percent correct trials was significantly greater during the vehicle control relative to the bilateral mPFC inactivation ($p < 0.01$; simple contrast) and to the bilateral PER inactivation ($p < 0.01$; simple contrast). Importantly, the amount of time it took rats to complete the 32 trials within a test session did not significantly vary between the MUS and vehicle control conditions for either the mPFC ($T_{[8]} = 1.53$, $p = 0.16$) or PER ($T_{[8]} = 1.36$, $p = 0.21$) inactivation. This observation indicates that the MUS infusions did not cause any overt sensorimotor or motivational impairments that influenced performance.

When the effect of drug condition on the side bias and object bias indices was quantified, there was a significant main effect of infusion condition on an animal's tendency to choose one side over another (i.e., the side bias; $F_{[2,12]} = 9.99$, $p < 0.01$). Planned comparisons indicated that, relative to the vehicle control, the side bias was greater for both the bilateral mPFC MUS ($p < 0.02$; simple contrast) and PER MUS ($p < 0.01$; simple contrast) conditions (Fig. 4B). In contrast, there was no main effect of infusion condition on the object bias ($F_{[2,12]} = 2.94$, $p = 0.09$; Fig. 4C). This suggests when either the mPFC or PER is inactivated, rats regress to their initial strategy during training in which they select one side, regardless of the object or arm of the maze. Together, these results are consistent with previous studies (Jo & Lee, 2010b; Lee & Solivan, 2008) and suggest that the PER and mPFC may need to interact in order to suppress non-adaptive object selection and use the object-in-place rule for optimal performance.

3.4. mPFC-PER disconnection impaired behavioral flexibility relative to ipsilateral inactivation

To investigate whether communication between the mPFC and PER is necessary for OPPA task performance, percent correct following contralateral MUS infusions was compared to ipsilateral MUS infusions and vehicle controls. Since inter-region projections are typically more extensive within same hemisphere compared to across hemispheres (Bedwell et al., 2015), this approach blocks communication between inactivated regions while leaving one hemisphere of each brain region intact to support behavior. For the contralateral infusions, MUS or saline was infused simultaneously into the mPFC of one hemisphere and the PER of the contralateral hemisphere. Ipsilateral mPFC and PER infusions of MUS

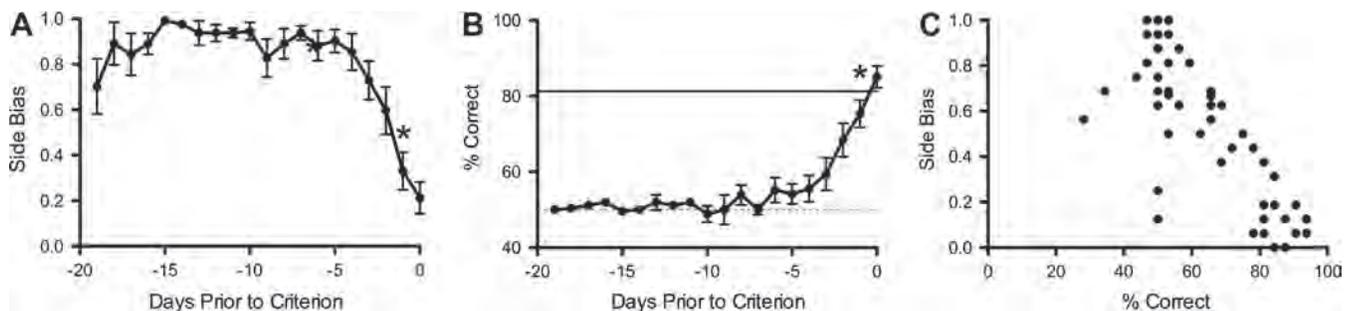


Fig. 3. Side bias during pre-training. (A) The mean side bias (Y axis) as a function of days before reaching criterion performance (X axis). During initial training, there was a response bias for a particular side. This bias did not significantly change across testing days until 1 day prior to hitting criterion performance ($p < 0.005$). (B) The percent correct responses (Y axis) as a function of days before reaching criterion performance (X axis). The mean percent correct did not significantly improve across testing days until one day prior to hitting criterion performance ($p < 0.001$). (C) There was a significant negative correlation over all days of testing between the side bias and percent correct on the OPPA task ($R^2_{[181]} = 0.71$, $p < 0.001$). Error bars are ± 1 SEM.

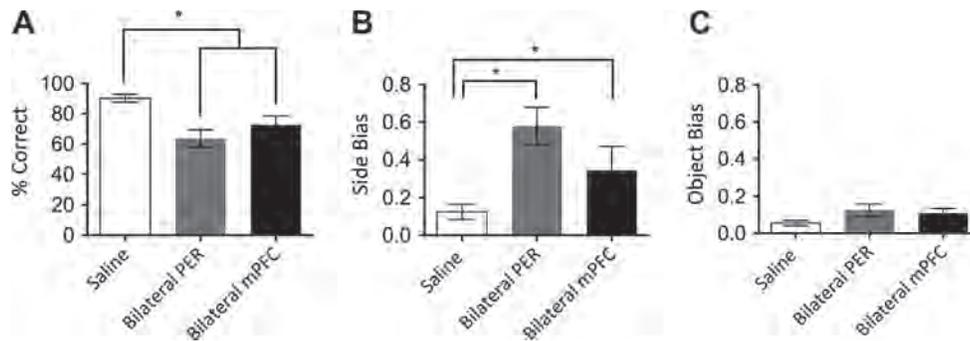


Fig. 4. Effects of bilateral mPFC or PER inactivation on OPPA task performance. (A) The Y axis shows percent correct for the bilateral MUS versus the vehicle control (saline) infusions (X axis). Bilateral mPFC and bilateral PER inactivation impaired performance relative to the vehicle control ($p < 0.01$ for both comparisons). (B) Side bias of rats' tendencies to choose one side over the other during the different infusion conditions. The side bias was significantly higher during both bilateral inactivation conditions relative to the vehicle control infusion ($p < 0.01$ for both comparisons). (C) Object bias during bilateral PER and mPFC MUS infusions and vehicle control infusions was not significantly different ($p = 0.09$). Error bars are ± 1 SEM.

were used to measure the effect of unilateral inactivation when mPFC-PER communication was left intact. Fig. 5A shows the percent correct trials following the different infusion conditions. During ipsilateral MUS infusions, rats maintained 77.29% correct (SD = 16.19), which was not significantly different from criterion ($T_{[8]} = 0.24$, $p = 0.81$). In contrast, contralateral MUS infusions resulted in a reduction of correct responses (59.79%; SD = 15.76). In fact, overall there was a significant main effect of infusion condition ($F_{[2,16]} = 23.62$, $p < 0.001$). Planned comparisons with a corrected α level of $p < 0.017$ (for 3 comparisons) were used to determine if there was a significant difference between different infusion conditions. The percent correct during contralateral mPFC-PER MUS infusions was significantly different when compared to the vehicle control ($p < 0.01$; simple contrast) as well as when compared with ipsilateral mPFC-PER MUS infusions ($p < 0.01$; simple contrast). There was a trend towards an effect of ipsilateral MUS inactivation relative to vehicle infusion that did not reach statistical significance ($p = 0.04$; simple contrast). This is consistent with several studies that have shown behavioral deficits following unilateral inactivation of higher-level association cortical areas (Poe et al., 2000; Tanninen, Morrissey, & Takehara-Nishiuchi, 2013; Wilson, Langston et al., 2013; Wilson, Watanabe, Milner, & Ange, 2013). Importantly, the amount of time it took rats to complete 32 trials within a testing session did not significantly vary between any of the infusion conditions (contralateral, ipsilateral, or vehicle control; $p > 0.13$ for all comparisons), indicating that MUS infusions did not cause sensorimotor or motivational impairments. Together these data show that blocking mPFC-PER communication impaired the ability of rats to update the selection of the correct object in the different spatial locations, compared to control conditions.

Fig. 5B and C shows the side and object biases, respectively, for the different infusion conditions. There was a main effect of MUS infusion compared to vehicle controls on side bias ($F_{[2,16]} = 19.56$, $p < 0.01$). Planned comparisons indicated that the side bias was greater for the ipsilateral ($p < 0.03$) and contralateral ($p < 0.01$) conditions relative to saline. There was no main effect of MUS infusions relative to saline controls on object bias during the task for either the ipsilateral or contralateral conditions ($F_{[2,16]} = 0.464$, $p = 0.64$). Importantly, these data indicate that, similar to the bilateral mPFC and PER infusions, disconnecting these regions also resulted in an inability to flexibly update response selection, with rats regressing back to the side bias that is observed early in training.

3.5. Side of infusion and repeated infusions did not alter behavioral performance

In order to examine whether or not different hemispheres were similarly affected by inactivation, the lateralization of infusions was compared. This analysis showed there was no significant effect of left versus right hemisphere ($T_{[6]} = 0.04$, $p = 0.97$; Fig. 6A). The two possible disconnection positions, left mPFC and contralateral PER and right mPFC and contralateral PER, were also found to not differ significantly ($T_{[6]} = 0.14$, $p = 0.89$; Fig. 6B).

The potential effect of repeating the infusions over days was also assessed. Multiple infusions did not adversely affect behavior over the course of testing. Specifically, performance on the OPPA task between infusion days remained above criterion with an average percent correct of 91.95% (SD = 0.10). Moreover, performance on the first non-infusion day was not significantly different than performance on the last non-infusion day ($T_{[7]} = 0.06$, $p = 0.96$),

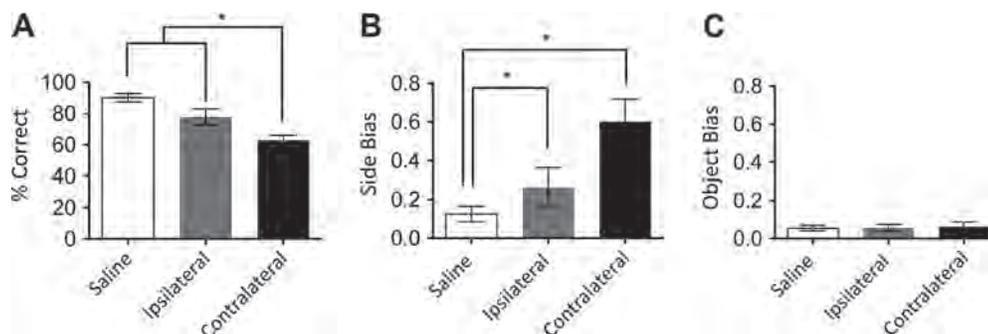


Fig. 5. Effects of contralateral mPFC-PER MUS infusions on OPPA task performance. (A) Percent correct (Y axis) for the disconnection versus ipsilateral and vehicle control infusion conditions. Contralateral infusions that disconnected the mPFC and PER impaired performance when compared to vehicle control or to ipsilateral inactivation ($p < 0.01$ for both comparisons). (B) The side bias during ipsilateral and contralateral MUS infusions was significantly greater compared to contralateral vehicle control infusions ($p < 0.05$ for both comparisons). (C) The object bias (Y axis) was not significantly different across infusion conditions (X axis; $p = 0.64$). Error bars are ± 1 SEM.

demonstrating that rats were not experiencing adverse effects of multiple infusion conditions. Additionally, there was not a significant difference between vehicle infusion and non-infusion days ($T_{[7]} = 0.64$, $p = 0.54$).

3.6. Contralateral inactivation did not impair performance on control conditions

Object-only and side-only control tasks were used to determine if communication between the mPFC and PER is needed for the individual elements of the OPPA task, namely the ability to discriminate between rewarded sides or objects, which does not require the animal to update their responses across trials and minimizes the working memory component across trials. There was no significant effect of contralateral MUS infusion on performance during the object-only ($T_{[6]} = 1.54$, $p = 0.18$) or side-only ($T_{[5]} = 0.35$, $p = 0.74$) control tasks when compared to the previous day of testing with no infusion (Fig. 7). Importantly, these data suggest that the behavioral deficit resulting from the mPFC-PER disconnection was selective to a behavior that required the integration of object and place information in order to facilitate rule shifting between different target objects.

4. Discussion

The current study examined the extent to which an animal's ability to update the selection of a rewarded object when the correct choice is contingent on spatial location requires communication between medial prefrontal (mPFC) and perirhinal cortices (PER). Blocking neural activity with muscimol (MUS) infusions into either region bilaterally, resulted in significant impairments. This

finding confirms previous reports that both regions are necessary for object-place paired association (OPPA) task performance (Jo & Lee, 2010a, 2010b; Lee & Solivan, 2008). The novel insight from the current data is that inactivation of one mPFC hemisphere and the contralateral PER resulted in a decline in OPPA task performance when compared to ipsilateral mPFC-PER inactivations and a regression back to the side bias that is observed early in training. This observation supports the conclusion that mPFC-PER communication is required for rule switching and retrieval of the appropriate biconditional response based on the current spatial location. As rats are able to use both intra- and extra-maze cues to aid response selection, these data are also consistent with previous work showing that communication across the mPFC and PER is necessary for an animal's ability to use spatial or contextual information to guide behavior (Barker et al., 2007), even when the hippocampus remains intact. Conversely, animals' performance on object-only and place-only discrimination tasks, which do not require the association of an object with a location or behavioral flexibility across trials, remained normal after mPFC-PER disconnection. This indicates that mPFC-PER communication is not necessary when the correct response only depends on either stimulus or spatial information and does not vary across trials.

Medial PFC and PER inactivation was verified by visualizing expression of the activity-dependent immediate-early gene *Arc* in rats that were ipsilaterally infused with MUS 30 min prior to testing on the OPPA task. Similar to previous reports (Kubik et al., 2012), blockade of *Arc* expression only occurred in the MUS infused hemisphere, as shown by the presence of *Arc* predominantly in the non-infused hemisphere. Moreover, *Arc* expression was not significantly blocked in brain regions adjacent to the MUS infusion site. Interestingly, in the current experiment, the proportion of *Arc* positive cells observed following OPPA behavior in the non-infused PER hemisphere was only 11%. A previous study reported that over 20% of cells were activated following object exploration (Burke, Hartzell et al., 2012). There are several possibilities for this apparent discrepancy. In the previous experiment, rats were presented with 5 objects, as opposed to 2 used in the current study. These data suggest that encountering a greater number of stimuli leads to greater PER activity. An alternative, but not mutually exclusive, possibility is that ipsilateral inactivation led to lower neuronal activity levels overall, which is consistent with the modest deficit in performance following ipsilateral inactivation. These possibilities will need to be explored with future experiments.

The mPFC supports OPPA task performance through several possible and potentially related mechanisms. First, the role of the mPFC may be to govern interactions among medial temporal lobe structures. The PER communicates heavily with the entorhinal cortex, both of which send projections to the hippocampus (Burwell & Amaral, 1998b). During distinct stages of learning, this interaction is facilitated by the mPFC (Paz et al., 2007). It could be that during the OPPA task, the mPFC updates representations in the rhinal cortices, based on reward prediction, to gate the flow of information between the hippocampus and neocortex. Consistent with this hypothesis is the observation that disconnection lesions of the PFC and inferotemporal cortex in monkeys impair delayed nonmatching-to-sample performance (Browning, Baxter, & Gaffan, 2013) and object-in-place scene memory (Wilson, Gaffan, Mitchell, & Baxter, 2007). Additional evidence for a unified mPFC-PER network that supports performance on the OPPA task is that the theta rhythm in the mPFC and hippocampus becomes more synchronized after acquisition of the OPPA task rule (Kim, Delcasso, & Lee, 2011). Because there are limited direct projections from mPFC to dorsal hippocampus, this synchrony may require that information flows through the rhinal cortices, thus making the PER an integral part of this circuit. The PER, however, is not the only structure reciprocally connected with both the hippocam-

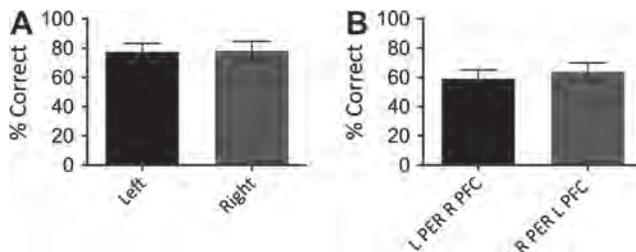


Fig. 6. Contralateral and ipsilateral infusion conditions did not show lateralization effects. (A) The Y axis shows percent correct on the OPPA task across the different hemispheres inactivated ipsilaterally (X axis). Left versus right hemisphere ipsilateral inactivation were not significantly different from each other ($p = 0.97$). (B) Percent correct (Y axis) on the OPPA task during left hemisphere mPFC/right hemisphere PER inactivation versus right hemisphere mPFC/left hemisphere PER inactivation (X axis). There was not a significant lateralization effect of infusion side between the disconnection conditions ($p = 0.87$). Error bars are ± 1 SEM.

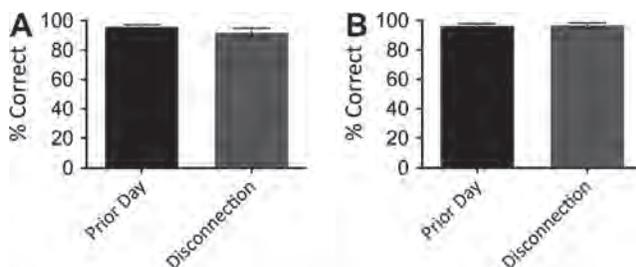


Fig. 7. Contralateral MUS infusions did not affect performance on side only or object only control tasks. Percent correct during an object-only control task (A) and a side-only control task (B) was not significantly different between the contralateral MUS inactivation compared to the previous non-infused day ($p > 0.18$ for both comparisons). Error bars are ± 1 SEM.

pus and prefrontal cortex. In fact, the nucleus reuniens of the ventral midline thalamus is also anatomically poised to functionally link the prefrontal cortex to the hippocampus (McKenna & Vertes, 2004; Vertes, 2002, 2006, 2015; Vertes, Hoover, Do Valle, Sherman, & Rodriguez, 2006; Vertes, Linley, & Hoover, 2015). Moreover, inactivation of the nucleus reuniens leads to deficits in strategy switching (Cholvin et al., 2013), suggesting that this brain area may be critical for flexibility by modulating prefrontal-hippocampal interactions. (Cassel et al., 2013). The fact that either PER or nucleus reuniens lesions produce behavioral flexibility deficits indicates that, while both regions are necessary for prefrontal-hippocampal interactions, neither structure alone is sufficient.

The mPFC has also been shown to be involved in the flexible control of behavioral responses (Beas et al., 2013; Chadick, Zanto, & Gazzaley, 2014; Ridderinkhof, Ullsperger, Crone, & Nieuwenhuis, 2004), as well as working memory (Sloan, Good, & Dunnett, 2006), both of which may be involved in OPPA task performance. Previous studies using MUS to inactivate the mPFC have reported deficits in rats' abilities to inhibit incorrect responses (Izaki, Maruki, Hori, & Nomura, 2001). Additionally, blocking glutamatergic transmission in the mPFC enhances impulsivity and leads to compulsive perseveration in rats (Carli, Baviera, Invernizzi, & Balducci, 2006). Rats show a significant side bias during the acquisition of the OPPA task and inhibiting this perseveration is essential to being able to learn the object-in-place rule (Hernandez et al., 2015; Lee & Byeon, 2014). Although rats may not stop displaying a side bias, in the days after acquisition of the rule, they may show inhibitory behavior towards the object on the preferred side. In line with this idea, mPFC neurons show selective firing for trials requiring this inhibition before object selection (Lee & Byeon, 2014). Thus, the importance of communication between the mPFC and PER may be to ensure that actions with unwanted outcomes are inhibited, enabling subjects to make the choice with a more desirable outcome. Additionally, the OPPA task requires some active maintenance of previously visited locations in order to correctly alternate. In fact, because the order of left versus right arm trials was not randomized in the current study, rats could have successfully performed the task by alternating between object selection regardless of arm location. Future experiments will randomize left versus right arm trials and increase the inter-trial interval to determine the relative contribution of working memory versus flexibility in OPPA performance.

A final possibility is that the mPFC is necessary for the expression of memory at remote time points (Takehara-Nishiuchi, Nakao, Kawahara, Matsuki, & Kirino, 2006). It is theorized that the mPFC initially relies on the hippocampus to form memories, but later may independently use previous experience to guide adaptive responses (Takehara-Nishiuchi & McNaughton, 2008). In line with this idea, hippocampal-dependent memory consolidation causes changes in the synaptic density in the mPFC that support the retrieval of remote memories (Insel & Takehara-Nishiuchi, 2013; Restivo, Vetere, Bontempi, & Ammassari-Teule, 2009). Thus, disconnecting the mPFC and PER could prevent the PER from having access to consolidated object-place associations. If the mPFC selectively supports remote memories that are dependent on the hippocampus shortly after acquisition, it is possible that infusing MUS into the mPFC during an earlier time point in training, or before acquisition, would not result in a deficit. This idea would predict that rats over-trained for a month on the OPPA task would be able to complete the task without the hippocampus. While previous bilateral inactivation of the hippocampus during the OPPA task has shown a performance deficit (Jo & Lee, 2010a; Lee & Solivan, 2008), the time frame of hippocampal involvement has not been explicitly examined.

One critical component of OPPA task performance is the ability to identify the different objects. The dense connectivity of the

PER with different sensory cortical areas enables it to link individual features of an object in order to identify it as a single entity (Murray & Bussey, 1999; Suzuki & Amaral, 1990, 1994). In fact, lesions to the PER cause impairments in object recognition, but not in spatial memory (Bussey, Muir, & Aggleton, 1999), and lesion data indicate the PER contributes to both object perception and memory (Buckley & Gaffan, 1998b; Murray & Bussey, 1999). Furthermore, a portion of PER neurons are selectively activated by different objects (Burke, Maurer et al., 2012; Deshmukh, Johnson, & Knierim, *in press*), even when the environment in which they are presented is changed (Burke, Hartzell et al., 2012). Although it is clear that the PER encodes object information, it is unlikely its contribution to the OPPA task is limited to object representations. Synaptic weight changes within the PER support associations between object pairs (Fujimichi et al., 2010; Higuchi & Miyashita, 1996; Murray & Richmond, 2001), and it is conceivable that this could extend across modalities. In fact, the PER is critical for linking visual to tactile information (Buckley & Gaffan, 1998a; Goulet & Murray, 2001; Jacklin, Cloke, Potvin, Garrett, & Winters, 2016; Parker & Gaffan, 1998; Reid, Jacklin, & Winters, 2014). A similar situation may occur when an object is associated with a place. While PER cells do not show spatial selectivity (Burke, Maurer et al., 2012; Burwell, Shapiro, O'Malley, & Eichenbaum, 1998), plasticity within PER may bias the retrieval of one object representation over another when the animal is in a specific location and this interaction could be modulated by projections from the mPFC (Paz et al., 2007). A critical issue not addressed by the current study is the necessity of mPFC-PER connectivity to acquire new object-place associations. Although not explicitly tested here, available data indicating that communication between these brain regions is necessary for an animal's ability to detect novel object-place associations (Barker & Warburton, 2008; Barker et al., 2007) suggest that this component of task performance would also be impaired by a mPFC-PER disconnection.

The mPFC-PER disconnection would disrupt any one of the aforementioned aspects of mPFC influence on the medial temporal lobe circuitry. Alternatively, it is possible that the deficit resulting from blocking communication across these regions is due to the inability to deal with proactive interference based on the rule used in the previous trial. This scenario, however, would predict that rats would default back to the correct choice on the previous trial and show an object bias. This behavioral outcome was not observed following the mPFC-PER disconnection, rather rats regressed to the side bias seen during pre-training.

It is likely that the role of the mPFC on the network is diverse and may encompass several aspects of learning and memory in regards to both behavioral flexibility and object-place associations. The observation that a contralateral deficit impaired performance on the OPPA task, which requires flexibility in selecting the correct object based on spatial location, but not the control tasks, is interesting in the context of aging and disease. The PER (Burke, Ryan, & Barnes, 2012; Khan et al., 2014; Reagh et al., 2016; Ryan et al., 2012) and mPFC (Griffith et al., 2014; Morrison & Baxter, 2012) are among the most vulnerable brain regions to age-related dysfunction. Even when these areas are compromised, however, aged animals are still able to perform object discriminations and differentiate the left from right side (Beas et al., 2013; Burke et al., 2011; Hernandez et al., 2015). Therefore, behaviors that require large-scale integration across different neural networks may be particularly sensitive to aging. Because individual brain regions may not age in the same manner, it is vital that potential treatments for cognitive aging and dementia not only alleviate dysfunction in an individual area, but also maintain the balance in global network interactions.

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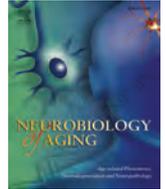
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Epigenetic regulation of estrogen receptor α contributes to age-related differences in transcription across the hippocampal regions CA1 and CA3

Lara Ivanov^{a,b}, Ashok Kumar^{a,*}, Thomas C. Foster^{a,b,*}

^a Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL, USA

^b Genetics and Genomics Program, Genetics Institute, University of Florida, Gainesville, FL, USA

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ABSTRACT

The expression of estrogen receptor alpha (ER α) varies across brain regions and changes with age and according to the previous history of estradiol exposure. ER α is regulated by a number of mechanisms including the level of mRNA (*Esr1*) expression. For this study, we took advantage of regional differences in hippocampal ER α expression to investigate DNA ER α promoter methylation at CpG dinucleotide sites as a potential epigenetic mechanism for regulating gene expression. Young and aged female Fischer 344 rats were ovariectomized, and *Esr1* expression and ER α promoter methylation were examined in hippocampal regions CA1 and CA3, either 3 or 14 weeks following surgery. The results indicate that reduced *Esr1* expression in region CA1 relative to CA3 was associated with an increase in DNA methylation in region CA1, particularly for the first CpG site. Additionally, differential methylation of distal CpG sites, 11–17, was associated with altered *Esr1* expression during aging or following long-term hormone deprivation. The results support the idea that methylation of site 1 may be the primary regulatory region for cross-regional patterns in ER α expression, while distal sites are modifiable across the life span and may act as a feedback mechanism for ER α activity.

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1. Introduction

Estradiol (E2) influences several biological processes that likely contribute to neuroprotection (Bean et al., 2014). The relative levels and subcellular distributions of estrogen receptor alpha (ER α) vary across brain regions and according to the previous history of E2 exposure (Mehra et al., 2005; Milner et al., 2001; Mitra et al., 2003; Mitterling et al., 2010). Differences in ER α expression/activity likely contribute to regional differences in vulnerability to ischemia and oxidative stress (Merchenthaler et al., 2003; Zhang et al., 2009). While the expression profile for ER α in the hippocampus is well characterized by age and hippocampal subregions, the molecular mechanisms that regulate estrogen receptor expression in the hippocampus are not well understood (Bean et al., 2014).

One mechanism for the regulation of gene expression is through methylation of cytosines in guanine-cytosine-rich areas

of the gene promoter region, termed CpG islands. In several tissues, ER α promoter methylation increases with age and is associated with decreased ER α expression and increased incidence of disease (Li et al., 2004; Post et al., 1999). Similarly, in the brain, ER α promoter methylation is associated with a decrease in ER α expression and underlies physiological and behavioral differences across the lifespan (Gore et al., 2011; Schwarz et al., 2010). We examined the DNA methylation status of the 17 CpG sites within the ER α promoter exon 1b region in ovariectomized female rats to test the hypothesis that CpG DNA methylation is an active epigenetic regulator of regional and age-related differences in the expression of ER α mRNA, *Esr1*. For this study, we took advantage of regional differences in hippocampal ER α expression, with increased expression in region CA3 relative to region CA1 (Mehra et al., 2005; Rune et al., 2002), and possible autoregulation of ER α promoter activity by E2 (Castles et al., 1997; Donaghue et al., 1999; Pinzone et al., 2004), which may underlie effects of hormone deprivation on ER α expression (Bean et al., 2014). The results suggest that differential methylation of sites within the ER α promoter may regulate transcription of *Esr1* across hippocampal regions and that DNA methylation of distal CpG sites may have a function in age-related expression changes relative to upstream sites in the promoter.

* Corresponding author at: Department of Neuroscience, McKnight Brain Institute, University of Florida, PO Box 100244, Gainesville, FL 32610-0244, USA. Tel.: (352) 394-0033; fax: (352) 392-8347.

E-mail addresses: Kash@ufl.edu (A. Kumar), foster1@ufl.edu (T.C. Foster).

2. Materials and methods

2.1. Animals

Procedures involving animal subjects have been reviewed and approved by the Institutional Animal Care and Use Committee and were in accordance with guidelines established by the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Female Fischer 344 rats of two ages, young (3 months, $n = 21$) and aged (18 months, $n = 22$), were obtained from National Institute on Aging colony at Charles River Laboratories, through the University of Florida Animal Care and Service facility. Animals were maintained on a 12:12 hours light schedule and provided ad lib access to food and water.

2.2. Surgery and tissue collection

Ovariectomy (OVX) was performed as previously described (Bean et al., 2015; Sharrow et al., 2002). Briefly, rats were handled for 5 minutes a day for at least 1 week prior to surgery. Female rats were ovariectomized under isoflurane (Piramal Healthcare) in oxygen using a VetEquip anesthesia system. Bilateral incisions were made to expose the ovaries, which were cleared from the fat tissue and dissected out. Subsequent to the closure of the incisions, buprenorphine (0.03 mg/kg) and saline (5–10 mL) were given by subcutaneous injection. Following OVX, the food of the animals was exchanged to a casein-based chow, which contains lower levels of phytoestrogens. Three weeks (wk) following OVX (young, $n = 10$ and aged, $n = 11$), rats were overdosed with CO₂, decapitated, and the hippocampi were dissected. The same procedure was repeated for the remaining animals after a 14-week period (young, $n = 11$ and aged, $n = 11$) (Fig. 1). Hippocampal regions (CA1 and CA3) were separated, placed in tubes, immediately frozen in liquid nitrogen, and stored in -80°C until processed.

2.3. RNA isolation and reverse transcription quantitative polymerase chain reaction

RNA was isolated from each hippocampal region ($n = 5$ –6 per region of each age and OVX duration group) using the RNeasy Lipid Tissue Mini kit (Qiagen, catalog number: 74804), and DNase digestion was performed with the RNase-Free DNase Set (Qiagen, catalog number: 79254). Following isolation, the concentration was measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific). Reverse transcription was performed using the QuantiTect Reverse Transcription kit (Qiagen, catalog number: 205311), and quantitative polymerase chain reaction was completed using the TaqMan Gene Expression Assays (*Esr1*: Rn01640372_m1, *Gapdh*: Rn01775763_g1) in a 7300 real-time

polymerase chain reaction (PCR) system with SDS software version 1.3.1 (Applied Biosystems). The $\Delta\Delta\text{CT}$ method (Livak and Schmittgen, 2001) was used to determine the relative cDNA levels, and the CA1 region from young short-term rats was used as the calibrator samples.

2.4. Sodium bisulfite sequencing

Genomic DNA was isolated from the CA1 and CA3 areas ($n = 5$ per age group and OVX time) using the DNeasy Blood and Tissue kit (Qiagen, catalog number: 69504). The DNA concentration was quantified using the NanoDrop 2000 spectrophotometer, and sodium bisulfite conversion was performed with the EZ DNA Methylation-Gold kit (Zymo Research, catalog number: D5005) according to the manufacturer's directions. The exon 1b promoter region of ER α (GenBank accession number: X98236) was amplified with the following modifications from previous reports (Champagne et al., 2006; Kurian et al., 2010). The thermocycler parameters included an initial denaturation cycle of 5 minutes at 94°C , 40 cycles of 1 minute at 94°C (denaturing), 3 minutes at 56°C (annealing) and 1 minute at 72°C (extension), followed by a final extension cycle of 15 minutes at 72°C . The following bisulfite sequencing PCR (BSP) primers were used: outer forward 5'TAGTATATTTGATTGTTATTTAT3'; outer reverse 5'CTAAACAAAAAATAAATTACTTTC3'. The PCR product was used for nested PCR with the same thermocycler parameters with the following BSP nested primers: forward 5'TTTATTTGTGGTTTATAGATATTT3' and reverse 5'ACAAAAAATAAATCAAAACAC3'. In addition, a preliminary check of bisulfite conversion efficiency of each sample was assessed by performing a separate nested PCR reaction. The same thermocycler conditions were used with wild-type sequence-specific primers, which map to the unconverted DNA sequence of the exon 1b promoter region of ER α (outer forward 5'CAGCACACTTTGACTGCCATTCTAC3'; outer reverse 5'CTAGGCAGAAAGGTAAGTTGCTTTC3'; nested forward 5'TTTATCTGTGGTTTACAGACATCT3'; nested reverse 5'ACAGAAAGAGGGAAATCAAAACAC3'). Amplification of the target sequence with wild-type primers would indicate incomplete bisulfite conversion. All samples demonstrated complete bisulfite conversion. Unconverted DNA was used as the positive control for the wild-type primers.

The nested product (459 bp) of each PCR reaction using BSP primers was cloned with the TOPO TA cloning kit for Sequencing (Life Technologies, catalog number: K4575-J10) with the following modifications to the transformation reaction: TOP10 cells were heat shocked for 45 seconds at 42°C and immediately transferred to ice for 7 minutes before the addition of S.O.C. medium. Positive clones were confirmed by colony PCR using nested BSP primers, and miniprep was performed on each positive clone (Pure-Linkquick plasmid DNA miniprep, Life Technologies, catalog number: K2100-10). The samples were sent for Sanger sequencing at the Interdisciplinary Center for Biotechnology Research, University of Florida. The DNA methylation status of all 17 CpG sites from each region was analyzed using BiQ analyzer (Bock et al., 2005) retaining the default parameters. All positive clones contained conversion rates from 97%–100%, and FASTA files which contained a gap in more than one CpG site were removed. After quality filtering, the average number of clones per animal/region was 10 (\pm standard error of the mean 2) and the average number of clones per age and OVX groups was 51 (\pm standard error of the mean 4). In addition, the total number of clones for each hippocampal region was 204 for CA1 and 203 for CA3. Hierarchical clustering and heatmap figures were generated in Partek Genomics Suite 6.6 (Partek Inc) using clones which contained at least one site methylated to illustrate the DNA methylation pattern in site 1 to sites 2–17.

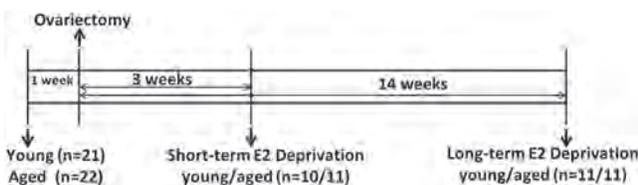


Fig. 1. Experimental research design. Young (3 months) and aged (18 months) F344 female rats were handled for 5 minutes a day for 1 week prior to OVX surgery and killed following a short-term E2 deprivation time (3 weeks) or a long-term E2 deprivation time (14 weeks). Abbreviation: OVX, ovariectomy.

2.5. Statistical analysis

All statistical analyses were performed using StatView 5.0 (SAS Institute Inc, NC, USA). Analyses of variance (ANOVAs) were used to determine significant main effects for RNA expression. For CpG methylation, repeated measures ANOVAs were employed to determine main effects of age and hippocampal region across CpG sites. Fisher's protected least significant difference post hoc comparisons with $p < 0.05$ were employed to localize differences related to differential expression across CpG sites. For interactions of CpG sites with age, OVX duration, or hippocampal region, post hoc ANOVAs were employed to localize differences within each site. A chi-square analysis was employed to examine the independence of methylation across CpG sites.

3. Results

3.1. Region and aging effects on *ERα* mRNA expression

Fig. 2 illustrates the expression of *Esr1* associated with age (young: 3 months, aged: 18 months), OVX duration (short term: 3 weeks, long term: 14 weeks), and region (CA1 or CA3). An increase in *Esr1* expression was observed in older animals (Fig. 2A). Similarly, expression was increased for the long-term OVX relative to short-term OVX (Fig. 2B). Finally, the largest difference was observed as a threefold increase in *Esr1* expression in CA3 relative to CA1 (Fig. 2C). A three-factor ANOVA for expression of *Esr1* confirmed

significant main effects of age [$F(1,38) = 33.14, p < 0.001$], OVX duration [$F(1,38) = 56.0, p < 0.0001$], and region [$F(1,38) = 155.42, p < 0.0001$]. Additionally, there was an interaction of OVX duration and age [$F(1,38) = 13.31, p < 0.001$], region and age [$F(1,38) = 9.25, p < 0.01$], and a tendency ($p = 0.063$) for a region by OVX duration interaction.

The interaction of age and OVX duration was due to increased *Esr1* expression limited to the short duration OVX (Fig. 2D). To examine the interaction of age and OVX duration, post hoc ANOVAs were conducted within respective OVX durations and revealed an age difference for short-term OVX [$F(1,18) = 40.27, p < 0.001$], with an age by region interaction [$F(1,18) = 9.52, p < 0.01$]. Examination of each region indicated that, for short-term OVX, an age-related increase in *Esr1* expression was observed in region CA1 [$F(1,9) = 27.6, p < 0.001$] and in region CA3 [$F(1,9) = 24.6, p < 0.0001$] (Fig. 2D).

Long-term OVX was associated with an increase in *Esr1* expression in CA1 and CA3 for younger animals. For older animals, long-term OVX increased expression only in region CA1 (Fig. 2D). To localize effects of E2 deprivation, effects of OVX duration within each region and each age group were examined. The results indicated that relative to young short-term OVX, *Esr1* expression increased in CA1 [$F(1,9) = 26.68, p < 0.001$] and CA3 [$F(1,9) = 60.6, p < 0.0001$] of young long-term OVX animals (Fig. 2D). For aged animals, long-term OVX increased *Esr1* expression [$F(1,10) = 10.45, p < 0.01$] in CA1 relative to aged short-term OVX rats (Fig. 2D). Thus, it appears that *Esr1* expression is increased due to age and

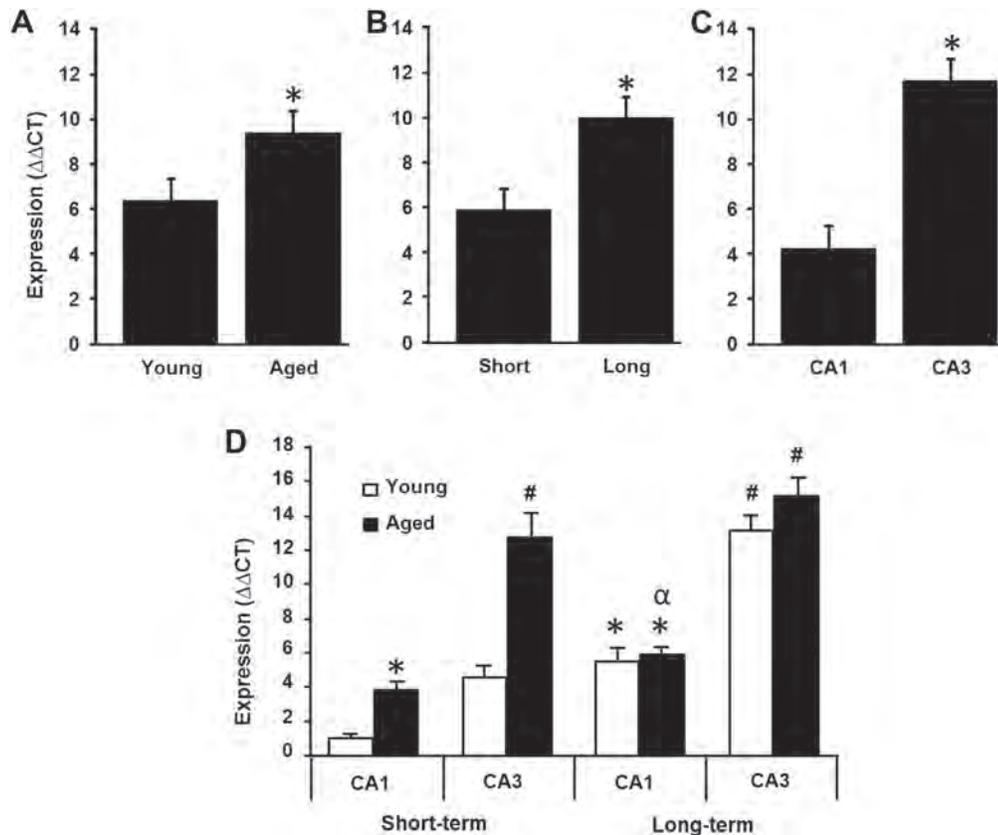


Fig. 2. Illustration of differences in *Esr1* expression. The CA1 region from young short-term rats was used as the calibrator sample in calculating the $\Delta\Delta\text{CT}$. For (A–C), each bar represents the mean (+SEM) of *Esr1* expression for the relevant variable, collapsed across all other variables. *Esr1* expression is increased with (A) age, (B) long-term OVX, and (C) in region CA3 relative to region CA1. Asterisks in (A–C) indicated a significant ($p < 0.001$) main effect. (D) Examination of interactions, where each bar represents the mean (+SEM) of *Esr1* expression for young ($n = 5–6$; open bars) and aged ($n = 6$; filled bars), across regions CA1 and CA3 according to OVX duration (short term: 3 week, long term: 14 weeks). Asterisk indicates a significant ($p < 0.05$) increase in region CA1 relative to young short-term OVX CA1. Pound sign indicates a significant ($p < 0.05$) increase in region CA3 relative to young short-term OVX CA3. For aged animals, long-term OVX was associated with increased expression relative to aged short-term OVX for region CA1 only ($\alpha, p < 0.05$). Abbreviations: OVX, ovariectomy; SEM, standard error of the mean.

long-term OVX deprivation. We confirmed this by comparing young short-term OVX relative to aged long term in CA1 [F(1,9) = 100.83, $p < 0.0001$] and in CA3 [F(1,9) = 75.80, $p < 0.0001$] (Fig. 2D).

3.2. Methylation of the ER α promoter

Considerable variability in methylation was observed across the 17 CpG sites of the ER α promoter region. In general, the pattern of methylation was similar across the two regions with the greatest methylation observed at site 1 and minimal methylation for sites 2–10. Modest methylation was observed for distal sites 11–17 (Fig. 3A). A repeated measures ANOVA was conducted across the 17 DNA CpG methylation sites examining main effects of

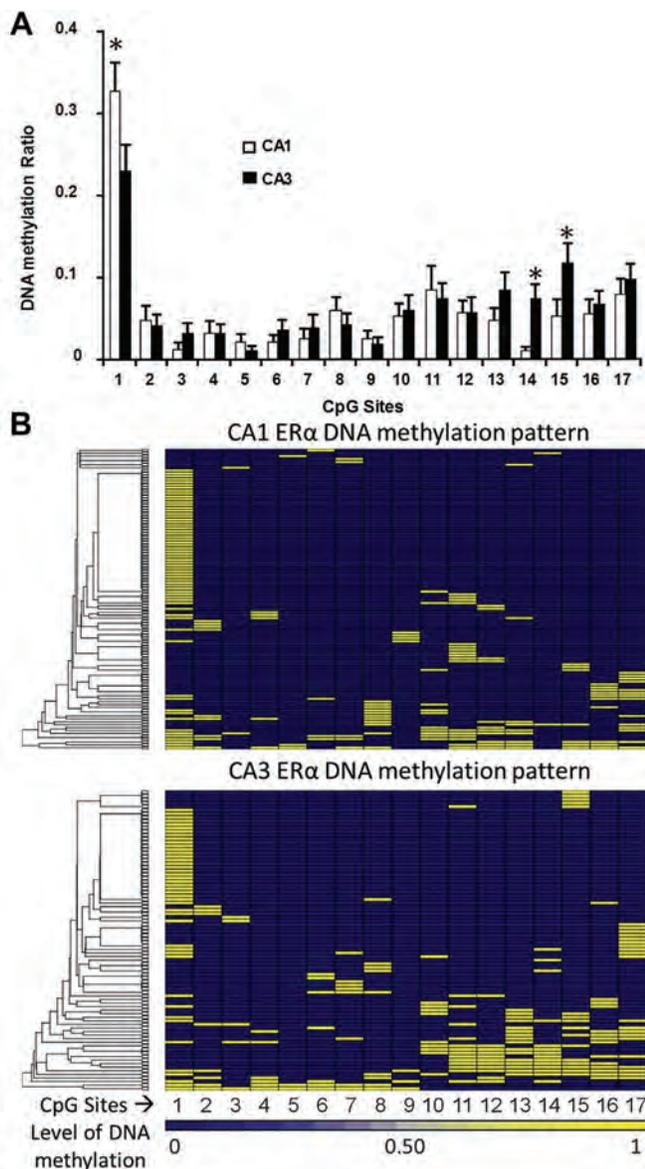


Fig. 3. Site-specific DNA methylation of CpG sites across CA1 and CA3 regions. (A) Each bar represents the mean (+SEM) DNA methylation ratio for sites 1–17 in CA1 (open bars) and CA3 (filled bars). Regional differences in CpG site methylation included increased methylation of site 1 in CA1 and sites 14 and 15 in CA3 ($n = 20$ per region). Asterisk indicates a significant ($p < 0.05$) increase in methylation. (B) Hierarchical clustering and heatmap of the clones which contained ≥ 1 methylated site [CA1: 104 clones (51%) and CA3: 84 clones (41%)]. Abbreviation: SEM, standard error of the mean.

region, age, and OVX duration. As expected, a significant difference in methylation was observed across CpG sites [F(16,512) = 26.91, $p < 0.0001$] indicative of the considerable variability in CpG methylation. Post hoc tests across all sites, collapsed across age, region, and OVX duration, indicated the first CpG site exhibited methylation that was significantly greater than all other sites. In addition, considerable methylation was observed for sites 11, 15, and 17, which were greater than sites 2–7, 9, and 14 (Table 1). No significance difference was observed for any of the main effects; however, there was an interaction of CpG site and region [F(16,512) = 2.49, $p < 0.005$] and a CpG site by age by region interaction [F(16,512) = 2.13, $p < 0.01$].

ANOVAs were conducted within each site to examine the site by region interaction. Increased methylation was observed in region CA1 for site 1 [F(1,38) = 4.30, $p < 0.05$], and methylation was increased in region CA3 for site 14 [F(1,38) = 10.34, $p < 0.005$] and site 15 [F(1,38) = 4.12, $p < 0.05$] (Fig. 3A). Due to the higher DNA methylation ratio on site 1 relative to downstream sites, a closer examination of the clones that contained at least one site methylated in the promoter was performed by chi-square analysis between DNA methylation in site 1 to sites 2–17.

For region CA1, chi-square analysis showed no significant associations between site 1 methylation and distal CpG methylation on sites 2–17, suggesting that methylation of these sites is independent of methylation of the first site in the exon 1b promoter (Fig. 3B). For region CA3, site 1 methylation was independent from the other sites with the exception of a significant association to DNA methylation in site 15. The analysis showed that when site 1 is not methylated, there is a higher probability of methylation on site 15 ($\chi^2 = 4.662$, $p < 0.05$).

To examine the site by age by region interaction, ANOVAs were conducted within each site and region to examine age effects (Fig. 4A and B). A significant age-related increase in methylation was observed for sites 11 [F(1,18) = 6.49, $p < 0.05$] and 12 [F(1,18) = 6.09, $p < 0.05$] in CA3. In contrast, young animals exhibited a significant increase in methylation at site 15 [F(1,18) = 4.76, $p < 0.05$] in CA1 and site 17 [F(1,18) = 5.57, $p < 0.05$] in CA3.

Because *Esr1* expression was increased in area CA1 and CA3 of young animals by long-duration OVX, age differences in DNA methylation may have been masked by effects of long-term OVX in young. Therefore, we separated young animals according to OVX duration and for sites 11–17, we compared young short-term OVX, young long-term OVX, and all aged animals. For region CA1, a significant group difference was observed for site 11 [F(2,17) = 3.96, $p < 0.05$] and site 14 [F(2,17) = 4.24, $p < 0.05$]. Post hoc tests indicated that in each case, methylation was increased in young short-term, relative to young long-term and aged animals (Fig. 4C and D). For region CA3, a significant group difference was observed for site 13 [F(2,17) = 3.80, $p < 0.05$] and post hoc tests indicated that methylation was decreased in young short-term OVX, relative to young long-term OVX (Fig. 4E).

Table 1
Fisher's PLSD for CpG sites in *Esr1* promoter

DNA methylation on CpG site	p -value
1 > 16 sites (2–17)	<0.05
8 > 1 sites (5)	<0.05
10 > 2 sites (5, 9)	<0.05
11 > 8 sites (2–7, 9, 14)	<0.05
12 > 3 sites (3, 5, 9)	<0.05
13 > 3 sites (3–7, 9)	<0.05
15 > 9 sites (2–9, 14)	<0.05
16 > 4 sites (3, 5, 6, 9)	<0.05
17 > 8 sites (2–7, 9, 14)	<0.05

Key: PLSD, protected least significant difference.

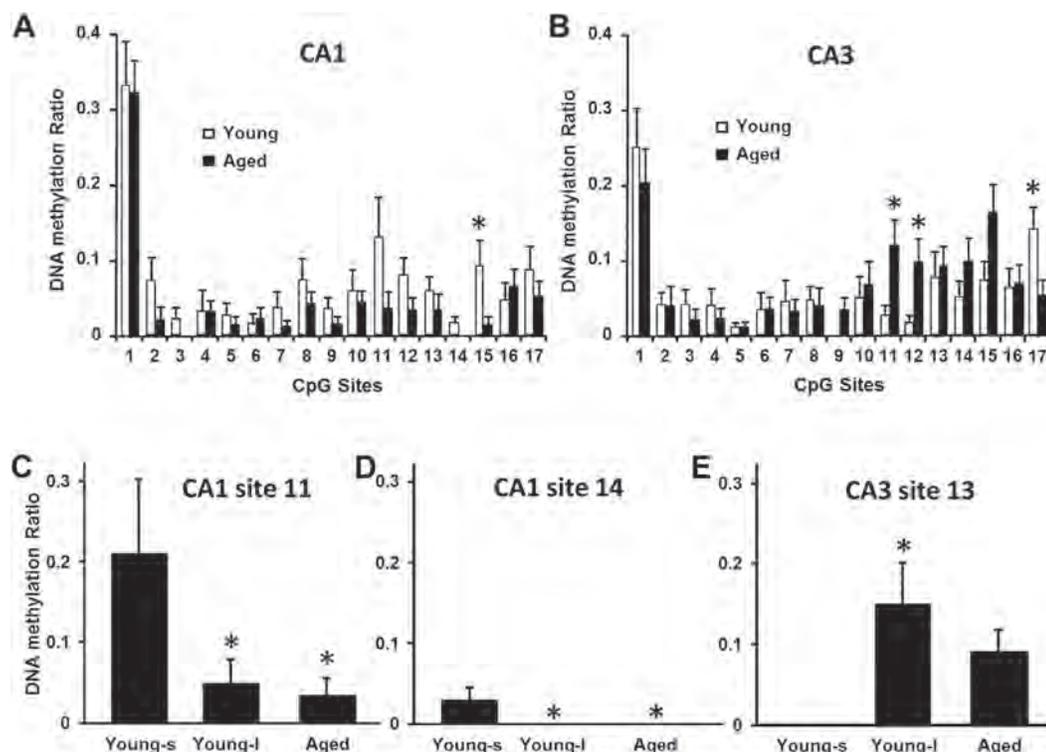


Fig. 4. ER α promoter DNA methylation is altered in regions CA1 and CA3 during aging. Age-related differences in CpG site methylation for (A) area CA1 were observed as an increase in DNA methylation for site 15 for young animals (open bars, $n=10$) relative to aged animals (filled bars, $n = 10$). (B) For area CA3, site 17 contained higher methylation in young rats, and sites 11 and 12 showed higher methylation in aged rats. Due to the influence of OVX duration on transcription, young animals were separated into short-term OVX (young-s) and long-term OVX (young-l) and compared to all aged animals for sites 11–17. Specific differences were observed in CA1 for (C) site 11 and (D) site 14. (E) For region CA3, a difference was observed for site 13. Bars represent the mean (\pm SEM) of the methylation ratio. Asterisks indicate a significant ($p < 0.05$) increase in (A) and (B) and a significant ($p < 0.05$) difference from young short-term in (C–E). Abbreviations: ER α , estrogen receptor alpha; OVX, ovariectomy; SEM, standard error of the mean.

4. Discussion

Previous research using rat models indicates region and age-related changes in ER α protein expression within the hippocampus, with elevated expression in region CA3 relative to CA1 and altered expression in both regions with age or E2 deprivation (Bohacek and Daniel, 2009; Mehra et al., 2005; Zhang et al., 2011). While the effect of age on *Esr1* expression is unclear (Ishunina and Swaab, 2007; Tohgi et al., 1995), *Esr1* messenger levels increase following E2 deprivation (Sarvari et al., 2014) and are higher in area CA3 relative to CA1 (Rune et al., 2002). In the current study, we confirmed that *Esr1* expression is elevated in region CA3 relative to region CA1, suggesting that transcriptional regulation contributes to differential expression of the receptor across the two regions. The age difference in *Esr1* expression was limited to the short-term OVX animals, with increased expression in all aged animals. Furthermore, expression in young animals was elevated in region CA1 following long-term hormone deprivation, such that expression was similar to that of aged animals. These results are consistent with work indicating that hormone state can regulate *Esr1* expression and indicate that long-term E2 deprivation may up regulate *Esr1*, possibly as a compensatory mechanism for a loss of ER α activity (Han et al., 2013). Nevertheless, it should be noted that the level of CA1 *Esr1* expression was consistently reduced relative to area CA3, regardless of age or OVX duration.

In other brain regions and tissues, DNA methylation of the ER α promoter is thought to contribute to differential *Esr1* expression. The promoter exon 1b region was chosen for examination as this has been shown to be the active promoter in the rat brain and contains a number of CpG sites previously associated with the

regulation of the *Esr1* gene (Champagne et al., 2006; Freyschuss and Grandien, 1996; Kurian et al., 2010). Considerable variability in methylation was observed across the 17 CpG sites located in this promoter region. In general, the greatest methylation was observed at site 1 and minimal methylation was observed for sites 2–10. Modest methylation was observed for sites 11–17 with sites 11, 15, and 17 exhibiting higher methylation relative to upstream sites 2–7.

DNA methylation is involved in heritable gene silencing or gene inactivation (Bird and Wolffe, 1999; Newell-Price et al., 2000). While promoter regions that are highly methylated tend to be less transcriptionally active, the relationship between DNA methylation and gene expression is far from clear. In the current study, the largest difference in *Esr1* expression was observed across hippocampal subregions and the site of the greatest DNA methylation, site 1, also exhibited differential methylation across subregions, with increased methylation in region CA1 associated with reduced *Esr1* expression. Similarly, the increase in mRNA expression in CA1 with age was associated with decreased methylation of site 15 in CA1, and the increase in *Esr1* for young long-term OVX and aged animals was associated with decreased methylation in CA1 of sites 11 and 14, compared to young short-term OVX animals. The results indicate that for sites with the greatest methylation (i.e., site 1), variability in methylation is associated with changes in *Esr1* expression. Furthermore, for more distal sites (i.e., 11, 14, 15), methylation is more modest and can be modified across the lifespan. Each of these points are addressed below.

The idea that increased methylation of site 1 is related to decreased mRNA expression is consistent with previous work in other brain regions (Kurian et al., 2010). However, we also observed increased methylation for sites 14 and 15 in region CA3 associated

with increased *Esr1* expression. Similarly, [Gore et al. \(2011\)](#) have reported that exposure to an estrogenic endocrine disruptor increased DNA methylation at one site, identified as site 14 of the *Esr1* promoter in the current study, which was associated with increased mRNA levels in the preoptic area ([Gore et al., 2011](#)). Thus, it should be emphasized that the variability in methylation, increasing or decreasing, only provides a correlate of mRNA expression. A mechanism through which differential DNA methylation might regulate *Esr1* expression remains to be elucidated.

The idea that methylation of these distal sites can be modified across the lifespan to regulate *Esr1* expression is supported by previous work, which demonstrates that maternal care and hormonal manipulations altered *Esr1* expression in the medial preoptic area and amygdala, and the changes in *Esr1* expression were associated with differential methylation of sites 11–16 ([Champagne et al., 2006](#); [Edelmann and Auger, 2011](#); [Gore et al., 2011](#)). Across hippocampal subregions, there is heterogeneity in DNA methylation, gene expression, and in the transcriptional response to aging ([Xu, 2015](#); [Zeier et al., 2011](#)). Previous studies have highlighted distinct patterns in DNA methylation and transcription across cell types including different neuronal cell types ([Angermueller et al., 2016](#); [Brunner et al., 2009](#); [Kozlenkov et al., 2014, 2016](#)), suggesting that variability in DNA methylation observed in the current study could be due to cell-type heterogeneity. However, it should also be noted that mRNA for ER α has been observed in both pyramidal cells and interneurons in the hippocampus and expression is higher in region CA3 relative to CA1 ([Rune et al., 2002](#)). Regardless, it will be important for future studies to determine if the relationship of DNA methylation and *Esr1* expression is specific to certain cell types.

Several transcriptional and post-translational feedback mechanisms control estrogen receptor expression ([Bean et al., 2014](#)). However, most of this work has been performed in breast cancer cell cultures, and the molecular mechanisms that regulate estrogen receptor expression in the hippocampus are not well understood. In mice, functional knockout of ER α or ER β induces a compensatory increase in hippocampal *Esr1* and *Esr2* transcription, respectively, suggesting a feedback mechanism ([Han et al., 2013](#)). The current study suggests that a shift in DNA methylation, particularly for distal sites, could be involved in feedback regulation of ER α expression. Previous studies have reported a link between ER α expression, DNA methyltransferase activity, and ER α promoter methylation during development, aging, and in disease states ([Wang et al., 2012](#); [Westberry et al., 2010](#); [Yang et al., 2001](#)). Methylation of the promoter may influence mRNA expression by regulating the binding of transcription factors. A number of putative transcriptional factors binding sites have been reported for the exon 1b region ([Gore et al., 2011](#)); however, only the binding of one transcriptional factor (Stat5b) has been found to be associated with ER α methylation ([Champagne et al., 2006](#)). In addition, DNA methyltransferase interacts with transcription repressor proteins (e.g., histone deacetylase) to alter chromatin structure and the pattern of DNA methylation ([Robertson, 2002](#)). Transcription repressors, Hdac2 and Sap18, influence *Esr1* transcription ([Bicaku et al., 2008](#); [Ellison-Zelski et al., 2009](#)) and E2 treatment decreases the expression of Hdac2 and Sap18 in the hippocampus ([Aenlle et al., 2009](#)). Thus, altered expression of transcription repressor proteins may interact with DNA methylation as part of a feedback mechanism.

Finally, the ratio of hippocampal ER α and ER β expression interacts with the level of E2 to influence transcription and synaptogenesis and a shift in the ER α /ER β ratio may determine the ability of E2 to influence cognition ([Bean et al., 2014, 2015](#); [Hall and McDonnell, 1999](#); [Han et al., 2013](#); [Pettersson et al., 2000](#)). The increase in *Esr1* and *Esr2* transcription in estrogen receptor

knockout mice is diminished for *Esr1*, but not *Esr2*, during aging ([Han et al., 2013](#)). It is also interesting to note that E2 treatment of aged animals increases hippocampal synaptic expression of ER β , but not ER α ([Waters et al., 2011](#)), suggesting differential regulation of estrogen receptors during aging. Due to the interaction of ER α and ER β in determining functional outcome, it will be important to map out differences in the regulation of these two estrogen receptors during aging.

In summary, transcriptional levels of *Esr1* were altered across hippocampal CA1 and CA3 subregions, with increased expression in region CA3 relative to CA1. In addition, an age-related increase in expression was found in region CA1 relative to young short-term OVX rats. Furthermore, the results support the idea that DNA methylation is an active epigenetic mechanism for the regulation of *Esr1* in the hippocampus, where methylation of site 1 may be the primary regulatory region for cross-regional patterns in ER α expression. Additionally, differential methylation of distal CpG sites, 11–17, was associated with aging or E2 deprivation, suggesting that these sites are modifiable across the life span and may act as a feedback mechanism for ER α activity.

Disclosure statement

The authors have no conflicts of interest to disclose.

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Movement Enhances the Nonlinearity of Hippocampal Theta

Alex Sheremet,¹ Sara N. Burke,^{3,4} and Andrew P. Maurer^{2,3}

¹Engineering School of Sustainable Infrastructure and Environment (ESSIE) and ²Department of Biomedical Engineering, University of Florida, Gainesville, Florida 32611, ³Department of Neuroscience, McKnight Brain Institute, College of Medicine, University of Florida, Gainesville, Florida 32610, and ⁴Institute of Aging, University of Florida, Gainesville, Florida 32603

The nonlinear, metastable dynamics of the brain are essential for large-scale integration of smaller components and for the rapid organization of neurons in support of behavior. Therefore, understanding the nonlinearity of the brain is paramount for understanding the relationship between brain dynamics and behavior. Explicit quantitative descriptions of the properties and consequences of nonlinear neural networks, however, are rare. Because the local field potential (LFP) reflects the total activity across a population of neurons, nonlinearities of the nervous system should be quantifiable by examining oscillatory structure. We used high-order spectral analysis of LFP recorded from the dorsal and intermediate regions of the rat hippocampus to show that the nonlinear character of the hippocampal theta rhythm is directly related to movement speed of the animal. In the time domain, nonlinearity is expressed as the development of skewness and asymmetry in the theta shape. In the spectral domain, nonlinear dynamics manifest as the development of a chain of harmonics statistically phase coupled to the theta oscillation. This evolution was modulated across hippocampal regions, being stronger in the dorsal CA1 relative to more intermediate areas. The intensity and timing of the spiking activity of pyramidal cells and interneurons was strongly correlated to theta nonlinearity. Because theta is known to propagate from dorsal to ventral regions of the hippocampus, these data suggest that the nonlinear character of theta decreases as it travels and supports a hypothesis that activity dissipates along the longitudinal axis of the hippocampus.

Key words: CA1; dorsoventral; oscillation; place cell; rat

Significance Statement

We describe the first explicit quantification regarding how behavior enhances the nonlinearity of the nervous system. Our findings demonstrate uniquely how theta changes with increasing speed due to the altered underlying neuronal dynamics and open new directions of research on the relationship between single-neuron activity and propagation of theta through the hippocampus. This work is significant because it will encourage others to consider the nonlinear nature of the nervous system and higher-order spectral analyses when examining oscillatory interactions.

Introduction

The nonlinear character of the brain (Buzsáki, 2006) has been recognized for >50 years (Ashby, 1947; Wiener, 1966) as the foundation of large-scale integration across local neuron structures (Steriade, 2001; Buzsáki and Draguhn, 2004) and for the metastable dynamics critical for rapid neuron organization in

support of behavior (Engström et al., 1996; Friston, 1997; Tognoli and Kelso, 2014). Understanding brain nonlinearity is therefore fundamental for determining how brain dynamics translate to behavior (Hasselmo, 2015; Marder, 2015), yet quantitative descriptions of brain nonlinearity are scarce (Buzsáki, 2006). Using a thermodynamics analogy, “understanding the brain” can be achieved by describing either its microscopic states (complete description of the state of each neuron) or its macroscopic states (sets of “statistically equivalent” microscopic states). Although the former does not seem achievable (Marder, 2015), even with new high-density measures of real-time neural activity (Ziv et al., 2013), a macroscopic model of brain dynamics could be built on existing measurement techniques, such as the local-field potential (LFP). The LFP reflects the activity of a larger number of neurons (Buzsáki, 2002; Buzsáki et al., 2012; Schomburg et al., 2012), providing a macroscopic signature of microscopic activity.

Although the characteristics of the hippocampal LFP in relation to behavior are well documented (Buzsáki, 2005), its nonlin-

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Correspondence should be addressed to Andrew P. Maurer, Department of Neuroscience, University of Florida College of Medicine, P.O. Box 100244, 1149 Newell Drive, McKnight Brain Institute, L1-100 Gainesville, FL 32610. E-mail: drewmaurer@ufl.edu.

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erity has not been examined explicitly. The goal of the current investigation was to quantify the nonlinearity of the hippocampal theta rhythm in relation to movement. Theta is a dominant feature of hippocampal LFP commonly reported as a 4–12 Hz oscillation associated with active exploration and REM sleep (Jung and Kornmüller, 1938; Green and Arduini, 1954; Vanderwolf, 1969) that may serve to organize hippocampal neuron firing. During movement, principal cells of the hippocampus express spatial receptive fields (O'Keefe and Dostrovsky, 1971). As an animal passes through a neuron's place field, the spike timing systematically shifts to earlier phases of theta between adjacent cycles (O'Keefe and Recce, 1993). The rate at which this precession occurs varies along the longitudinal axis of the hippocampus (Maurer et al., 2005). Interestingly, differences between dorsal and more ventral regions of the hippocampus are also observed in theta amplitude and the firing rate of neurons, such that firing rates and theta power in intermediate CA1 show less velocity modulation than in dorsal CA1 (Maurer et al., 2005). This suggests that dynamic interactions between hippocampal neurons and LFP may reflect the behavioral state of an animal, but this varies according to longitudinal position.

In addition to amplitude, the shape of the theta wave is sensitive to movement, with a transition from a sinusoid to sawtooth shape at faster running speeds (Buzsáki et al., 1983; Terrazas et al., 2005). Moreover, a 16 Hz oscillation has been observed to develop as a function of velocity (Czurko et al., 1999; Terrazas et al., 2005), described as the second harmonic of theta (Harper, 1971; Coenen, 1975; Leung et al., 1982; Leung, 1982). The standard technique used to investigate theta, however, has typically been based on Fourier power-spectrum analysis. Because it ignores phases, this approach is fundamentally linear. Therefore, the nonlinear mechanisms underlying the relationship between the 16 Hz oscillation and the sawtooth shape of theta in relation to running speed (Skaggs, 1995) and dorsoventral position is not well understood.

The theta rhythm is also known to propagate along the hippocampal longitudinal axis (Lubenov and Siapas, 2009; Patel et al., 2012), losing amplitude as it moves into more ventral regions (Maurer et al., 2005; Royer et al., 2010). It is not known, however, how this relates to longitudinal differences in the modulation of neuron activity by velocity. To understand the nature of the sawtooth shape of theta and the 16 Hz frequency component in the hippocampus, we investigated the nonlinear character of the theta rhythm in the dorsal and intermediate regions of the hippocampal CA1 subregion. The nonlinearity was quantified in terms of phase coupling between different Fourier frequency bands that were estimated using third-order Fourier statistics.

Materials and Methods

Subjects and behavior. Neurophysiology data were obtained from three Brown Norway/Fisher-344 hybrid male rats between 8 and 12 months of age. The rats were housed individually and maintained on a 12 h light/dark cycle. Recordings took place during the dark phase of the cycle. Surgery was conducted according to the National Institutes of Health's guidelines for rodents and approved Institutional Animal Care and Use Committee protocols.

Before surgery, the animals were food deprived to 85% of their *ad libitum* weight. During this time period, the rats were trained to run on circular tracks for food reinforcement. Food was given on either side of the barrier and at the 180° opposite point. Rats ran for ~20 min, resulting in a variable number of laps per session depending on motivation and other factors. Each track running session was flanked by a rest period in which the rat rested in a towel lined pot located near the track. For the

present analyses, only data obtained during running conditions are presented.

Surgical procedures. Neuronal recordings were made in the dorsal and intermediate CA1 subregion of the hippocampus. Before surgery, rats were administered ampicillin (Bicillin; Wyeth Laboratories; 30,000 U, i.m., in each hindlimb). The rats were implanted, under isoflurane anesthesia, with an array of 14 separately movable microdrives ("hyperdrive"). This device, implantation methods, and the parallel recording technique have been described in detail previously (Gothard et al., 1996). Briefly, each microdrive consisted of a drive screw coupled by a nut to a guide cannula. Twelve guide cannulae contained tetrodes (McNaughton et al., 1983; Recce and O'Keefe, 1989), four-channel electrodes constructed by twisting together four strands of insulated 13 μm nichrome wire (H.P. Reid). Two additional tetrodes with their individual wires shorted together served as an indifferent reference and an EEG recording probe. A full turn of the screw advanced the tetrode 318 μm .

For 1 rat, the tetrodes were divided into 2 groups of 7 ("split bundle drive"), permitting recording simultaneously from the septal (3.0 mm posterior, 1.8 mm lateral to bregma) and middle (6 mm posterior, 5.0 mm lateral to bregma) regions of the hippocampus. For the other 2 rats, recordings were made sequentially, first from the intermediate (5.7 mm posterior, 5.0 mm lateral to bregma). After intermediate recordings were completed, the implant was removed. Rats were then implanted with a new array in dorsal/septal (3.0 mm posterior, 1.4 mm lateral to bregma) regions. In all cases, the implant was cemented in place with dental acrylic anchored by dental screws. A ground lead was connected to one of the jeweler's screws placed in the skull. After surgery, rats were orally administered 26 mg of acetaminophen (Children's Tylenol *Elixir; McNeil). They also received 2.7 mg/ml acetaminophen in the drinking water for 1–3 d after surgery and oral ampicillin (Bicillin; Wyeth Laboratories) on a 10 d on/10 d off regimen for the duration of the experiment. Data from these animals have been used in other unrelated analyses that have been published previously (Maurer et al., 2005; Maurer et al., 2006a, 2006b; Maurer et al., 2012), drawing from a database of ~900 well isolated pyramidal neurons.

Electrophysiological recording. Twelve tetrodes were lowered after surgery into the hippocampus, allowed to stabilize for several days just above the CA1 hippocampal subregion, and then gradually advanced into the CA1 stratum pyramidale. Another probe was used as a neutral reference electrode and was located in or near the corpus callosum. The final probe was used to record theta field activity from the vicinity of the hippocampal fissure. Each tetrode was attached to four separate channels of a 50-channel unity-gain head stage (Neuralynx). A multiwire cable connected the head stage to digitally programmable amplifiers (Neuralynx). The spike signals were amplified by a factor of 1000–5000, band-pass filtered between 600 and 6 kHz, and transmitted to the Cheetah Data Acquisition system (Neuralynx). Signals were digitized at 32 kHz and events that reached a predetermined threshold were recorded for a duration of 1 ms. Spikes were sorted offline on the basis of the amplitude and principal components from the four tetrode channels by means of a semiautomatic clustering algorithm (BBClust; P. Lipa, University of Arizona, Tucson, AZ, and KlustaKwik; K.D. Harris, Rutgers University, Newark, NJ). The resulting classification was corrected and refined manually with custom-written software (MClust; A.D. Redish, University of Minnesota, Minneapolis, MN), resulting in a spike-train time series for each of the well isolated cells. No attempt was made to match cells from one daily session to the next, so the numbers of recorded cells reported does not take into account possible recordings from the same cells on consecutive days. However, because the electrode positions were adjusted from one day to the next, recordings from the same cell over days were probably relatively infrequent. Putative pyramidal neurons were identified by means of the standard parameters of firing rate, burstiness, spike waveform shape characteristics (Ranck, 1973), and the first moment of the autocorrelation (Csicsvari et al., 1998).

Theta activity in the CA1 layer was taken from the tetrode that collected the most pyramidal neurons while the fissure LFP was recorded from a separate probe that was positioned 0.5 mm below the CA1 pyramidal layer. LFP signals were band-pass filtered between 1 and 300 Hz and sampled at 2.4 kHz, amplified on the head stage with unity gain, and

then amplified again with variable gain amplifiers (up to 5000). Several light-emitting diodes were mounted on the head stage to allow position tracking. The position of the diode array was detected by a television camera placed directly above the experimental apparatus and recorded with a sampling frequency of 60 Hz. The sampling resolution was such that a pixel was 0.3 cm.

Time-series analysis. Throughout the current study, the phrase “time series” will be used in its traditional sense of a sequence of unprocessed measurements (numbers) indexed in time, synonymous to raw, unfiltered voltage traces of the EEG. This study assumes that EEG time series express essential properties of the underlying physical processes of the brain; that is, the physiological interactions contributing to the extracellular transmembrane currents. In principle, these processes could be represented using a mathematical formalism that uses a set of differential equations to describe their time evolution. The term “system” will therefore be used as it is in “dynamical systems” theory, to denote the differential equations that describe the brain processes and the time evolution of their solutions. Therefore, “system” can be thought of as a mathematical abstraction completely describing the brain processes.

In the mathematical description introduced above, EEGs are functions of, and carry information about, the state of the underlying system. The central focus of this study is the nonlinear character of the system and the central observation that the nonlinearity of the brain is reflected in the EEG time series. If the system is linear, then known solutions can be added to construct new solutions; that is, the solution space is a linear space. Under quite general uniformity conditions, sinusoids form a basis of the solution space, the Fourier basis. The general solution is a superposition of sinusoids with different amplitudes (which include the initial phase) and thus completely defined by the distribution of amplitudes. The stochastic general solution is a superposition of sinusoids with random amplitudes. The joint probability density of the amplitude set defined completely the statistics of the solution. Therefore, the geometry of the solution space of a linear system is trivial: any point in the space spanned by the Fourier basis is a solution to the system and the general solution is completely characterized by the projections on the elements of the basis (amplitudes).

In contrast, in the case of nonlinear systems, two solutions cannot be added to construct a new solution, which makes them rather intractable unless some simplifying assumptions can be made. Our central assumption is that the brain is a weakly nonlinear system and, for weakly nonlinear systems, the general solution can still be represented, in the leading order, as a superposition of sinusoids. However, the amplitudes cannot be constant (otherwise, the system would be linear) and therefore have to evolve. Indeed, decomposition on a linear basis (e.g., the Fourier one) yields a system of equations that describes the evolution of amplitudes through mutual interactions.

The geometry of the nonlinear solution space becomes nontrivial: a general solution is a trajectory in the space spanned by the Fourier basis. The stochastic solution will therefore be characterized by statistically correlated amplitudes and phases. Phase correlations are expressed in the peculiar appearance of the shape of the solution, for example, in the development of time series asymmetries. It follows that such a solution is not completely defined by its power spectrum and knowledge about the phase correlations is essential.

The analysis of the theta spectral band EEG time series used in the current study was based on standard techniques used for stationary signals (Priestley, 1981; Papoulis and Pillai, 2002). We assume that the EEG time series $g(t)$ is a stochastic process, stationary in the relevant statistics, and decompose it using the discrete Fourier transform (DFT) as follows:

$$g_j = \frac{1}{N} \sum_{n=1}^N G_n \exp(2\pi f_n t_j); \quad G_n = \sum_{j=1}^N g_j \exp(-2\pi f_n t_j); \quad (1)$$

Where $g_j = g(t_j)$ is a sequence of N points collected at times $t_j = j\Delta t$, with $j = 1, 2, \dots, N$, Δt is the sampling interval, and $G_n = G(f_n)$ is the sequence of complex Fourier coefficients corresponding to the following frequencies:

$$f_n = \frac{n}{N\Delta t}, \quad \text{with } n = 1, 2, \dots, N. \quad (2)$$

The family of sequences $s_n(t_j) = \exp(-2\pi f_n t_j)$ form the Fourier basis, also called modes. The second-order (linear) statistics of the Fourier spectrum of time series g are characterized by the spectral density as follows:

$$S_n = S(f_n) = \mathbf{E}[G_n G_n^*] \quad (3)$$

Where G_n is the series of complex Fourier coefficients of process g , f_n is a frequency band in the Fourier representation (Eq. 2), and $\mathbf{E}[\dots]$ is the expected-value operator. Spectral densities describe the frequency distribution of variance. If the process g is linear, it is completely characterized by its variance and consequently its variance density S_n . A realization of the process can be derived by assigning a set of random phases (uniformly distributed in $[-\pi, \pi]$) to modal amplitudes defined e.g., as $\sqrt{S_n}/2$. Note that the spectral density contains no information about phases, their correlation, and, therefore, about the nonlinearity of the process.

The nonlinear character of the system, expressed in phase correlations across spectral components, is described in the lowest order by the bispectrum, first proposed for ocean waves by Hasselmann et al. (1963) and further developed by Rosenblatt and Van Ness (1965) and others (Kim and Powers, 1979; Masuda and Kuo, 1981; Elgar, 1987), as follows:

$$B_{n,m} = B(f_n, f_m) = \mathbf{E}[G_n G_m G_{m+n}^*], \quad (4)$$

Where f_n and f_m are two frequencies in the Fourier sequence in Equation 2. In other words, in this notation, f_n and f_m are waves of different frequencies. When the bispectrum is derived from a single time series, the result is described as the auto-bicoherence (simply referred to as bicoherence in the present study). The bispectrum is statistically zero if the Fourier coefficients are mutually independent; that is, for a linear system. For nonlinear systems, the bispectrum will exhibit peaks at triads (f_n, f_m, f_{n+m}) that are phase correlated, measuring the degree of three-wave coupling. The real and imaginary part of the bispectrum B provide measures of third-order statistics (Masuda and Kuo, 1981; Elgar, 1987), as follows:

$$\sigma^{-3} \sum_{n,m} B_{n,m} = \zeta + iA \quad (5)$$

Where ζ is the skewness and A is the asymmetry of the process (Haubrich and MacKenzie, 1965; Masuda and Kuo, 1981) and σ is its SD, with the sign of ζ and A (\pm) indicating the direction of the skew or asymmetry. Both ζ and A are “global” measures of the nonlinearity of the process (Fourier modes obviously have zero skewness and asymmetry). Meaningful skewness/asymmetry estimates can be defined for a frequency band only if it is wide enough (see below) to contain all of the relevant phase-coupled modes.

To eliminate the distortion induced by the variance distribution, the bispectrum can be normalized (Haubrich and MacKenzie, 1965) as follows:

$$b_{n,m} = \frac{B_{n,m}}{\left(\mathbf{E} \left[\left| G_n G_m \right|^2 \right] \mathbf{E} \left[\left| G_{n+m} \right|^2 \right] \right)^{1/2}}. \quad (6)$$

The squared modulus and the phase of the normalized bispectrum are called bicoherence and biphas. Although definition 5 does not insure that $|b_{n,m}| < 1$ (see, e.g., Kim and Powers, 1979; Elgar and Guza, 1985), it was adopted here for its simplicity and ease of implementation. Equation 4 implies that the real and imaginary part of the normalized bispectrum can be interpreted as the “frequency distribution” of skewness and asymmetry. In the sequel, we will refer to the real and imaginary parts of the normalized bispectrum as skewness and asymmetry distributions as follows:

$$\zeta_{n,m} = \mathbf{R}\{b_{n,m}\}; \quad A_{n,m} = \mathbf{I}\{b_{n,m}\}, \quad (7)$$

Where $\mathbf{R}\{z\}$ and $\mathbf{I}\{z\}$ are the real and imaginary parts of the complex number z , respectively.

The bispectrum (and thus the normalized bispectrum) has well known symmetries with respect to its arguments (Rosenblatt and Van Ness,

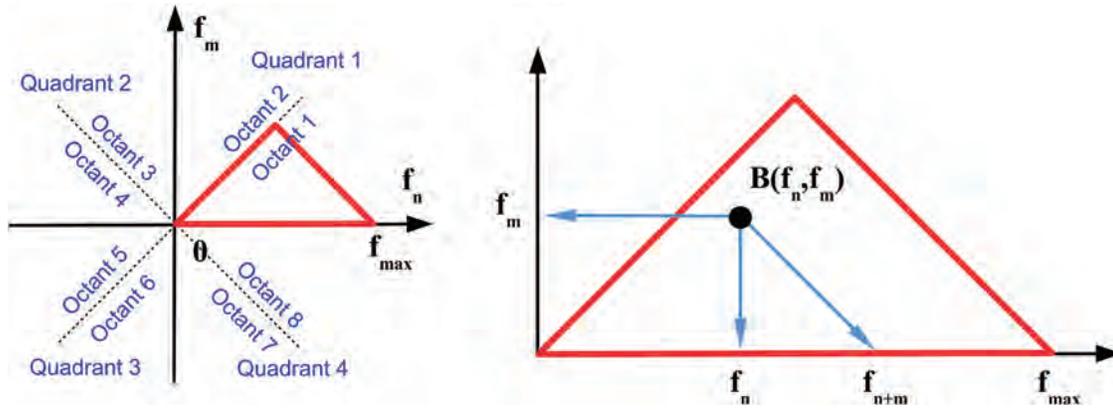


Figure 1. Bispectral symmetries (see Eq. 7–9). Left, Quadrant and octants of the frequency plane. Red triangle represents the area containing nonredundant information for the DFT. Right, Peak in the bispectral estimate (represented by the black dot) represents a phase-coupled triplet (f_n, f_m, f_{n+m}) , where f_n, f_m are two frequencies in the Fourier representation sequence defined in Equation 1. If a bispectral peak (black dot) is on the first diagonal, then $m = n$, meaning that frequency f_n is phase correlated to its second harmonic $f_{2n} = 2f_n$.

1965; Fig. 1), which is equivalent to saying that different regions in the plane spanned by (f_n, f_m) contain equivalent (redundant) bispectral information, as follows:

$$B(-f_n, -f_m) = B^*(f_n, f_m) \quad \text{Quadrants 1 – 3 and 2 – 4} \\ \text{are equivalent.} \quad (8)$$

$$B(f_m, f_n) = B(f_n, f_m) \quad \text{Octants 1 – 2 are equivalent.} \quad (9)$$

$$B(f_n, f_m) = B^*(f_n, -f_m, f_m) \quad \text{Octants 1 – 8 and 2 – 7} \\ \text{are equivalent.} \quad (10)$$

From rules 8–10, it follows that the first octant of the plane (f_n, f_m) contains all nonredundant bispectral information. For the DFT, the nonredundant domain reduces to a segment of the first octant bounded by a line parallel to the second diagonal passing through the maximum (Nyquist) frequency. The nonredundant domain in the (f_n, f_m) plane is illustrated in Figure 1. The figure contains a basic, minimal receipt for understanding bispectral distributions. At any point in the (f_n, f_m) plane, the value of the bispectrum $B(f_n, f_m)$ represents the phase correlation between the Fourier modes with frequencies f_n, f_m , and $f_{n+m} = f_n + f_m$. In other words, the triad contains the two coordinates of the point and the frequency defined by the intersection of the second diagonal with the horizontal axis.

Implementation of bispectral analysis. To estimate second-order Fourier statistics, the time series were de-meant, linearly de-trended, and divided into 50% overlapping segments of ~4 s windows (2^{13} -point windows for the sampling rate $f_s = 1988$ Hz for rats 7951 and 8042; 214 for $f_s = 936$ Hz for rat 7805), with a frequency resolution of $\sim \Delta f = 0.25$ Hz. The total time intervals for analysis were chosen so as to yield a number of degrees of freedom (DOF) >170 for all data analyzed and DOF = 300 for the data presented. The statistics of the bispectrum of stationary processes are well understood (Haubrich and MacKenzie, 1965; Elgar and Guza, 1985; Elgar and Sebert, 1989; and many others). Briefly, for the normalization used here (Eq. 6), the probability density function (PDF) is approximated by the noncentral χ^2 distribution, where $|b|^2$ is the mean value of the bicoherence, and the parameters n and α are given by the following (equations 6–8 in Elgar and Sebert, 1989).

$$f\left(\frac{|b|^2}{\alpha}\right) \approx \chi^2(|b|^2, n) \quad \text{with } n = \frac{DOF |b|^2}{2(1 - |b|^2)^3}, \text{ and } \alpha = \frac{|b|^2}{n} \quad (11)$$

Knowledge of the theoretical PDF allows for estimating confidence limits for any mean value of the bicoherence. Because zero mean that bicoherence is meaningful for distinguishing between linear and nonlinear stochastic processes, an important consequence of the above discussion is

that the zero-mean bicoherence is χ^2 distributed with $n = 2$. From this, a confidence level can be derived. With DOF = 300, the zero-mean bicoherence $|b| < 0.05$ at 90% confidence level and bicoherence $|b| < 0.1$ at 95%. This is consistent with Elgar and Guza’s (1985) estimate of $|b| < \sqrt{\frac{6}{DOF}} \approx 0.15$ at 99% (Haubrich and MacKenzie, 1965 gives this relation for a different bispectral normalization and for the confidence level of 95%).

The time–frequency representation (windowed Fourier transform; Mallat, 1999) was used to represent the time evolution of the EEG frequency content (2^{12} -point window at 90% overlap). Bispectral analysis was used to examine the nonlinearity of the EEG time series and was implemented using a lower-frequency resolution and increased DOF by using 210-point windows (0.5 s) segments.

To compare the nonlinear character of theta across hippocampal regions and velocity conditions, we defined a global nonlinearity measure as the square root of the bicoherence integrated over the frequency domain (Sheremet et al., 2002), as follows:

$$\phi = \left[\sum_{n,m=1}^n 8 \left| \bar{b}_{n,m} \right|^2 \Delta f^2 \left(1 - \frac{1}{2} \delta_{n,m} \right) \right]^{\frac{1}{2}} \quad (12)$$

where

$$\bar{b}_{n,m} = \begin{cases} b_{n,m} & \text{if } b_{n,m} > 0.1 \\ 0 & \text{otherwise} \end{cases} \quad (13)$$

The value 0.1 is the 95% confidence level of $|\bar{b}_{n,m}| = 0$, where $|b_{n,m}|$ is the normalized bispectrum matrix (Eq. 6); $\Delta f = \frac{1}{N\Delta t}$ is the frequency increment, δ is the Kronecker symbol, as in the following:

$$\delta_{n,m} = \begin{cases} 1 & \text{if } n = m \\ 0 & \text{otherwise} \end{cases}, \quad (14)$$

and the rest of the symbols have the same meaning as in Equation 6. The measure ϕ is proportional to the Euclidean norm of the matrix $|b_{n,m}|$, where the coefficient $\left(1 - \frac{1}{2} \delta_{n,m} \right)$ accounts for the symmetries of $b_{n,m}$, that is, for the diagonal appearing only four times in the full matrix.

All calculations were coded in MATLAB using its implementation of the DFT. The bispectral estimates were computed using code based on modified functions of the HOSA toolbox (Swami et al., 2000).

Spike spectrogram. To determine the frequency in which neuronal spiking occurred, we implemented spectral analyses on spike trains (Leung and Buzsáki, 1983). First, action potentials were sorted based upon the velocity of the rat. Spikes between either 10–20 cm/s or 60–70

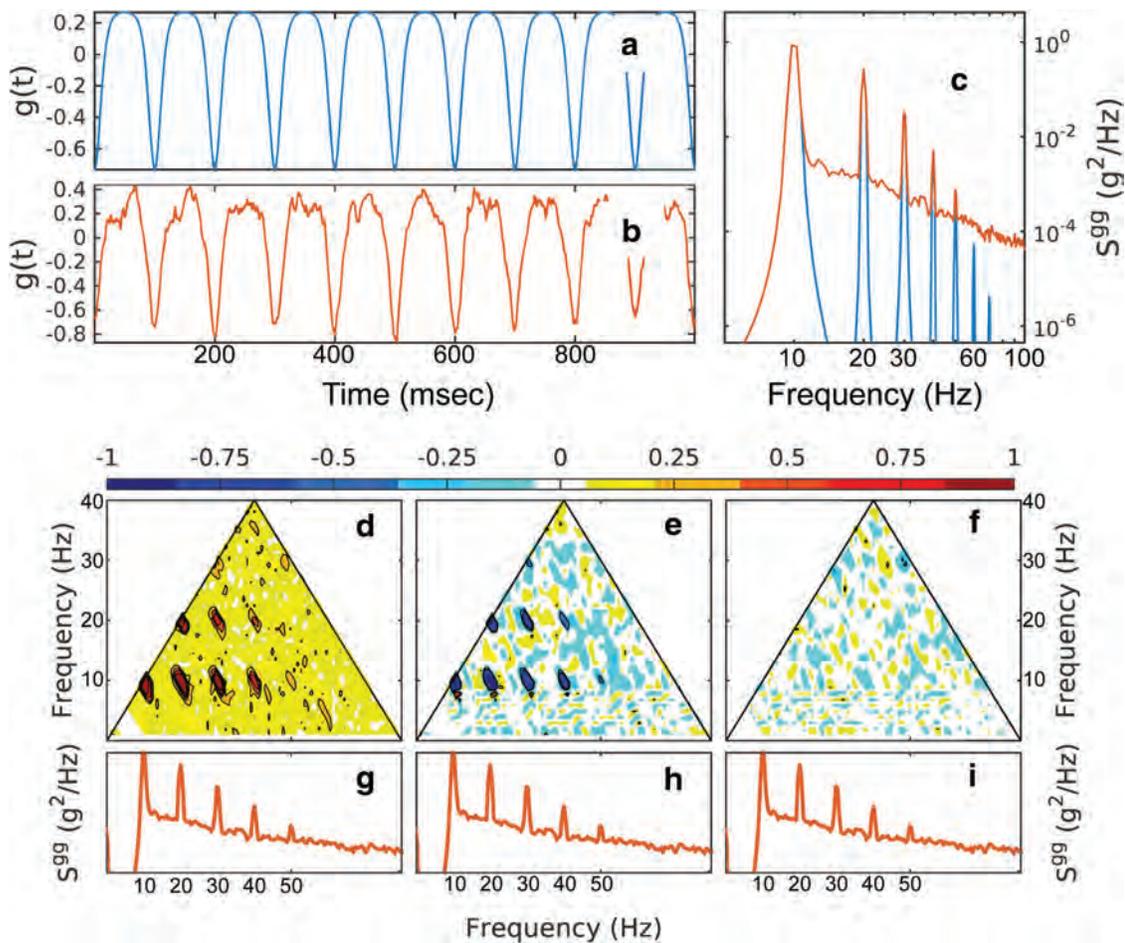


Figure 2. Bispectral analysis of a noisy time series with negative skewness. *a*, Signal based on a cnoidal function. *b*, Signal with added f^{-2} Gaussian noise with at 20% signal variance. *c*, Spectra of the two time series. *d–f*, Normalized bispectrum of the noisy time series depicting the modulus (*d*), the real part (*e*; skewness distribution), and the imaginary part (*f*; asymmetry distribution). The power spectra of the signal with added f^{-2} Gaussian noise are repeated in *g–i* for reference.

cm/s were analyzed separately, placing the spikes for each velocity segment into 1 ms bins for the duration of the recording. This often resulted in a sparse, but periodic vector of spike counts. The power spectra of the spike trains were then calculated via the Thomson multitaper method.

Results

Simplified nonlinear EEG model

Here, we constructed a simplified model that illustrates the bispectral signature of stochastic processes with measurable global skewness and asymmetry. The model consists of two nonlinear time series (Figs. 2, 3) constructed using periodic analytical functions (period 1 s) based on elliptic Jacobi functions (Whitham, 2011) deformed to have controllable asymmetry (Figs. 2*a*, 3*a*). The process analyzed in Figure 2 has negative skewness and zero asymmetry; the process analyzed in Figure 3 has zero skewness and positive asymmetry (Figs. 2*a*, 3*a*). For a realistic aspect, a Gaussian noise process with a spectral law of f^{-2} (Figs. 2*b,c*, 3*b,c*) is added to the analytic functions. The spectra of the time series (both analytic and “noisified”) exhibit peaks at the harmonics of the fundamental frequency of 10 Hz. Note that the DOF values for the model are arbitrary, so the statistics of the processes represented in Figures 2, 3, and 4 are arbitrarily close to the theoretical ones.

Different components of the bispectrum of the noisified time series are shown in Figures 2*d–f*, 3*d–f*, and 4*d–f*. The modulus of the normalized bispectrum (Eq. 6, Fig. 2*d*, 3*d*) exhibits seven, but

potentially more, distinct peaks. Based on the diagram in Figure 1, these indicate the frequency triplets that are strongly phase coupled. One can distinguish coupling up to the sixth harmonic of the peak: $(f_p, f_p, 2f_p)$, $(f_p, 2f_p, 3f_p)$, $(f_p, 3f_p, 4f_p)$, $(f_p, 4f_p, 5f_p)$, $(2f_p, 2f_p, 4f_p)$, $(2f_p, 3f_p, 5f_p)$, $(2f_p, 4f_p, 6f_p)$, as well as $(3f_p, 3f_p, 6f_p)$. For the first nonlinear time series (Fig. 2), the skewness distribution γ (real part of the normalized bispectrum, Eq. 6, Figs 2*e*, 3*e*) shows the same peaks, but negative, in accordance with the global skewness of the time series. Because the time series has no asymmetry, the asymmetry distribution α is statistically zero (Fig. 2*f*). Alternatively, for the nonskewed but asymmetric time series (Fig. 3), the skewness distribution is statistically zero (Fig. 3*e*), but the asymmetry distribution is positive (Fig. 3*f*).

The contrast spectral and bispectral analysis and the effectiveness of the bispectral components in detecting nonlinearity is illustrated in Figure 4. Here, a linear time series is constructed by assigning Fourier modes random phases uniformly distributed in $[-\pi, \pi]$ to amplitudes derived from the spectral density of the negatively skewed, symmetric time series (Fig. 2*b*), as described above. Importantly, a simple spectral density analysis does not distinguish between the linear and the nonlinear time series because these two oscillations have the same spectral density characteristics (Fig. 4*c*). This illustrates the point that spectral density estimators contain no information about phase correlation across the spectrum, and thus no information about the nonlin-

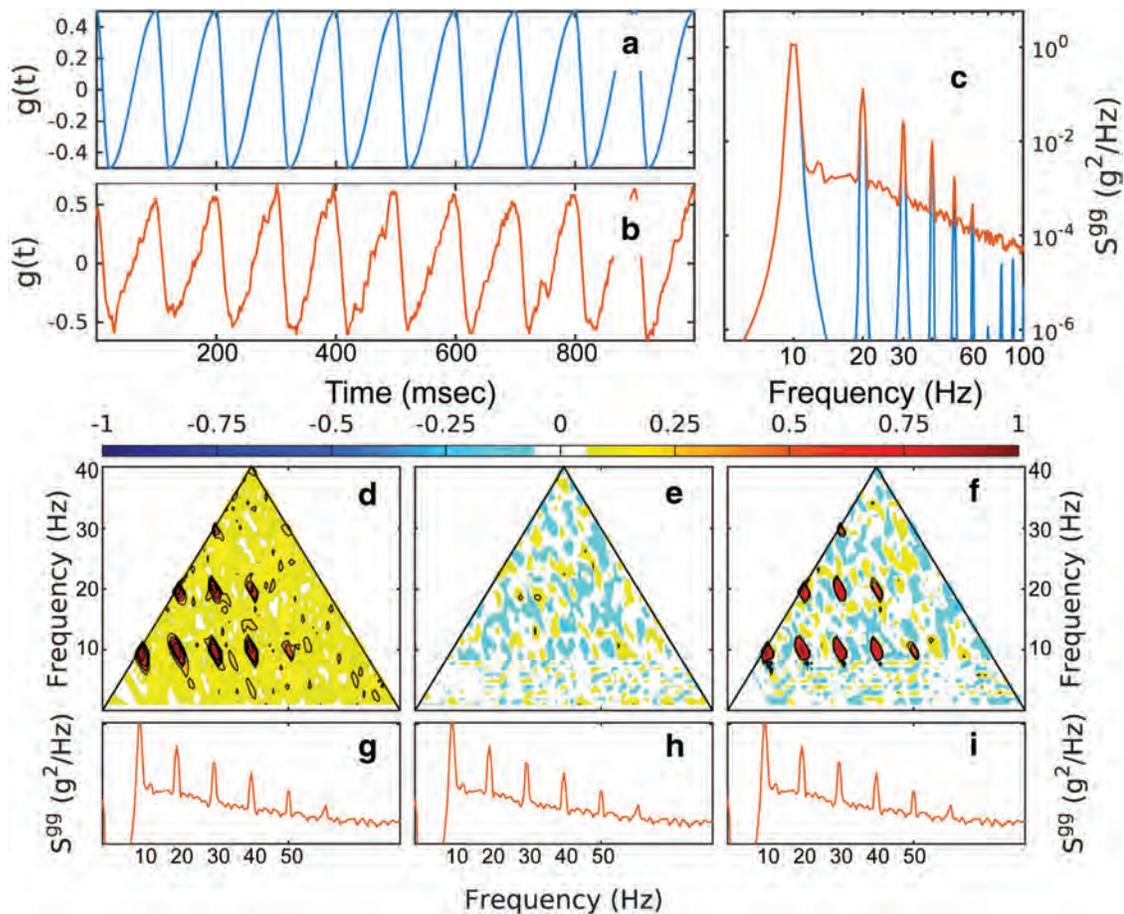


Figure 3. Bispectral analysis of a noisy time series with positive asymmetry. *a*, Signal based on a sinusoidal function. *b*, Signal with added f^{-2} Gaussian noise with at 20% signal variance. *c*, Spectra of the two time series. *d–f*, Normalized bispectrum of the noisy time series depicting the modulus (*d*), the real part (*e*; skewness distribution, and the imaginary part (*f*; asymmetry distribution). The power spectra of the signal with added f^{-2} Gaussian noise are repeated in *g–i* for reference.

ear character of the process. Bispectral analysis (cf. Figs. 2*d–f*, 4*d–f*), however, reveals clearly the linear character of the linear time series in Figure 4*b*. The normalized bispectrum and all of its components are statistically zero.

This simple example makes several points: (1) the bispectrum can identify phase coupling, (2) the different components of the bispectrum are useful to identify the effect of the phase coupling on the skewness and asymmetry of the process, and (3) the presence of phase coupling can be detected even in the presence of Gaussian noise and below the noise level. Finally, this analysis suggests a more general meaning for the concept of harmonics. Harmonics can be defined in several ways. A mode G_{kn} with frequency $f_{kn} = kf_n$, with k integer, is called the k th harmonic of mode G_n . This definition is trivial and not useful: because spectral estimates are never exactly zero at any frequency, such harmonics always exist but have no statistical relationship with the mode G_n . It is tempting to describe spectral distributions such as that in Figure 3 as fundamental frequency and its harmonics.

The inability of the spectral density estimators to distinguish between linear and nonlinear systems makes another important point: the presence of peaks in spectral densities at multiples of a given frequency (harmonics) might have different meanings. For linear systems, harmonics are statistically independent and their presence does not modify the statistics of the process. For nonlinear systems, the presence of harmonics might be accompanied by phase correlations that have significant and fundamental effects on the shape and statistics of the process.

Based on this discussion, hereafter, we reserve the term “harmonic” only for cases in which there is a phase correlation between the harmonics and the fundamental frequency (mode). The determination of whether certain modes are or not harmonics of a spectral peak has to involve an examination of the bispectral characteristics.

Relation between theta and velocity in EEG measured in the dorsal and intermediate CA1

An examination of LFP time series showed obvious changes in the shape of the hippocampal theta (Fig. 5), in agreement with previous studies (Harper, 1971; Coenen, 1975; Leung et al., 1982; Leung, 1982; Terrazas et al., 2005). The unfiltered LFP trace was well approximated by a near-sinusoidal 6–8 Hz band-pass signal for running speeds <20 cm/s (Fig. 5*a,c*). At running speeds >20 cm/s (Fig. 5*b*), the dorsal LFP trace became asymmetric and skewed, departing significantly from the 6–8 Hz signal. Note that the departure of the raw LFP from the 6–8 Hz filter was attenuated in the intermediate hippocampus, even at fast running speeds (Fig. 5*d*). These data suggest the development of cycle skew and asymmetry may be associated with the 16 Hz frequency noted in prior investigations.

Velocity and the CA1 spectrogram

To examine the effect of running speed on the LFP, the average shape of the power spectrogram as a function of running speed was calculated (Fig. 6). At low speeds, the 4–60 Hz frequency

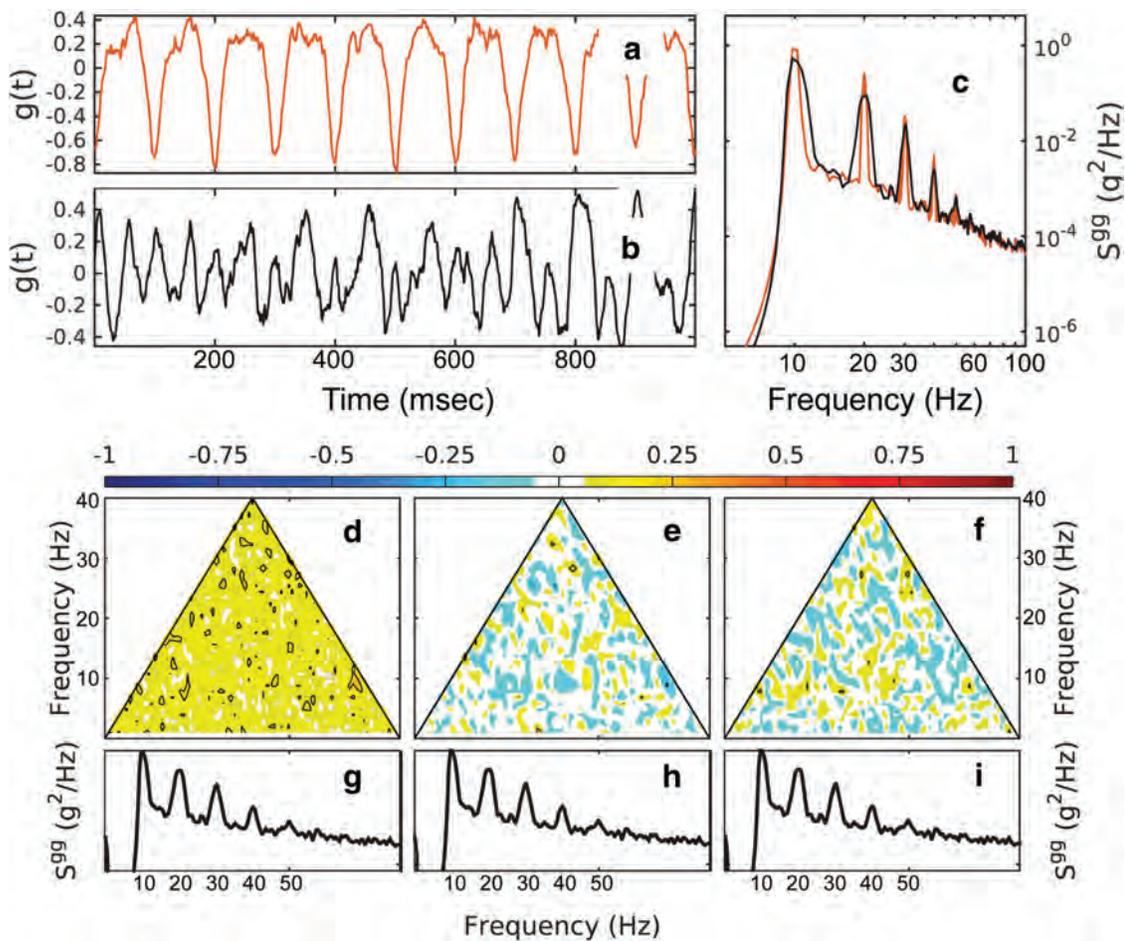


Figure 4. Bispectral analysis of a linear and nonlinear time series. *a, b*, Nonlinear signal (*a*; red) and linear signal (*b*; black) constructed from the spectrum of the nonlinear signal using random phases uniformly distributed in $[0, 2\pi]$. *c*, Spectra of the nonlinear and linear time series (red and black, respectively). *d–f*, Normalized bispectrum of the linear time series (cf. Figs. 2, 3) for the modulus (*d*), the real part (*e*; skewness distribution), and imaginary part (*f*; asymmetry distribution). The power spectra of the linear decomposition are repeated in *g–i* for reference.

band was dominated by a single spectral peak located approximately between 6 and 7 Hz (Fig. 6*a–d*). As the speed increased, the peak quickly narrowed, became more prominent, and shifted toward 8 Hz while additional peaks developed at frequencies $n f_\theta$ ($n > 1$ integer) where $f_\theta = 8$ Hz. For simplicity, we will call the $n f_\theta$ peaks putative theta “harmonics” (see simulation above). The dominance of the putative harmonics increased with movement speed at the expense of the bands separating them. This effect was more pronounced in the dorsal region of the hippocampus, where harmonics could be identified up to $n = 6$ (Fig. 6*a,b*), whereas the intermediate region developed no more than $n = 2$ (Fig. 6*c,d*).

Phase dependence of spectral peaks

The development of spectral peaks in the 16 Hz range and higher have considerable overlap with beta (10–30 Hz) (Penttonen and Buzsáki, 2003) and low-gamma (25–50 Hz) (Colgin et al., 2009; Belluscio et al., 2012). Therefore, the development of higher frequencies at faster velocities may be due to the appearance of rhythms other than the evolution of theta harmonics. Beta and low-gamma, however, are meaningful as distinct rhythms only if they are statistically independent of the theta rhythm. Therefore, the identity of harmonics can be tested simply by checking their statistical relation to theta. In the lowest order, this can be achieved by using bispectral analysis. Compared with other methods for examining frequency coupling between two differ-

ent frequencies (Lachaux et al., 1999), this approach has the advantage that it provides direct measures of phase coupling between Fourier frequency bands with no *a priori* (arbitrary) band identification (filtering). Bispectrum components provide complementary information about the stochastic process analyzed. The absolute value of the normalized bispectrum (bicoherence; Fig. 2) is a measure of the nonlinearity of the signal and is statistically zero for independent frequency bands (stochastic process with uncorrelated phases). The real and imaginary parts provide measures of the contribution of different bands to the skewness (Fig. 3) and asymmetry (Fig. 4) of the total signal. For low-velocity conditions (Fig. 7*a*), dorsal CA1 bicoherence estimates (Fig. 7*a,d*) were statistically zero over the entire Fourier space (no phase correlation). This implies that the asymmetry and skewness measures were also statistically zero (Fig. 7*b,c*). That is, the LFP process was nearly sinusoidal. This agreed with the general aspect of the trace (Fig. 5*a,c*). In contrast, bispectral measures estimated for high-velocity conditions exhibited a distinct pattern of peaks, coinciding with the location and development of the harmonics (see Fig. 1 for instructions on how to read the plots). The bicoherence showed significant phase coupling between theta and the harmonics, with correlations distinguishable for up to the sixth harmonic in dorsal CA1. The bicoherence peaks were reproduced in the skewness and asymmetry distributions shown in Fig. 7, *e* and *f*, where the negative sign indicates the type of skewness and asymmetry produced by the peaks. Remark-

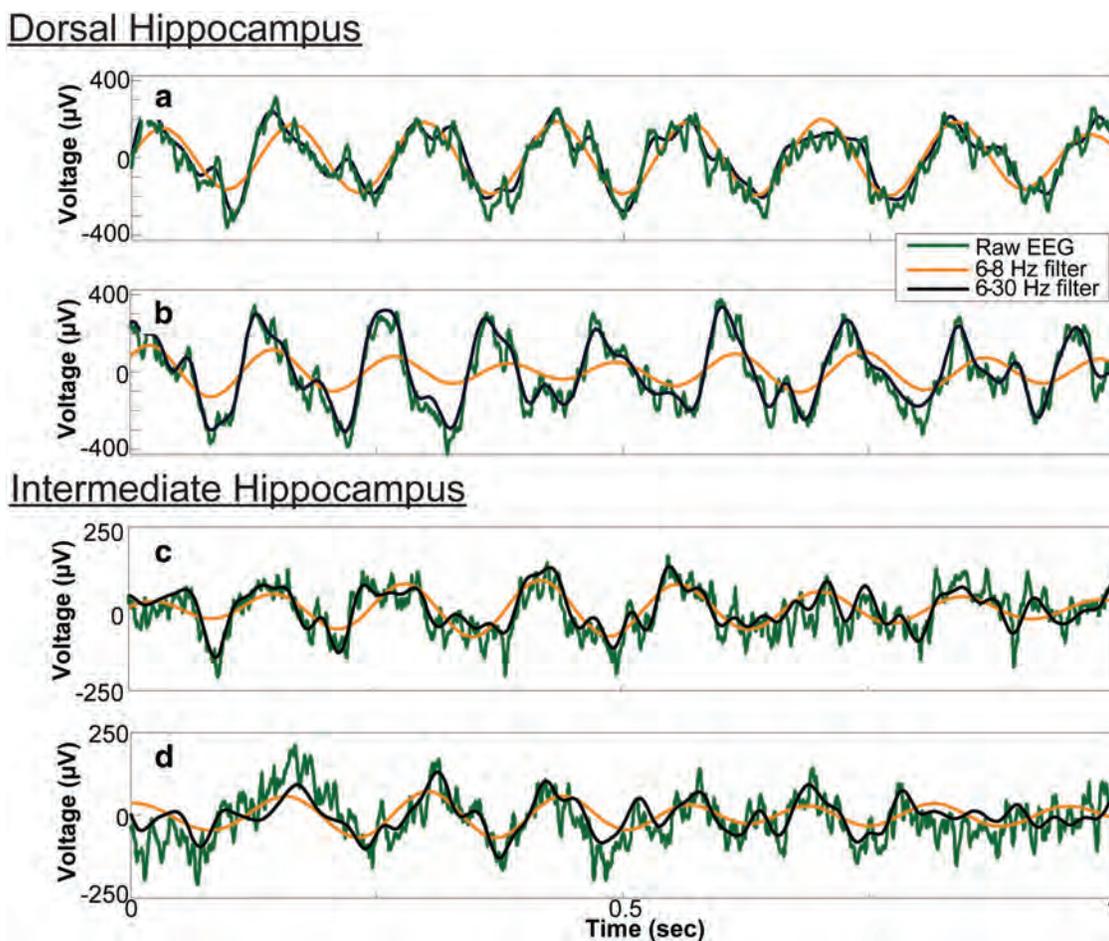


Figure 5. Change of LFP shape as a function of rat running speed. Representative examples of LFP during low running speeds (<20 cm/s; *a, c*) and high running speeds (>20 cm/s; *b, d*) showing the raw data (green), narrow filter (orange), and wide-band filter (black) that captures the asymmetry that developed as a function of running velocity in the dorsal hippocampus.

ably, the nonlinearity of theta was modulated across the hippocampal regions. The same analysis performed for intermediate CA1 (Fig. 7*j–r*) exhibited weaker nonlinearity, with coupling only between theta and the second harmonic clearly distinguishable.

To compare the magnitude of the EEG nonlinearity across hippocampal regions and velocity conditions directly, the mean global nonlinearity measure (Eq. 12–14) for each rat in the dorsal and intermediate hippocampus for the low and high velocities was calculated. Figure 8 illustrates the effects of speed on theta nonlinearity for the three rats tested. The nonlinearity measure φ is consistently higher for higher speeds, with the nonlinearity of the dorsal CA1 region dominating the nonlinearity of the intermediate CA1 region. This comparison revealed that there was a significant main effect of hippocampal region ($F_{(1,4)} = 30.6, p < 0.01$; repeated measures) on the magnitude of the nonlinearity such that the EEG in the dorsal hippocampus was more nonlinear than the intermediate. Although there was no overall main effect of velocity (low vs high) on nonlinearity ($F_{(1,4)} = 5.9, p = 0.08$; repeated measures), the interaction between hippocampal region and velocity was significant ($F_{(1,4)} = 9.1, p < 0.05$; repeated measures). *Post hoc* analysis indicated that this interaction effect was due to a significant difference in EEG nonlinearity across velocity conditions in the dorsal hippocampus ($p < 0.05$), but not in the intermediate hippocampus ($p = 0.2$). Together, these data indicate that EEG in the dorsal hippocampus is more nonlinear and more sensitive to changes in speed of movement relative to the intermediate hippocampus.

Neuronal activity and theta nonlinearity

Because theta has been hypothesized to organize spike timing of neurons (O’Keefe and Recce, 1993; Lisman and Idiart, 1995; Skaggs et al., 1996; Harris et al., 2003), we examined action potential frequency as a function of velocity for single cells in dorsal and intermediate CA1 using spectral density estimates (Fig. 9). The spectral power of spiking in the theta band demonstrated that dorsal neurons were sensitive to changes in velocity. There was a significant increase in both frequency ($t_{(5)} = 7.75, p < 0.001$) and amplitude ($t_{(5)} = 3.58, p < 0.02$) between slow and fast velocities, which is consistent with a previous report (Geisler et al., 2010). This was not the case in the intermediate hippocampus, in which neither the frequency ($t_{(5)} = 0.48, p = 0.66$) nor the amplitude ($t_{(5)} = 1.12, p < 0.33$) was significantly affected by velocity. Second harmonic peaks were seen to develop for the pyramidal cell action potential spectrograms in the dorsal and intermediate region of the hippocampus, but not for the interneurons. The arrows in Figure 9 indicate a second harmonic peak that is significantly different from 0 ($p < 0.05$ for both comparisons) for the pyramidal cell spike spectrogram in high-velocity conditions only. Given the appearance of harmonics exclusively in principal neurons, it suggests that harmonic activity may be related to rebound activity after inhibition (Cobb et al., 1995; Diba et al., 2014). Specifically, velocity-dependent increases of interneuron activity may invoke rebound excitation, leading to pyramidal cells firing two distinct bursts within a single theta cycle (see Discussion).

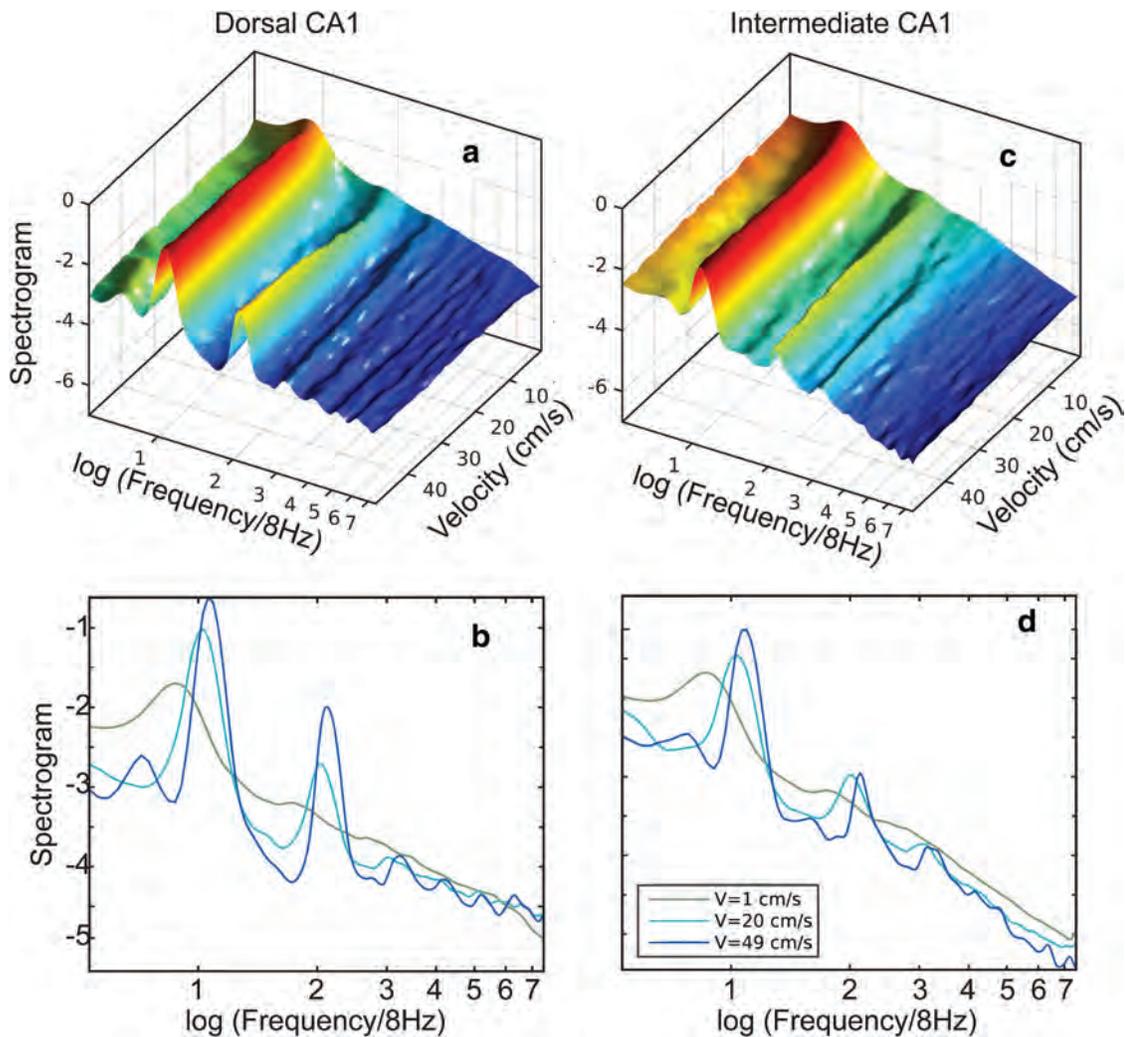


Figure 6. Power spectral density as a function of movement velocity. Normalized spectral density (logarithmic scale) as a function of velocity averaged over four rats (see Materials and Methods) for dorsal CA1 (**a**) and intermediate CA1 (**c**). The frequency axis was normalized by 8 Hz. Spectral densities (logarithmic scale) are shown for three velocities for dorsal CA1 (**b**) and intermediate CA1 (**d**).

Discussion

Using bispectral analysis, the current study documents the novel findings that (1) the CA1 subregion of the hippocampus has harmonics that are statistically dependent on theta; (2) higher-order harmonics emerge as a function of running speed in dorsal CA1 and, to a lesser extent, in intermediate CA1; and (3) harmonics were the source of the overall deformation of the LFP shape (negative skewness and asymmetry). Therefore, theta harmonics are in fact the spectral signature of the shape change of the LFP and express the increased nonlinearity of the theta rhythm in response to faster running speeds and increased neural activity. The harmonics cannot be regarded as a distinct (statistically independent) set of oscillations and are therefore not related to the beta and low-gamma rhythms.

These results show that the theta rhythm changed in response to running behavior, becoming more nonlinear as the animal's movement velocity increased. In the time domain, this is expressed as the development of skewness and asymmetry in the theta shape; in the spectral domain, it is expressed as the development of a chain of harmonics statistically phase coupled to the theta oscillation. This evolution is reflected in the spiking activity of principal cells (Maurer et al., 2005) and modulated across

regions of the hippocampus: it is stronger in the dorsal CA1 than in the intermediate CA1. The current findings add to our understanding of how dynamic activity patterns vary as a function of anatomical position along the longitudinal axis of the hippocampus. Because theta is known to propagate from dorsal to ventral regions (Petsche and Stumpf, 1960; Lubenov and Siapas, 2009; Patel et al., 2012), these data suggest that the nonlinear character of theta decreases as it travels and suggests a hypothesis that activity during movement dissipates along the longitudinal axis of the hippocampus. Future studies will need to test this idea directly.

Although the potential mechanisms that attenuate theta nonlinearity as it travels are not known, the dorsal and ventral regions of the hippocampus are dissociated in terms of patterns of gene expression and the behavioral impact of lesions (Nadel, 1968; Stevens and Cowey, 1973; Sinnamon et al., 1978; Moser et al., 1993; Moser et al., 1995; Long and Kesner, 1996; Moser and Moser, 1998; Kjelstrup et al., 2002; Burton et al., 2009; Wang et al., 2015). In fact, it has been proposed that the hippocampal longitudinal axis is functionally organized along a gradient (Strange et al., 2014; Long et al., 2015) and others have even proposed that the dorsal and ventral hippocampus should be

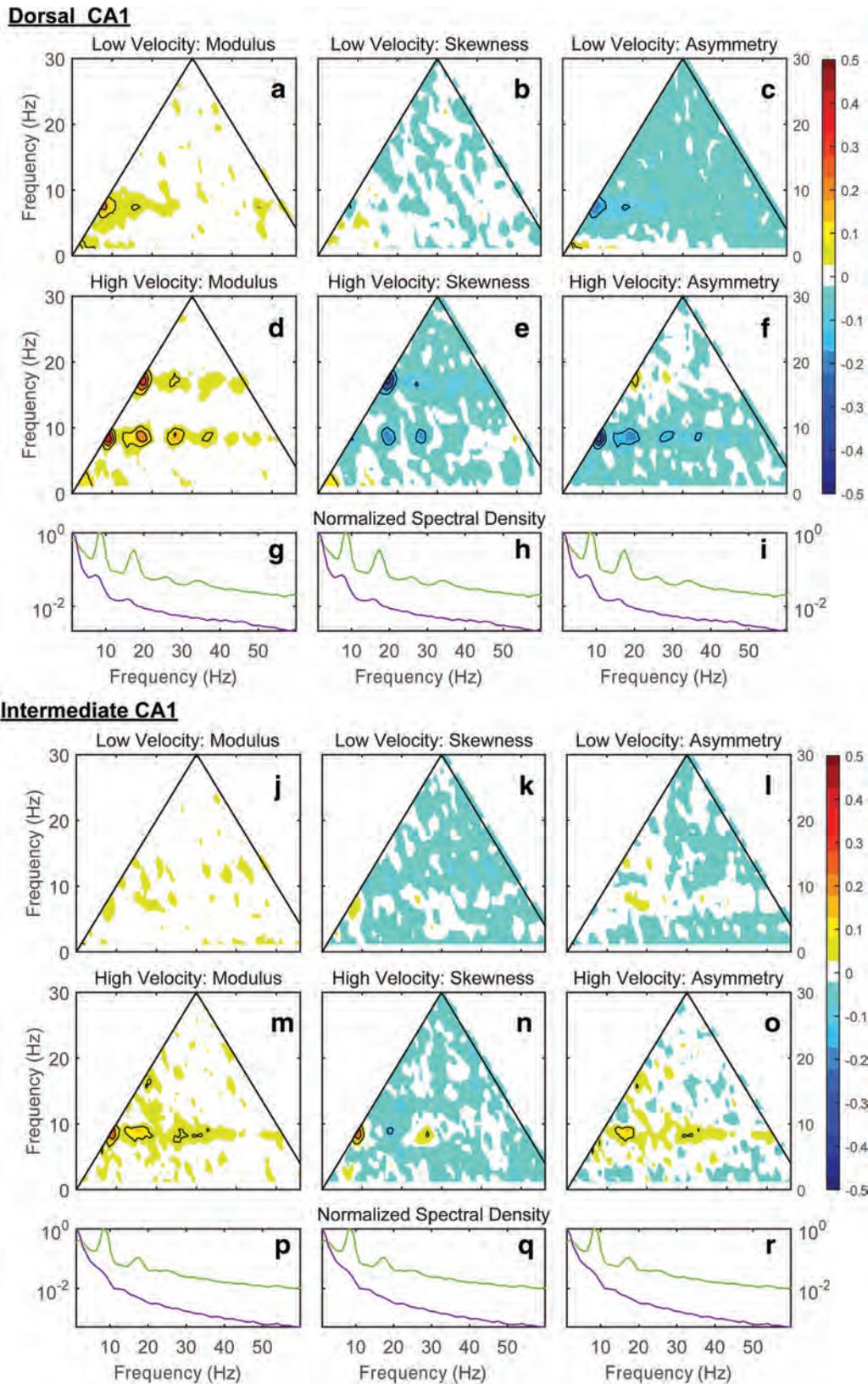


Figure 7. Representative bispectral properties of hippocampal LFP in the dorsal and intermediate CA1 region from rat 7805. The variability of the normalized bispectral distribution as a function of running speed (*a–c*: speed < 10 cm/s; *d–f*: speed > 30 cm/s) for the modulus (*a, d*), the real part (*b, e*; skewness distribution), and the imaginary part (*c, f*; asymmetry distribution). *g, h*, Normalized power spectral densities (maximum value = 1) are provided for reference (red, low speed; blue, high speed). Note that, in *d*, the contour lines outline 6 distinct regions of triad phase correlations (using the format f_r, f_m, f_{n+m} depicted in Fig. 1: [8 Hz, 8 Hz, 16 Hz], [16 Hz, 8 Hz, 24 Hz], [24 Hz, 8 Hz, 32 Hz], and [32 Hz, 8 Hz, 40 Hz], [16 Hz, 16 Hz, 32 Hz], [24 Hz, 16 Hz, 48 Hz]). *j–r* are equivalent to *a–f* except that data were obtained from intermediate CA1. Bispectral maps are colored for absolute values >90% (>0.05) and contoured for absolute values >95% (>0.1). Contour lines are drawn with an increment of 0.05.

considered distinct structures (Fanselow and Dong, 2010). The observation that the theta rhythm is more nonlinear in the dorsal relative to intermediate hippocampus supports the idea that information may be processed differently along the dorsoventral axis. It also leaves open the question of whether different behavioral parameters could more strongly modulate theta nonlinearity in the intermediate and ventral areas. Specifically, whereas spatial cognitive behaviors are attributed to the dorsal hippocampus, the ventral hippocampus is often associated with emotional processing, stress and affect (for review, see Fanselow and Dong, 2010). This presents the possibility that LFP nonlinearity in more ventral regions of the hippocampus might be influenced by the emotional valence of an experience rather than by the animal's movement.

The development of the theta harmonic with faster running speeds was reflected in the spectrogram of principal cell firing. Although the correlation between theta nonlinearity and neuron spiking is obvious, establishing causality is more difficult. One can make the argument that spiking activity contributes to the background field (Geisler et al., 2010) and, therefore, that altered spiking dynamics with velocity would change the LFP shape. This argument, however, may be circular in that the evolution of theta with velocity alters the spike timing by mechanisms such as ephaptic coupling (Buzsáki et al., 1991; Anastassiou et al., 2011). Perhaps more importantly, theta is not carried by the spiking of neurons, but rather is the summed activity of excitatory and inhibitory postsynaptic potentials (for review, see Buzsáki, 2005). Because hippocampal pyramidal neurons project to local interneurons (Csicsvari et al., 1998), which in turn provide dense feedback inhibition to several hundred principal neurons (Sik et al., 1995), local recurrent dynamics have been proposed to support the local generation of theta (Leung, 1998). In the model of Leung (1998), two inputs are responsible for the theta dipole across the CA1 layer: one onto the soma and proximal dendrites of pyramidal neurons carried by rhythmic basket cells and the other from input onto distal apical dendrites. Moreover, the interneurons across these regions exhibit differences in their phase preference (Klausberger et al., 2003; Somogyi and Klausberger, 2005), providing structured IPSPs along different pyramidal neuron domains (Allen and Monyer, 2015). Therefore, the consequence of two inhibitory rhythmic inputs at ~ 8 Hz

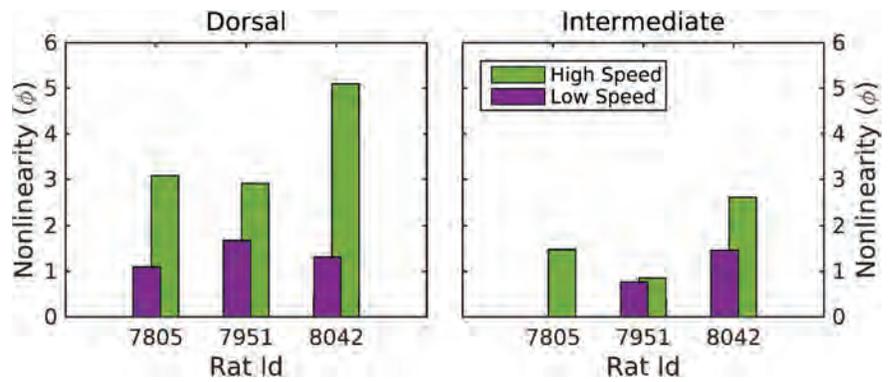


Figure 8. Theta nonlinearity of the three rats tested in the dorsal (left) and intermediate (right) CA1 regions and low (green) and high (purple) running velocities. The nonlinearity measure ϕ (Eq. 12) is defined only for bispectral values exceeding the 95% confidence level of the zero modulus of the normalized bispectrum (Eq. 13): the missing low-speed bar for rat 7805 for intermediate CA1 indicates that, at low speed, the normalized bispectrum is statistically zero. There was a significant main effect of hippocampal region ($F_{(1,4)} = 30.6, p < 0.01$; repeated measures) on the magnitude of the nonlinearity such that the EEG in the dorsal hippocampus was more nonlinear than the intermediate. There was not a significant main effect of velocity (low vs high) on nonlinearity ($F_{(1,4)} = 5.9, p = 0.08$; repeated measures), but the interaction between hippocampal region and velocity was significant ($F_{(1,4)} = 9.1, p < 0.05$; repeated measures). *Post hoc* analysis indicated that this interaction effect was due to a significant difference in EEG nonlinearity across velocity conditions in the dorsal hippocampus ($p < 0.05$), but not in the intermediate hippocampus ($p = 0.2$).

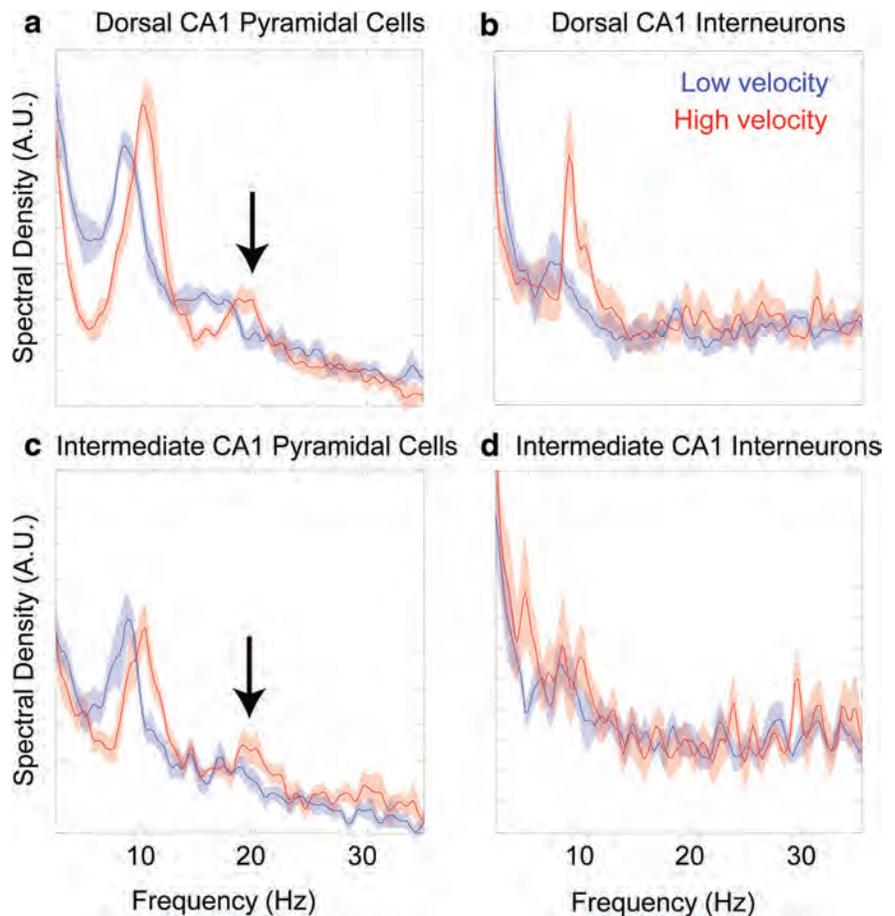


Figure 9. Spike spectrogram versus velocity. *a, b*, Dorsal CA1: pyramidal cells (*a*) and interneurons (*b*). *c, d*, Intermediate CA1: pyramidal cells (*c*) and interneurons (*d*). Because the analysis was conducted on binary spike trains, power units are arbitrary, although the axes are constant across respective cell classes. Low-velocity epochs are 10–20 cm/s and high-velocity epochs are 60–70 cm/s. Values are mean and SEM. Note the presence of a significant fundamental harmonic frequency, ~ 8 Hz, evident in the spectrogram of the spikes for the pyramidal cells at high velocities (arrows).

across distinct subcompartments of CA1 pyramidal neurons will interact, providing the basis for the 16 Hz modulation seen in Figure 9. Because there are fewer neurons active in the intermediate region of the hippocampus at any given moment in time (Maurer et al., 2006a) and these cells are less sensitive to changes in velocity (Maurer et al., 2005), fewer excitatory and inhibitory events contribute to the theta rhythm and the harmonic. Nonetheless, the intermediate pyramidal cells are still influenced by the structured inhibition, yielding a harmonic in the firing patterns.

This brings to the forefront the question of the role of the theta oscillation in the architecture of the brain. Although axons tend to propagate in a hierarchical manner (Felleman and Van Essen, 1991), the brain's architecture is more akin to a recurrent network in which neurons generate, as well as add, their own information to the activity propagating through the networks (Buzsáki, 2006). Because the theta rhythm is theorized to play a role in the coordination of brain activity, the nonlinear nature of wave propagation may govern the organization of spike timing. This idea is supported by the brain's capacity for large-scale integration of small components (Steriade, 2001; Buzsáki and Draguhn, 2004) and self-organized dynamics (Ashby, 1947; Kelso, 1997), as well as the simple observations that neurons fire during sleep and quiescent periods, when integration is largely disabled. The self-organized emergence of these oscillatory patterns, which reflects the excitatory (or inhibitory) state of the local groups of neurons (Haken, 1984), allows the firing patterns among assemblies of neurons to be "constrained" or timed via fluxes in the extracellular ionic concentration that modifies ionic driving force (Anastassiou et al., 2011). Although there have been significant theoretical advancements in describing self-organized, nonlinear dynamics in the nervous system (Amari, 1982; Haken, 1984; Freeman, 1992; McKenna et al., 1994; Kelso, 1997), the majority of our knowledge has been gained via linear methods, which ignore important elements of the complexity of the brain (Buzsáki, 2006). Future research attempting to untangle the dynamics of the nervous system should integrate nonlinear methods to understand the interaction between anatomy and activity.

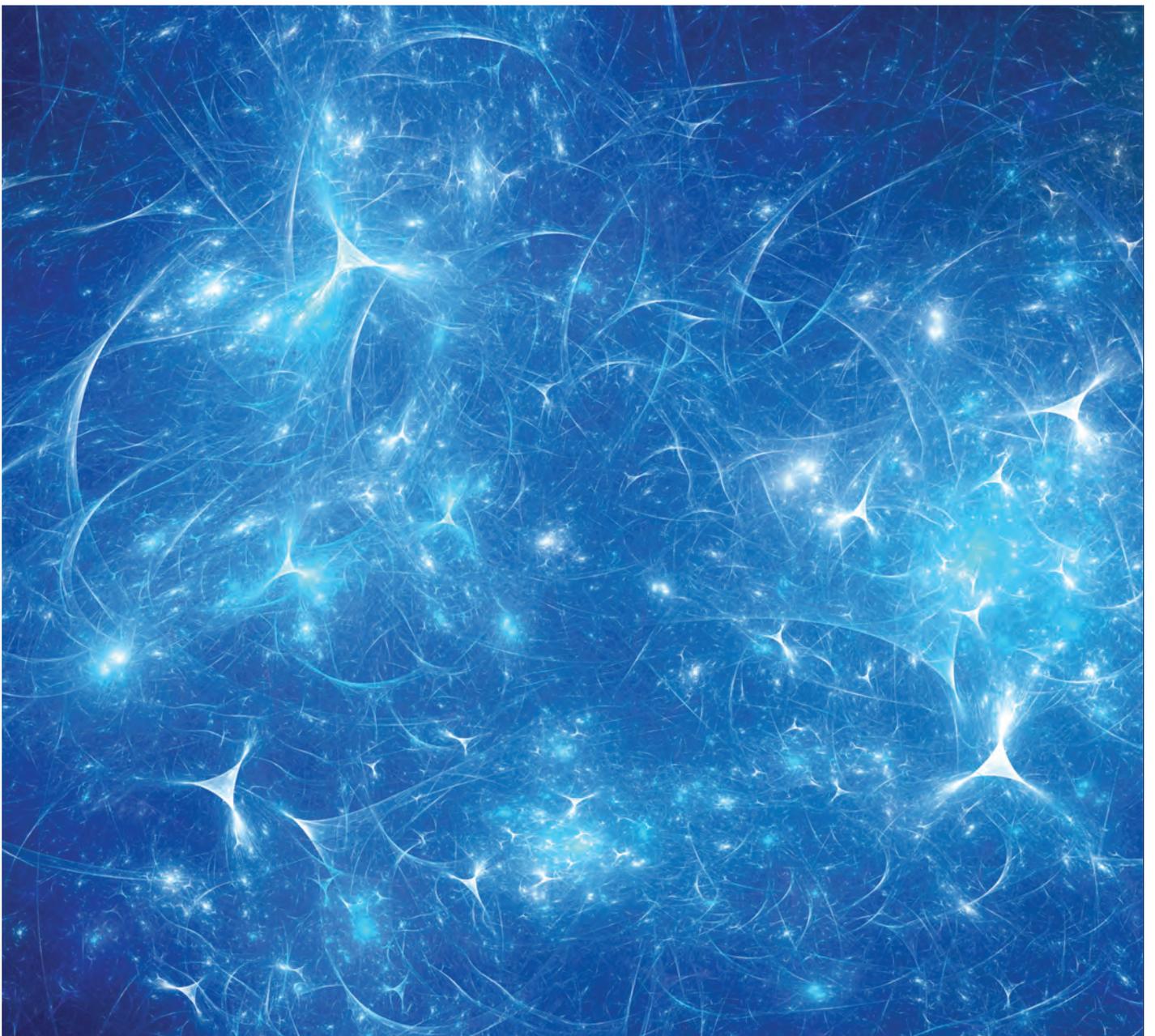
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Statistical Approaches for the Study of Cognitive and Brain Aging

Huaihou Chen^{1,2*}, Bingxin Zhao¹, Guanqun Cao³, Eric C. Proges², Andrew O'Shea², Adam J. Woods² and Ronald A. Cohen²

¹ Department of Biostatistics, University of Florida, Gainesville, FL, USA, ² Department of Aging and Geriatric Research, Center for Cognitive Aging and Memory, Institute on Aging, McKnight Brain Institute, University of Florida, Gainesville, FL, USA, ³ Department of Mathematics and Statistics, Auburn University, Auburn, AL, USA

Neuroimaging studies of cognitive and brain aging often yield massive datasets that create many analytic and statistical challenges. In this paper, we discuss and address several limitations in the existing work. (1) Linear models are often used to model the age effects on neuroimaging markers, which may be inadequate in capturing the potential nonlinear age effects. (2) Marginal correlations are often used in brain network analysis, which are not efficient in characterizing a complex brain network. (3) Due to the challenge of high-dimensionality, only a small subset of the regional neuroimaging markers is considered in a prediction model, which could miss important regional markers. To overcome those obstacles, we introduce several advanced statistical methods for analyzing data from cognitive and brain aging studies. Specifically, we introduce semiparametric models for modeling age effects, graphical models for brain network analysis, and penalized regression methods for selecting the most important markers in predicting cognitive outcomes. We illustrate these methods using the healthy aging data from the Active Brain Study.

Keywords: semiparametric model, graphical model, penalized regression methods, structural covariance, functional connectivity

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Biotechnology, Greece
Alex Zhavoronkov,
The Biogerontology Research
Foundation, UK

*Correspondence:

Huaihou Chen
huaihouchen@ufl.edu

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INTRODUCTION

Multimodal neuroimaging collected in cognitive aging studies provides a noninvasive way to investigate brain changes in structure, function, and metabolism as people age, and thus helps us to understand age-related cognitive changes. However, the high-dimensionality and complex structure of those multimodal neuroimaging data raise statistical challenges. Additionally, the age range is large in aging studies and very often the age effects may not be linear in the large age interval. For instance, participant's age ranges from 50 to 90 in the Active Brain Study, a successful aging cohort. To efficiently analyze those data, there is a strong need for introduction of advanced statistical methods. We will elaborate on the limitations of several existing methods and introduce three advanced statistical methods in sequence.

First, age is a complex variable and often has a nonlinear effect on the outcomes of interest. In developmental studies, flexible semiparametric models have been well used, because it is well-known that growth curves are nonlinear. However, in aging studies, linear or quadratic models are often used to characterize age-related changes. Although a majority of aging research treats aging as a linear process (constant rate of change) and linear models are often considered the gold standard method for evaluating aging effects, this approach may not be the most effective method for representing the complexity of aging data. For instance, Raz et al. (2010) used linear

mixed effects models to characterize the age-related brain structural changes in a longitudinal neuroimaging study with 76 participants whose age ranges from 49 to 85. Similarly, Resnick et al. (2003) also used linear mixed effects models to show the age-related brain structural changes in the longitudinal Baltimore study. However, as noted in Fjell et al. (2010), Gogtay et al. (2004), and Thompson et al. (2011); brain structure may show complex age-related nonlinear changes, and could be misspecified by a linear or quadratic model. We have shown that misspecified linear models can result in biased estimates and low powers in statistical tests (Chen et al., 2012). As a nonparametric method, a spline model is recommended for its flexibility and robustness. To accurately model the age trajectories of the neuroimaging markers, we will introduce a spline-based semiparametric model in the methods section and illustrate these methods using the structural neuroimaging data from the Active Brain Study in the example section. The semiparametric model excels at determining rates of global and regional brain atrophy and identifying vulnerable regions of interest (ROIs) susceptible to aging.

Second, marginal correlations are often used in brain network analyses. For example, structural covariance was studied in Mechelli et al. (2005) and Alexander-Bloch et al. (2013), which may be related to structural and functional connectivity. There is also a large literature on Pearson correlation based functional connectivity analysis, where the correlation between two functional magnetic resonance imaging (fMRI) time series [that is the blood-oxygen-level dependent (BOLD) signal] is computed. However, marginal correlation between two brain ROIs is indirect and weak in the sense that all the components in a system are correlated to some degree. Two regions can be indirectly associated with each other due to their correlation with a third region. Moreover, when the number of ROIs is large, the sample covariance/correlation matrix is unstable, as the number of parameters increases quadratically with the number of ROIs. Alternatively, graphical models are attractive for inferring brain connectivity due to their advantages over conventional marginal correlation based analysis (Lauritzen, 1996; Yuan and Lin, 2007; Koller and Friedman, 2009). Graphical models can generate either partial correlations or a binary undirected graph. Sparse penalty is used to regularize the loglikelihood function and make the solution robust. Partial correlation is a desirable measure, as it quantifies the conditional association between two ROIs given the rest of ROIs. Partial correlation can be interpreted as the adjusted correlation. Preliminary applications to neuroimaging data can be found in Salvador et al. (2005), Valdés-Sosa et al. (2005), and Smith (2012). In the methods section, we will introduce two graphical methods for brain network analysis. We will apply these methods to the cortical thickness data from the Active Brain Study for building cortical networks.

Third, the high dimensional neuroimaging markers may provide informative early signs of age-related cognitive and functional decline. For example, brain atrophy in the basal ganglia, hippocampus, and prefrontal areas often precedes the clinical diagnosis of cognitive impairment (Amieva et al., 2005; Grober et al., 2008; Jedynak et al., 2012). It is of great interest to select the informative neuroimaging markers for predicting

cognitive decline. However, the high-dimensionality of the neuroimaging markers posit challenges on how to efficiently pick up the informative subset of the markers. Traditional backward or forward variable selection methods are computationally inefficient given the large number of neuroimaging markers. Also neuroimaging markers are often highly correlated with each other. The unpenalized least square based estimates often suffer from high variability or instability (that is with large variance). Moreover, when the number of neuroimaging markers is larger than the sample size, the design matrix is singular and not invertible, and thus there is no unique estimate. In contrast, penalized regression methods can lead to stable solutions and are computationally efficient by using advanced algorithms (Tibshirani, 1996; Fan and Li, 2001; Zou, 2006; Meinshausen and Bühlmann, 2010). Penalized regression methods can simultaneously select and estimate the effects of the predictors. The variable selection is achieved by the sparsity penalty. In the methods section, we will introduce penalized regression methods for selecting the optimal subset of neuroimaging biomarkers for predicting cognitive outcomes. We will illustrate those methods using structural neuroimaging and cognitive data from the Active Brain Study.

The rest of the paper is structured as follows. In the methods section, we introduce the three sets of methods including the spline-based semiparametric model, graphical models, and penalized regression methods. In the examples section, we apply those methods to the data from the Active brain study. We end our paper with general discussions.

METHODS

Semiparametric Models and Methods

We first introduce some notations. Let n be the number of subjects and let R be the number of ROIs. For the i th participant, denote t_i as the age, denote Y_{ir} as the structural/metabolic imaging markers [for instance, volume, fractional anisotropy (FA), myo-inositol (MI)] at the r th ROI, and denote Z_i as other predictors such as education and sex that we want to study. To accurately and efficiently model the age effects, we introduce the following semiparametric model (1) for neuroimaging markers in cross-sectional studies.

$$Y_{ir} = \mu_r(t_i) + \mathbf{Z}_i \boldsymbol{\beta}_r + \epsilon_{ir}, \quad i = 1, \dots, n, \quad r = 1, \dots, R, \quad (1)$$

where $\mu_r(t)$ is the unspecified aging trajectory for the older people at the r th ROI evaluated at age t , and $\boldsymbol{\beta}_r$ are the regression coefficients of the other predictors at the ROI. The measurement errors ϵ_{ir} are assumed to be independently and identically distributed and follow a normal distribution $N(0, \sigma_r^2)$ with mean zero and variance σ_r^2 . Model (1) consists of both the nonparametric part $\mu_r(t)$ and the parametric part $\mathbf{Z}_i \boldsymbol{\beta}_r$, and thus it is called semiparametric model. The semiparametric model is a parsimonious way to both capture the potential nonlinear age trajectory and investigate the effects of other predictors. Notably, the traditional linear model is a special case of model (1), where the function $\mu_r(t)$ is specified as a linear function $\beta_{0r} + \beta_{1r}t$. Extension of model (1) to longitudinal data

case is straightforward, which can be accomplished by either introducing subject-specific random effects or using generalized least square methods (Wood, 2006; Wu and Zhang, 2006).

For estimation, we use spline basis functions to approximate the unspecified function $\mu_r(t)$. Particularly, we assume $\mu_r(t) = B(t)\theta_r$, where $B(t)$ is a set of B-spline basis functions and θ_r is the associated spline coefficients (de Boor, 1978). The B-spline basis functions are piecewise polynomial functions in the age interval. A smoothing penalty $\lambda \int [\mu_r''(t)]^2 dt$ is used to achieve smoothness of the fitted function $\hat{\mu}_r(t)$, where $\mu_r''(t)$ is the second derivative function of $\mu_r(t)$, and $\lambda \geq 0$ is a smoothing parameter controlling the degree of smoothness. The tuning parameter λ is crucial for the estimation and inference and is often chosen by data driven methods. By minimizing the penalized log-likelihood function, we can obtain the estimate for these parameters including θ_r and β_r . Compared to traditional linear model and methods, spline method offers flexible estimation of these functions. Based on the semiparametric model (1), we will be able to more accurately delineate the aging trajectories and their derivative functions and get unbiased estimates for the parametric part.

The spline-based semiparametric model and methods have been implemented in several R packages (R Core Team, 2012) including the *mgcv* package (Wood, 2006). The *gam* function in *mgcv* can output the estimates and inferential results for both the parametric and nonparametric parts. Specifically, for the parametric part, estimates of the regression coefficients and *p*-values are provided which is similar to a linear regression model. For the nonparametric part, the procedure provides the estimate and pointwise confidence intervals for the estimated function and a *p*-value for testing the function as a constant. The 95% point-wise confidence interval $[\mu_r^L(t), \mu_r^U(t)]$ for $\mu_r(t)$ provides the variability at the age *t* in the *r*th ROI, in addition to the magnitude. The first derivative function of $\mu_r(t)$ indicate the rate of brain atrophy, where in the linear case is the slope of the line. The first derivative functions are can be easily obtained using $B'(t)\theta$, where $B'(t)$ are the first derivative functions of the B-spline basis functions. Based on the first derivative functions of the aging trajectories, ROIs/markers show early atrophy/abnormality could be candidate biomarkers for early diagnosis of diseases. We adjust for multiple comparison by controlling the *false discovery rate* (FDR) (Benjamini and Hochberg, 1995; Benjamini and Heller, 2007).

Remark 1 Misspecified linear models could introduce bias for the estimates of $\mu_r(t)$ and β_r , that is for both the nonparametric and parametric parts.

Remark 2 To achieve good approximations of these unspecified functions, enough number of basis functions should be used for the penalized splines. If the procedure leads to an oversmooth case, one can fit a regression cubic spline with fixed number of knots without penalty, thus the degree of freedom is fixed.

Remark 3 Computing time is not a concern for ROI-level data. Some statistical packages such as the *vows* have implemented massive parallel algorithm for voxel-level data (Reiss et al., 2014).

Graphical Model and Methods

We first define a graph $G = (V, E)$, where V is a set of vertices/nodes, and E is a set of edges connecting pairs of

nodes in V . An adjacency matrix of a graph is a binary matrix indicating the connection between the nodes. We introduce graphical models for brain structural and functional network analysis. In the past decade, *Gaussian graphical model* (GGM) has been a hot topic in statistics as a tool for complex system analysis. The GGM has many advantages over the traditional marginal correlation based analysis including resulting in partial correlations, i.e., direct dependency/independence, and sparse networks. Let Y be an R -dimensional random variable following a multivariate Gaussian distribution $N(\mu, G^{-1})$ with mean μ and covariance G^{-1} . G is a precision matrix (inverse covariance), and the *i, j*th component of G , $g_{rs} = 0$ indicates *conditional independence* between ROIs *r* and *s* given all the other ROIs $\{1, \dots, R\}/\{r, s\}$. The partial correlation between ROIs *r* and *s* is defined as $\rho_{rs} = -g_{rs}/\sqrt{g_{rr}g_{ss}}$ (Lauritzen, 1996). We obtain a sparse graph by minimizing the following penalized loglikelihood function (Yuan and Lin, 2007):

$$\arg \min_{G \in \mathbb{G}} -\log |G| + \frac{1}{n} \sum_{i=1}^n (Y_i - \mu)^T G (Y_i - \mu) + \lambda \sum_{r \neq s} |g_{rs}|, \quad (2)$$

where argmin stands for argument of the minimum, \mathbb{G} is the set of $R \times R$ positive definite matrices, and $\lambda \geq 0$ is the tuning parameter chosen by a data-driven method. The lasso penalty (Tibshirani, 1996) is used to regularize the loglikelihood function and achieve a sparse solution. This method is often called graphical lasso (glasso) in the statistical literature. Along the same line, Meinshausen and Bühlmann (2006) proposed the node-wise regression based approach for obtaining a binary graph. Both the glasso and the node-wise regression methods have been implemented in the R package *huge* with computational efficient algorithms (Zhao et al., 2012). The *huge* package can provide estimate for the precision matrix or adjacency matrix of an undirected graph. The stability selection method (Meinshausen and Bühlmann, 2010) is preferred for the selection of the tuning parameter, which controls the sparsity of the estimated precision/adjacency matrix. A large tuning parameter will penalize the loglikelihood function heavily and shrink the small elements in the precision matrix/regression coefficients to zero, while a smaller tuning parameter will barely penalize the loglikelihood function and thus leads to many tiny elements in the precision matrix/regression coefficients.

Once the graph is obtained, graph summary statistics such as centrality measures and clustering coefficient can be computed. For visualizing and summarizing graphical objects, the R package *igraph* provides a set of sophisticated tools (Csardi and Nepusz, 2006).

Penalized Regression Methods

To utilize high-dimensional markers for predicting cognitive outcomes, we introduce penalized regression methods for linear models. Penalized regression methods can reduce the dimensionality of the predictors by automatically selecting the optimal subset. The variable selection is achieved by a sparsity penalty such as lasso (Tibshirani, 1996), adaptive lasso (Zou, 2006), elastic net (Zou, 2006), SCAD (Fan and Li, 2001), or by stability method (Meinshausen and Bühlmann, 2010). For the

i th participant, let Y_i be the cognitive outcome, and let X_i be the stacked $p \times 1$ vector of neuroimaging markers and other covariates. We consider the following linear model and penalized method.

$$Y_i = X_i\beta + \epsilon_i, \quad i = 1, \dots, n, \quad (3)$$

$$\beta = \arg \min_{\beta \in \mathbb{R}^p} \sum_{i=1}^n (Y_i - X_i\beta)^2 + \lambda\phi(|\beta|), \quad (4)$$

where β are the coefficients for neuroimaging markers and covariates, and $\phi(\cdot)$ is a penalty function of the regression coefficients β . By minimizing the penalized least squares (4), we can obtain the penalized estimator $\hat{\beta}$. The sparsity penalty shrinkages those small regression coefficients to zeros, thus the procedure automatically leads to a subset of the predictors. If $\hat{\beta}_j = 0$, then the j th predictor X_j is not selected. The sparsity of the estimate is controlled by the tuning parameter $\lambda \geq 0$, which is usually chosen by data driven methods such as cross-validation or generalized cross-validation.

Thanks to the implementation of efficient algorithms, current software package can handle thousands of predictors simultaneously for a medium sample size such as $n = 80$. In general the computational time is moderate and depends on the size of the data that is the sample size n and the number of predictors p . One of the popular R package *glmnet* has implemented a few penalized methods including lasso and elastic net. The *glmnet* function in the *glmnet* package provides all the coefficient solution paths as functions of the tuning parameter λ . To get the optimal solution, the user needs to use the cross-validation method to select the optimal tuning parameter with the smallest mean squared error (MSE).

Remark 4 The penalized regression methods are applicable to generalized outcomes including binary and count data as well. For example, we can use penalized logistic regression methods to select informative neuroimaging markers in predicting risk of mild cognitive impairment (MCI).

Remark 5 Because the penalty shrinkages those regression coefficients toward to zero according to their magnitude, large differences in the original scale of those predictors can mess up the selection. Therefore, it is recommended to standardize the predictors and make all the variables in the same scale.

EXAMPLES: THE ACTIVE BRAIN STUDY

We illustrate the introduced methods using the data from the Active Brain Study. The aim of the study is to investigate the brain changes associated with age-related cognitive decline via multimodal neuroimaging. We consider $n = 114$ participants with structural imaging. Among them 68% are female. The mean age of the sample is 72.3 with the standard deviation (SD) 10. Those participants are well educated as can be seen from the mean education years = 16.2 ($SD = 2.6$). They also show high cognitive performance with mean Montreal Cognitive Assessment (MoCA) score 25.7 ($SD = 2.6$). The structural imaging was processed using standard procedures implemented

in Freesurfer version 5.3 (Dale et al., 1999; Fischl et al., 2002). For a more detailed description of the Freesurfer processing methods used by our group see Szymkowicz et al. (2016). Brain volumetric indices including regional and global volumes of cortical and subcortical structures as well as cortical thickness were generated. Particularly, we used the anatomical cortical parcellation in Desikan et al. (2006), which generated 34 ROIs in each hemisphere. Similarly, the subcortical segmentation of a brain volume is based on the existence of an atlas containing probabilistic information on the location of structures (Fischl et al., 2002).

Aging-Related Trajectories of Brain Regional Volumes and Areas

We are interested in delineating the aging trajectories for the regional volumes and areas, while adjusting for sex, education, and the total intracranial volume (ICV). For normalization purpose, the regional volumes are divided by the ICV. To check the nonlinearity of the age trajectories of the regional volumes and areas, we first applied the loess (locally weighted scatterplot smoothing) method using the R function *loess*, which is a popular exploratory tool for checking nonlinear pattern. As a lot of ROIs show nonlinear age trajectories of brain regional volumes, we fit a semiparametric model for the normalized volume at each ROI with nonparametric age trajectory and parametric effects for sex and education using the *gam* function in the *mgcv* package. Similarly, we fit a semiparametric model for the area at each ROI with nonparametric age trajectory and parametric effects for sex, education and ICV. Penalized cubic B-splines with 10 basis functions are used to fit the age trajectories. The restricted maximum likelihood (REML, Reiss and Todd Ogden, 2009) method is used to select the tuning parameters. For comparison, we also fit linear and quadratic models for the age trajectories. The quadratic age term is centered to achieve robustness. Alternatively, orthogonal polynomial model can be used to avoid multicollinearity problem.

We choose the normalized volume of the lateral ventricle and putamen for illustration. **Figure 1** shows the estimated age trajectories (the solid lines) using different methods, and the 95% pointwise confidence intervals (the shaded area) for the B-spline fits. The lateral ventricle displays considerable expansion especially after age 70, while the putamen shows a large amount of decline especially before age 75. Both the lateral ventricle expansion and the putamen volume shrinkage indicate brain atrophy as people age. We notice that both the loess and the semiparametric fits indicate nonlinear age patterns. As displayed in **Figure 1**, linear models are not flexible enough to capture the nonlinear age trajectories. Linear models assume the rate of age-related change is constant as people age, which may not be true for all the ROIs. The deviation of the linear fits from the semiparametric model fitted curves are large in the two ends and the middle part of the interval, that is less than 60, greater than 80, and around 70. The quadratic age trajectories show agreement with the B-spline fits around the middle of the age interval [60, 80], but not in the two ends

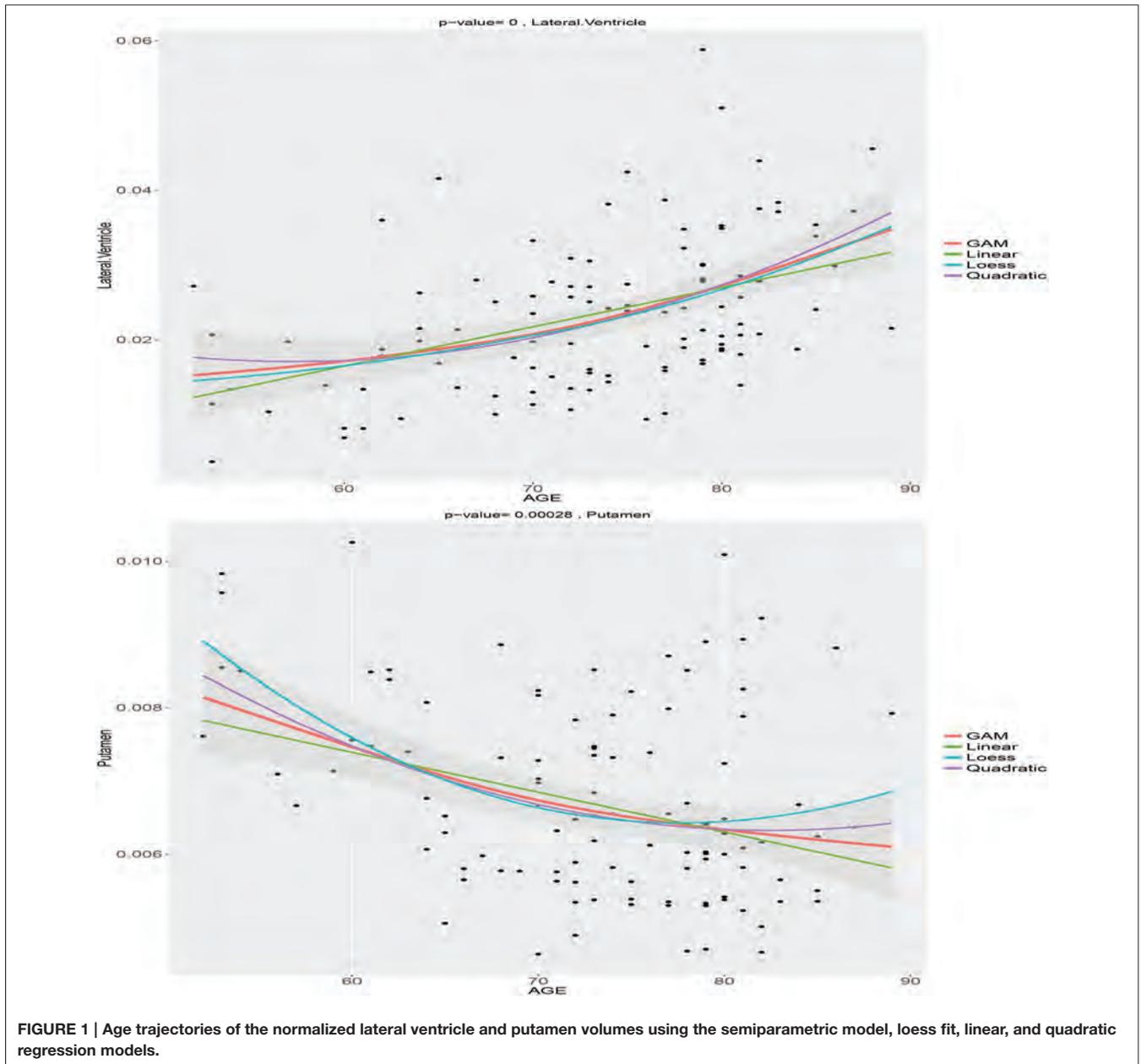


FIGURE 1 | Age trajectories of the normalized lateral ventricle and putamen volumes using the semiparametric model, loess fit, linear, and quadratic regression models.

either. The loess fits are exploratory without adjusting for sex and education. Interestingly the B-spline fits agree with the loess fit for the lateral ventricle volume but not the putamen volume.

Overall, age has significant effects on almost all of the cortical and subcortical regional volumes in both hemispheres after FDR correction. Particularly, the cortical frontal, temporal, parietal, occipital, cingulate lobes are significantly impacted by aging except the left caudal anterior cingulate, bilateral entorhinal, pericalcarine, and frontal pole. The insula shows a marginally significant age effect. The ventricle, subcortical regions, and corpus callosum are significant except for the bilateral caudate. Our findings are consistent with the literature that as people age,

the brain regional volumes shrink, while the ventricle system and CSF considerably expand. We also observe that older females tend to have less brain atrophy compared to older males after FDR correction. Education does not have a significant effect on any of those regional volumes after FDR correction. Additionally, age shows similar effects on the cortical regional areas. However, after adjusting for the ICV, neither sex nor education has an effect on the cortical regional areas.

In summary, the linear/quadratic model due to its parametric nature, may not be flexible enough to capture age-related brain changes as people age. A nonparametric/semiparametric model should be used if there is a convincingly nonlinear pattern as suggested by an exploratory loess fit.

Cortical Thickness Based Cortical Network

Structural covariance has been used in the literature for studying cortical networks and patterns of neurodegeneration (Mechelli et al., 2005; Alexander-Bloch et al., 2013). Here, we are interested in applying graphical models to investigate the cortical network using the cortical thickness data from the Active Brain Study. We consider the cortical thickness at 34 ROIs in each of the hemispheres. For summary purpose, we group the 68 cortical ROIs into six lobes including the frontal, temporal, parietal, occipital, cingulate, and insula. We first compute the marginal Pearson correlation for the structural covariance/correlation. We then use the *huge* function to obtain the partial correlation (based on the precision matrix) and a binary undirected graph (or equivalently the adjacency matrix) using the *glasso* and *node-wise* regression respectively. The tuning parameters are selected by the stability method (Liu et al., 2010; Meinshausen and Bühlmann, 2010).

The results are summarized in **Figure 2**. The top two patterns in **Figure 2** display the thresholded marginal/partial correlation map for the 68 cortical ROIs (34 ROIs per hemisphere). The bottom two patterns display the undirected graph and the frontal subgraph plotted using function in the *igraph* package. The marginal correlation map is cut by 0.3. The marginal correlation and partial correlation show very different patterns. The range of the marginal correlation is much larger compared to the partial correlation. The two graphical methods share some similarity. The left and right correlation are strong even conditional on all the other ROIs. There are both inter- and intra-hemisphere correlation. Based on the bottom adjacency matrix plot, we observe that the frontal ROIs tend to be conditionally correlated (see also the bottom right panel in **Figure 2**). Other graph summary statistics can be easily calculated using functions in the *igraph* package such as degree of centrality.

In summary, the marginal correlation and partial correlation map often show different patterns. The interpretation of the two are also different. The marginal correlation between two ROIs does not account for the involvement of other ROIs, while the partial correlation between two ROIs adjusts for other ROIs. Due to the lasso penalty, the partial correlation map and the adjacency matrix are sparse that is some of the partial correlations/elements of the adjacency matrix are estimated to be zeros.

Predicting MoCA Using Brain Regional Volumes

In this section, we aim to select informative brain regional volumes in predicting the cognitive outcome MoCA. We first normalize regional volumes via dividing by the estimated intracranial volume (ICV), then standardize the variables by subtracting the sample mean and divided by sample standard deviation to make the variables comparable. The predictors we consider include the cortical and subcortical regional volumes, age, sex, and education. We used the *glmnet* function in the R package *glmnet* with both lasso and elastic net penalties. We choose the tuning parameters by 10-fold cross-validation.

Table 1 summarizes the selected variables and their coefficients using both penalties. The selected variables

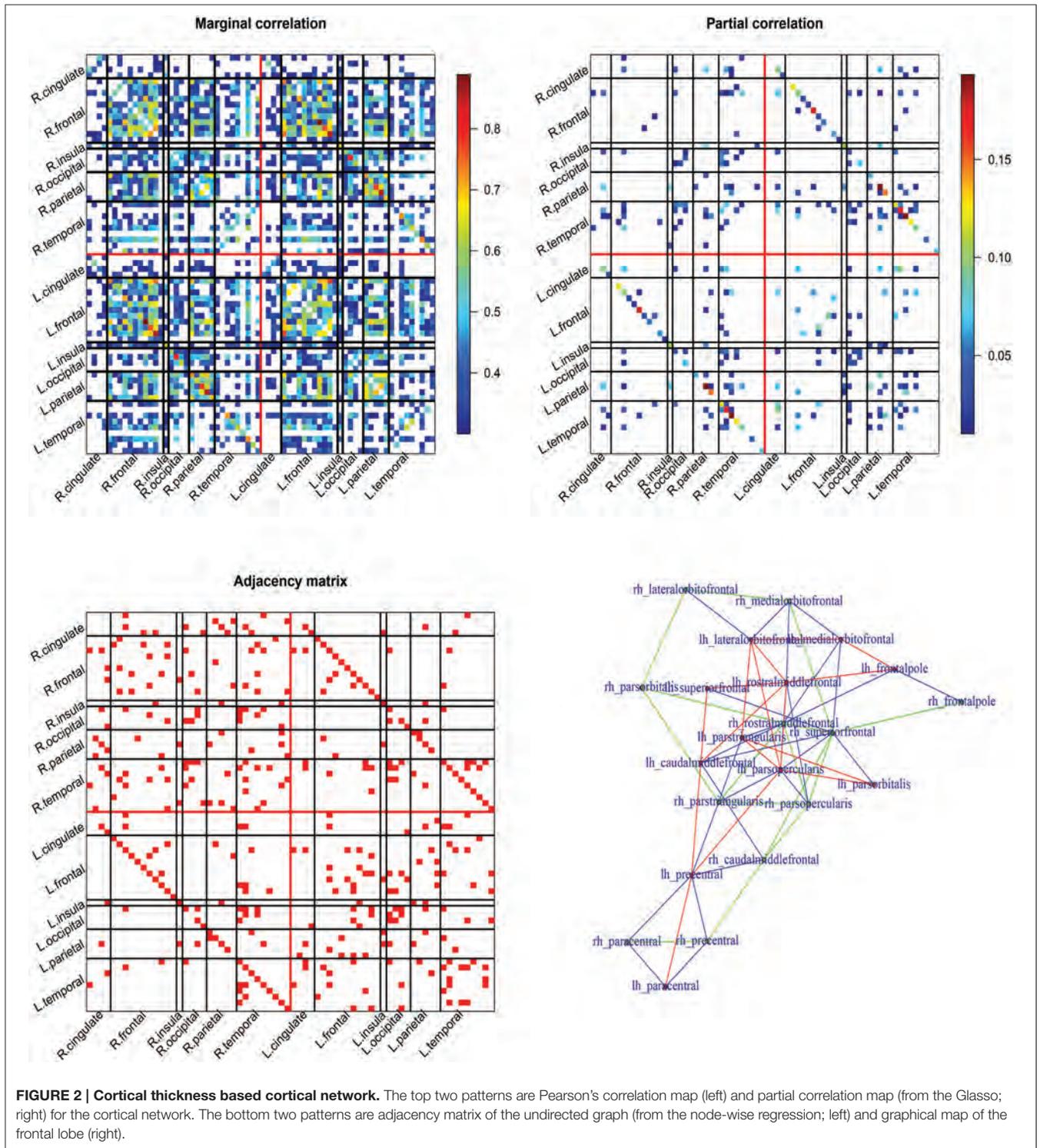
include regional volumes from the frontal and temporal lobes, subcortical regions, and demographic variables. Compared to the elastic net penalty, the lasso penalty tends to choose a small subset of correlated predictors. For example, the left pars opercularis (Brodmann area 44) was selected but not the right one. Consistent with the findings in the literature, we found that volumes of subcortical and cortical ROIs including the left accumbens, middle temporal, pars opercularis, temporal pole, right entorhinal, medial-orbito-frontal, pars opercularis are positively associated with MoCA.

The *glmnet* function can output the whole solution path. **Figure 3** displays the whole solution path for all the coefficients as functions of the logarithm tuning parameter λ for the lasso penalty. The vertical line corresponds to the optimal λ selected by cross-validation. When the tuning parameter λ is small (that is with less penalization), the magnitudes of the coefficients are large and the variability is large. The traditional least square estimate is similar to the small penalization case, which is not stable. As the tuning parameter increases, the variability of the coefficients declines. The regularization achieves the small variance at the cost of introducing bias. The cross-validation criterion selects the tuning parameter by balancing the variance and bias.

To check the performance of the selected subset of the regional volumes, we refit a model with the volume of the selected ROIs and compare to the model with only age, sex, and education. The selected regional volumes from the elastic net penalty explains additional 19% variance in MoCA, where R^2 increases from 16 to 35%. We conduct an ANOVA test to compare the two models, where the p -value is less than 0.001.

DISCUSSIONS

Misspecified linear models are not uncommon in the literature, which may lead to biased results and misleading conclusions. Based on our previous neuroimaging analysis (Chen et al., 2014, 2015), linear models may not always be appropriate for characterizing the age-related brain changes, although it is the default method due to its simplicity. In practice, cautions need to be raised for the potential nonlinear age-related changes. To minimize the potential bias, we introduced the spline-based semiparametric models, which are more flexible and able to capture the underlying age trends in the data. Notably a linear model is a special case of the semiparametric model. When the underlying trend is linear, semiparametric model agrees with the linear model. Semiparametric methods have been implemented in many statistical softwares such as R. One of the popular implementations is the *gam* function in the *mgcv* package. The *gam* function provides the estimated curves and inferential results for both the parametric and the nonparametric parts. Extension of the basic semiparametric model (1) has been extensively studied in the past few decades in the statistical literature. More sophisticated models such as varying coefficient models and additive models have also been developed (Wood, 2006; Wu and Zhang, 2006). All these semiparametric methods are scalable and applicable to voxel level data as well. The



R package *vows* has implemented semiparametric models for voxel-level data. A parallel algorithm is implemented to speed up the computational procedure.

In the neuroimaging literature, network analysis provides a systematic way to study the brain structural and functional changes. The use of network analysis is a remarkable progress

from the pairwise relationship between ROIs. However, researchers often compute the marginal correlation then threshold the correlation matrix to obtain the graph/network. It is well known that sample covariance/correlation matrix is highly instable when the number of ROIs is large. The pairwise nature of the marginal correlations hurts and limits the interpretation of

TABLE 1 | Selected brain regional volumes and covariates in predicting MoCA.

	lh.middle.temporal (temporal lobe)	lh.pars.opercularis (frontal lobe)	lh.pars.orbitalis (frontal lobe)	lh.temporal.pole (temporal lobe)	rh.entorhinal (temporal lobe)	rh.medial.orbito.frontal (frontal lobe)
Elastic-net	0.047	0.345	-0.320	0.145	0.188	0.048
LASSO	0.004	0.433	-0.343	0.154	0.193	0.005
	rh.pars.opercularis (frontal lobe)	lh.Accumbens	Age	Sex (F vs. M)	Education	
Elastic-net	0.031	0.309	-0.231	0.331	0.177	
LASSO	-	0.358	-0.225	0.329	0.177	

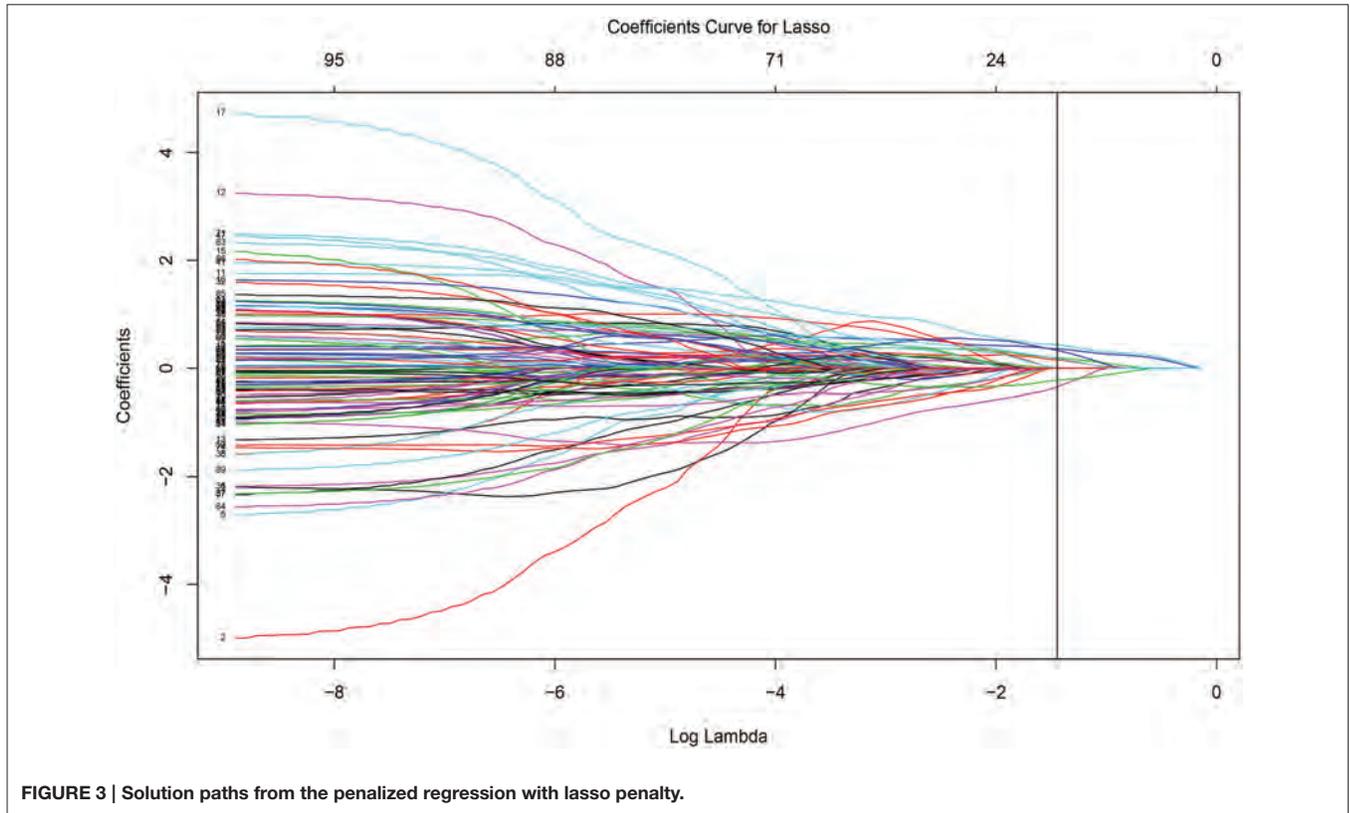


FIGURE 3 | Solution paths from the penalized regression with lasso penalty.

the subsequent network based results. To address the limitation of the marginal correlation, we introduced two Gaussian graphical models, which can generate either partial correlations or an undirected graph. Under the multivariate Gaussian assumption, a zero partial correlation for two ROIs given all the other ROIs is equivalent to conditional independence between the two ROIs. Similarly, for an undirected Gaussian graph, the edges indicate conditional dependence between ROIs. We illustrated the graphical models using the cortical thickness data, where the generated cortical networks may be related to the cortical structural connectivity. The application of graphical model to fMRI for investigating functional connectivity is straightforward, but need to be modified to account for the temporal correlation within each time series. Besides partial correlation in the time domain, there is a

few works on correlation measure in the frequency domain such as the total independence (Wen et al., 2012) and partial correlation for multivariate time series (Fried and Didelez, 2005).

For testing the brain network differences between groups such as young vs. older, there are three levels of tests including the edge-level, node-level, and subgraph-level (Nichols and Holmes, 2002; Kim et al., 2014, 2015; Narayan and Allen, 2016). The edge-level testing approach first tests the group differences at the edges one by one, then applies multiple correction for the *p*-values such as FDR correction. The node-level testing method investigates the group differences in graph summary statistics at each node such as degree of centrality. The subgraph-level testing aims to detect either topologically connected cluster difference (Zalesky et al., 2010) or differences in graph overall

metrics such as clustering coefficient. The three levels of testing approaches provide complementary ways of testing the brain network differences.

Efficiently and accurately predicting cognitive decline is a central topic in cognitive and brain aging studies. In practice, very often an *a priori* subset of neuroimaging biomarkers are used to predict cognitive outcomes, which are based on the predetermined hypothesis. Hypothesis-driven methods are a recommended way to conduct research that can generate reproducible results. However, by chance, we may miss important neuroimaging markers that could indeed be predictive for cognitive decline. We introduced penalized regression methods for incorporating a large amount of neuroimaging biomarkers in predicting cognitive outcomes, where the number of predictors can be close to or even larger than the number of subjects. These data-driven methods can simultaneously estimate the regression coefficients and select a subset of the high-dimensional predictors. We illustrated those methods using the brain regional volumes in predicting MoCA outcomes. Moreover, these methods are applicable to categorical cognitive impairment outcomes such as a variable with three nominal levels: normal, mild cognitive impairment, and dementia. In addition to the penalized regression methods, some machine learning type of methods such as penalized support vector machine (SVM) can be used for building prediction/classification rule based on high dimensional neuroimaging biomarkers (Zhu et al., 2004; Zhang et al., 2006; Wu and Liu, 2007; Robinson et al., 2015). For long term followup longitudinal studies, penalized mixed effects model can be used to improve the prediction accuracy by incorporating both the individual trajectories and baseline

or longitudinal neuroimaging biomarkers (Bondell et al., 2010; Ibrahim et al., 2011).

Neuroimaging data collected in studies of cognitive and brain aging raise statistical and analytic challenges due to the high dimensionality and complex structure. Fortunately, advanced statistical methods developed in the past few decades for high dimensional data and complex structured data could be applied for leveraging the multimodal neuroimaging analysis. These approaches provide a good starting point for analyzing such data. However, there is a strong need for developing new statistical methods that are specific to the multimodal neuroimaging analyses in cognitive and brain aging studies.

AUTHOR CONTRIBUTIONS

The first two authors HC and BZ conducted the analysis and initiated the paper, while the other five coauthors GC, EP, AO, AW, and RC contributed significant components for the presentations of the models and methods and the general discussions. The last two authors AW and RC are the PIs of the Active Brain Study.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Archival Report

Frontal Gamma-Aminobutyric Acid Concentrations Are Associated With Cognitive Performance in Older Adults

Eric C. Porges, Adam J. Woods, Richard A.E. Edden, Nicolaas A.J. Puts, Ashley D. Harris, Huaihou Chen, Amanda M. Garcia, Talia R. Seider, Damon G. Lamb, John B. Williamson, and Ronald A. Cohen

ABSTRACT

BACKGROUND: Gamma-aminobutyric acid (GABA), the brain's principal inhibitory neurotransmitter, has been associated with perceptual and attentional functioning. Recent application of magnetic resonance spectroscopy (MRS) provides in vivo evidence for decreasing GABA concentrations during adulthood. It is unclear, however, how age-related decrements in cerebral GABA concentrations contribute to cognitive decline, or whether previously reported declines in cerebral GABA concentrations persist during healthy aging. We hypothesized that participants with higher GABA concentrations in the frontal cortex would exhibit superior cognitive function and that previously reported age-related decreases in cortical GABA concentrations continue into old age.

METHODS: We measured GABA concentrations in frontal and posterior midline cerebral regions using a Mescher-Garwood point-resolved spectroscopy (MEGA-PRESS) ¹H-MRS approach in 94 older adults without history or clinical evidence of mild cognitive impairment or dementia (mean age, 73 years). We administered the Montreal Cognitive Assessment to assess cognitive functioning.

RESULTS: Greater frontal GABA concentrations were associated with superior cognitive performance. This relation remained significant after controlling for age, years of education, and brain atrophy. GABA concentrations in both frontal and posterior regions decreased as a function of age.

CONCLUSIONS: These novel findings from a large, healthy, older population indicate that cognitive function is sensitive to cerebral GABA concentrations in the frontal cortex, and GABA concentration in frontal and posterior regions continue to decline in later age. These effects suggest that proton MRS may provide a clinically useful method for the assessment of normal and abnormal age-related cognitive changes and the associated physiological contributors.

Keywords: Aging, Cognition, GABA, γ -Aminobutyric, MEGA-PRESS, MRS

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Gamma-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the human nervous system and plays a fundamental role in central nervous system function (1). GABA neurotransmission is involved in nearly all neuronal coding and processing throughout the brain. It directly influences membrane potentials through ionic GABA_A receptors and modulates both short- and longer-term neuronal activity via G-protein coupled GABA_B receptors, modifying synaptic and network plasticity (2–6). Given this connection to synaptic plasticity, GABA has been studied in the context of the aging brain. Recent work demonstrates that GABA concentrations decline with age (7), and rodent models have shown age-related decreases in a GABA synthetic enzyme, glutamic acid decarboxylase (8). However, the relation between these long-term decreases in GABA concentrations and age-related declines in cognitive function has yet to be determined.

A large body of GABA studies relies on examination of downstream pharmacological effects of GABAergic agents (e.g., benzodiazepines) and animal models. This work links GABA to age-related cognitive decline in rodents (9), specifically

noting the importance of GABA as a modulator of memory encoding (10,11). Although such studies provide a strong foundation for investigations into the relation between GABA and cognition, these methods make extensions of their results to more broad discussions of human cognition challenging. Relevant to the question at hand, then, is the development of Mescher-Garwood point-resolved spectroscopy (MEGA-PRESS) (12,13) for GABA-edited magnetic resonance spectroscopy (MRS) (12,14,15). This acquisition sequence allows for relatively rapid and reliable quantification of GABA concentrations in the brain of awake humans. Because these GABA concentrations are experimentally mutable (16,17), MEGA-PRESS more directly enables research into the regionally variable role of GABA in behavior and cognitive function.

This approach has proven to be a flexible and powerful tool for examining GABA, facilitating investigations of GABAergic contributions to specific behaviors and pathological disorders and differences in GABA concentrations between populations. Broadly, researchers have used this approach to demonstrate

that GABA concentrations correlate with other measures of brain activity, including functional magnetic resonance imaging indices (18,19), cerebral blood flow (19), and motor cortex gamma oscillations (20). Specifically applying this work to the intersection between GABA and cognition, several MRS studies have examined the role of GABA in sensory and motor functioning in healthy populations. Often, these studies delineate the differential importance of GABA in various brain regions for multiple sensorimotor or cognitive functions. For example, associations between sensorimotor GABA concentrations and tactile sensitivity have been demonstrated in sensorimotor cortices (21,22). GABA concentrations in the occipital cortex have been shown to relate to visual orientation discrimination (23), whereas frontal GABA concentrations correspond with working memory performance (24). Thus, some degree of specificity between cortical GABA concentration and cognitive ability seems likely. Less clear, however, is the relation between GABA and higher-order cognitive functioning and its decline in healthy aging.

Notably, although GABA concentrations tend to be stable over the short term (25), they do change over longer periods of time. A recent cross-sectional study of adults (20–76 years of age) indicated that GABA concentrations decrease with age after adolescence. This report specifically found an approximate 5% reduction in GABA concentrations with age per decade in the frontal cortex (7). Because the frontal cortex is important for numerous cognitive domains, notably those related to executive function (26–29), such a decline might correlate or even underlie alterations in related domains of cognitive function. The functional significance of these age-associated changes in GABA is not well established.

Given these considerations, the present study examined the relation between frontal and posterior GABA concentrations and cognitive function in the context of normal cognitive aging. We sought to extend previous work relating GABA and cognitive function in modality-specific cortices (e.g., occipital lobe) by investigating higher-order cognition with a general cognitive screening measure, the Montreal Cognitive Assessment (MoCA) (30). This tool, widely used in clinical settings, taps several cognitive domains, including attention/working memory, verbal memory, naming, and fluency. Because a number of these domains fall under the umbrella of executive functions, the MoCA is quite sensitive to frontal dysfunction in general (31). Convergently, older adults demonstrate changes in both frontal activation and frontally mediated cognitive functions. Thus, we placed our primary MRS voxel of interest in the frontal lobe. We predicted that GABA concentrations would continue to decrease in advanced age. We also predicted that the relation between concentrations of GABA in the frontal regions would predict general cognitive performance on the MoCA. We additionally placed a voxel in the posterior cortex to serve as a control. We predicted that, although GABA in this region would decline with age, there would be no association between GABA concentrations and global cognitive performance.

METHODS AND MATERIALS

Population

Ninety-four older volunteers (54 women, 40 men; age [mean \pm SD], 73.12 \pm 9.9 years; years of education, 16.25 \pm 2.8 years;

MoCA scores, 25.5 \pm 2.5) were recruited from the local community. Subjects with a self-reported history of neurological or psychiatric disease on comprehensive medical questionnaires or magnetic resonance imaging (MRI) prescreening forms were excluded from the study. Subjects reported abstaining from alcohol on the day of MRS data collection. Of the 94 subjects, 89 had the frontal voxel collected, and 90 had the posterior voxel collected (due to time constraints in the imaging sequence, 5 participants had only a frontal voxel collected and 4 participants had only a posterior voxel collected). Ethical approval for the study was obtained via the University of Florida's Institutional Review Board, and all participants signed an informed consent form after discussion of the study with a study coordinator and review of the document.

MoCA

The MoCA is a one-page cognitive assessment that takes approximately 10 minutes to administer. A score of 0–30, reflecting general cognitive function, is derived from performance on tasks assessing the following cognitive domains: verbal memory, visuospatial abilities, executive functions, attention, working memory, naming, verbal fluency, repetition, and orientation to time and place (30). One point was added to the scores of participants who had 12 years of education or less (30). Using the MoCA total score in this analysis has a number of advantages. First, the MoCA is a widely used clinical tool with good psychometric properties (e.g., test-retest reliability and internal consistency). It has better sensitivity to mild cognitive impairment and other forms of cognitive decline, including Korsakoff's syndrome, than the Mini-Mental State Examination (32). Thus, a comparison between MoCA performance and GABA concentration allows for a discussion of these mechanisms in a translational context. Precisely because this measure is both sensitive and quick to administer, the MoCA is an efficient test to use in the clinical space. This analysis, then, allows for extension of previous GABA studies to a highly clinically relevant tool. The MoCA, however, does present a notable disadvantage. This instrument is useful when interpreted as a whole, but is not as useful at the level of subscale analysis, because the domains frequently are probed with three to five questions. This small range limits variability, and in healthy populations, many domains experience a ceiling effect. Therefore, the utility of this instrument is somewhat limited to general cognitive performance.

MRS Acquisition and Analysis, ¹H-MRS Spectroscopy, Spectrum Editing, and Volume-of-Interest Refraction

All scanning was performed on a 3T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) using a 32-channel head coil. A T1-weighted anatomical image (magnetization-prepared rapid gradient-echo; repetition time/echo time = 8 ms/3.7 ms, 1-mm³ isotropic voxels) was acquired for MRS voxel placement and segmentation. GABA-edited MRS data were acquired using the MEGA-PRESS sequence (12). PRESS localization was achieved with minimum-phase amplitude-modulated excitation pulses (2-kHz bandwidth) and amplitude-modulated refocusing pulses (bandwidth, 1.3 kHz), as shown in Figure 3 of Mullins *et al.* (14). Editing was performed with 14-ms

sinc-Gaussian pulses applied at 1.9 ppm in the on experiment and 7.46 ppm in the off experiment. This editing scheme co-edits approximately 50% macromolecules at 3 ppm, which are coupled to spins at 1.7 ppm also inverted by editing pulses. Therefore, all GABA values reported refer to GABA + macromolecules. Acquisition variables were repetition time/echo time of 2 s/68 ms; 320 transients with on-off scans alternating every 2 transients; a 16-step phase cycle (with steps repeated for on and off); 2048 data points acquired at a spectral width of 2 kHz; and variable pulse power and optimized relaxation delays (VAPOR) water suppression (33). Sixteen transients of water-suppressed data were also acquired for quantification using the same acquisition variables. All voxels were $3 \times 3 \times 3 \text{ cm}^3$. Representative voxel locations are shown in Figure 1A. Voxel locations were verified after data collection to identify placement errors. Quantitative analysis was performed using the Gannet program (version 2.0) (34). All time domain data were frequency- and phase-corrected using spectral registration (35), filtered with a 3-Hz exponential line broadening and zero-filled by a factor

of 16. The 3-ppm GABA peak in the difference spectrum was fit using a five-parameter Gaussian model and quantified relative to water (fit with a Gaussian-Lorentzian model) in institutional units. To correct for tissue-related factors, controlling for cerebrospinal fluid (CSF) content in the voxel is the most common approach (36) and has been applied in populations in whom voxel tissue composition may vary (37,38). This correction involved the generation of a binary mask of the MRS voxel created with the same imaging matrix as the T1-weighted anatomical image, using Gannet's (34) integrated voxel-to-image coregistration. Segmentation of the anatomical image was performed using Segment in SPM12 (39). The voxel fraction that was CSF, gray matter, and white matter was calculated. In addition, all multiple regression models were rerun, replacing CSF with gray matter and white matter in the model. In all models, all factors significant for the CSF approach were also significant for the gray matter and white matter approach and vice versa. Thus, we only report the CSF fraction because it makes fewer assumptions as to tissue-specific

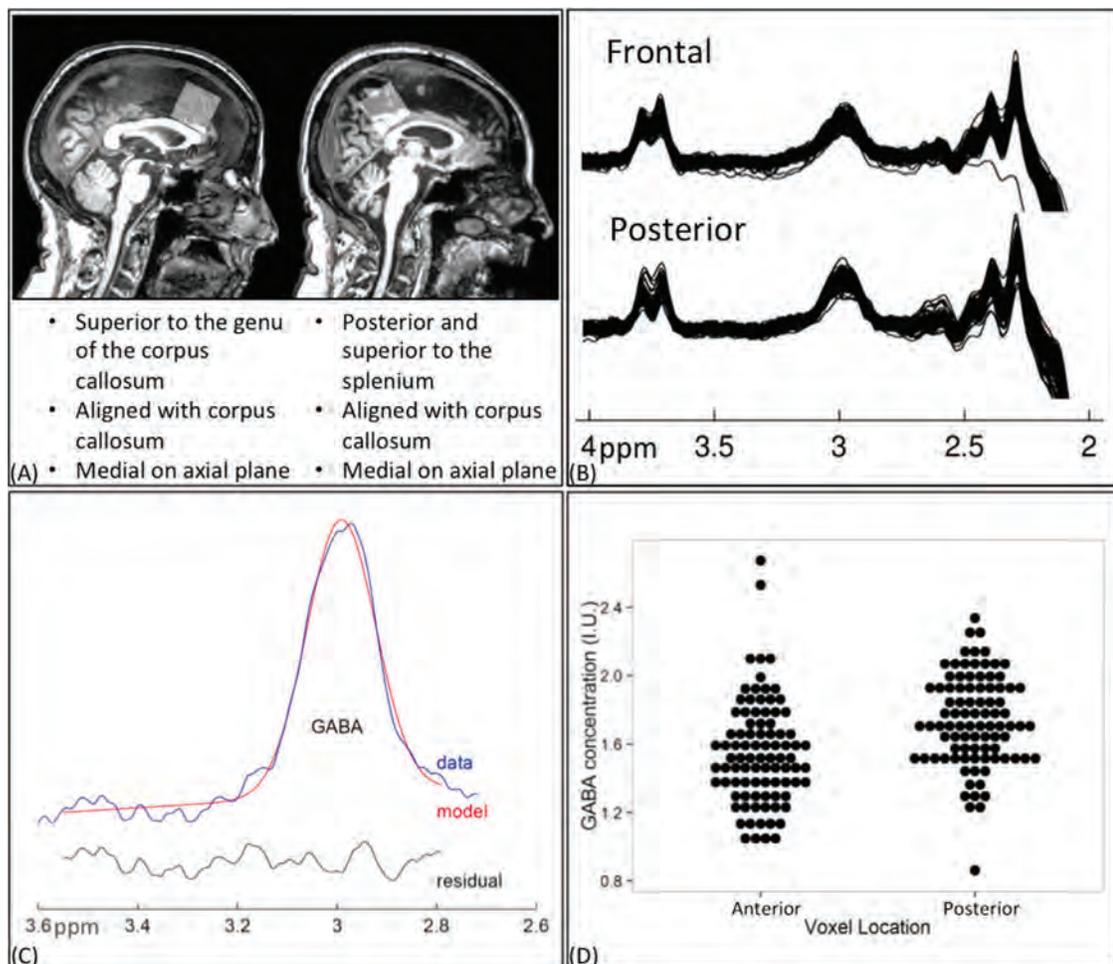


Figure 1. (A) Voxel locations in the frontal and posterior regions of the brain. The gray box represents the location of the $3 \times 3 \times 3 \text{ cm}$ voxel collected using Mescher-Garwood point-resolved spectroscopy. (B) Edited spectra from the frontal and posterior voxels for all subjects. Gamma-aminobutyric acid (GABA) peak is at 3.02 ppm. (C) Representative Gannet GABA model fit. (D) Stacked dot plot demonstrating greater GABA concentrations in the posterior voxel. I.U., institutional unit.

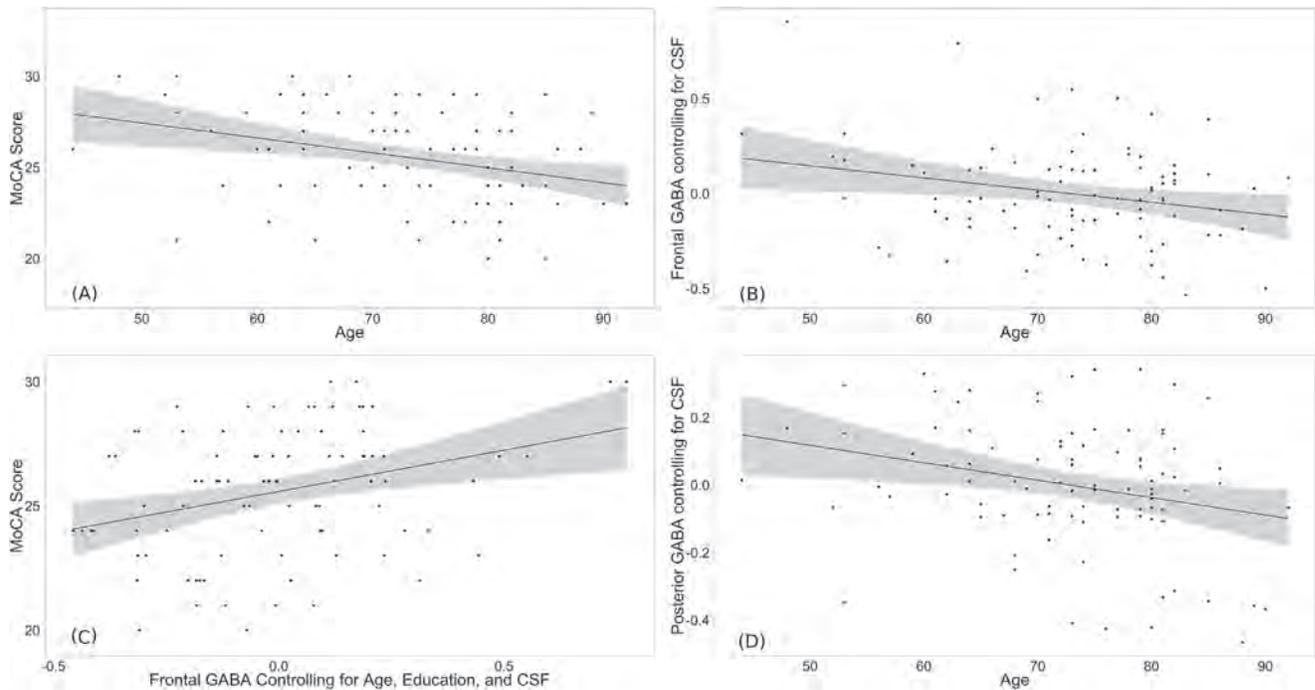


Figure 2. Confidence intervals are 95% for the regression line. **(A)** Plot demonstrating greater participant age associated with lower performance on the Montreal Cognitive Assessment (MoCA). **(B)** Plot demonstrating the age-related decrease in frontal gamma-aminobutyric acid (GABA) concentrations. **(C)** Plot demonstrating the age-related decrease in posterior GABA concentrations. **(D)** Plot demonstrating the relation between frontal GABA concentrations and MoCA scores. The relation remains significant when the two highest GABA data points are removed. CSF, cerebrospinal fluid.

GABA concentrations (36) and is more consistent with previously published approaches to age-related changes in GABA (7).

RESULTS

MoCA as a Function of Demographic Variables

MoCA scores were initially investigated with relation to the demographic variables. Multiple regression was used to analyze the predictive value of age and education for overall cognitive performance. This regression demonstrated that these demographic variables accounted for a significant proportion of variance in cognitive performance ($R^2 = .1103$, $F_{2,91} = 5.64$, $p < .005$). Within this model, age significantly predicted score, such that older participants had lower scores ($B = -.08$, $p < .005$). Figure 2A displays the relation between age and MoCA score. Education was not associated with scores ($B = .08$, $p = .33$).

GABA Concentrations as a Function of Brain Region

A significant difference was found for GABA concentrations between the frontal (1.546 ± 0.305) and posterior ($.7339 \pm 0.264$) voxels ($t_{84} = -5.3373$, $p < .001$), with a greater concentration of GABA in the posterior voxel.

GABA Concentrations as a Function of Age

Frontal Voxel. With the use of a linear regression model, lower GABA concentrations significantly predicted increased age ($R^2 = .25$, $F_{1,87} = 29.56$, $p < .001$, $B = -16.42$, $p < .001$).

Given that this decrease may have been a function of age-associated atrophy, rather than GABA-specific changes per se, we conducted a multiple regression, including CSF fraction and GABA concentration as predictors of age ($R^2 = .38$, $F_{2,86} = 26.19$, $p < .001$). CSF concentrations were positively associated with age ($B = 57.41$, $p < .001$), and GABA concentrations were negatively associated with age ($B = -9.378$, $p < .01$). Notably, even when accounting for CSF fraction, GABA concentration remained a significant predictor of age. This association is depicted in Figure 2B.

Posterior Voxel. Linear regression revealed that lower GABA concentrations in the posterior voxel significantly predicted increased age ($R^2 = .15$, $F_{1,88} = 15.26$, $p < .005$, $B = -14.28$, $p < .001$). To account for age-related atrophy, a multiple regression was conducted with CSF fraction and GABA concentration as predictors of age ($R^2 = .38$, $F_{2,86} = 26.19$, $p < .001$). Greater age was related to lower GABA concentrations ($B = -13.95$, $p < .01$); no relation was found between CSF concentrations and age ($B = 1.65$, $p = .93$). This association is depicted in Figure 2C.

Cognitive Performance as a Function of GABA Concentrations

Frontal Voxel. The relation between frontal GABA concentration and the MoCA score was investigated using linear regression. The results of this regression indicated that GABA in this region accounted for a significant amount of variance in MoCA performance, such that higher concentrations of GABA

predicted better cognitive functioning ($R^2 = .18$, $F_{1,87} = 18.95$, $p < .001$, $B = 3.5$, $p < .001$). On visual inspection, two data points appeared to be outliers. Although they did not fall beyond our outlier cutoff of 3 SDs above or below the mean, we reran the analyses without these participants. When their data were removed, the relation remained significant ($R^2 = .12$, $F_{1,85} = 11.63$, $p < .001$), and GABA concentration continued to predict cognitive functioning ($B = 3.3$, $p < .001$).

Next, the relation between GABA concentration and MoCA score was queried, controlling for age, education, and CSF fraction. The results of this multiple regression demonstrated that the four predictors accounted for a significant amount of the variance in cognitive performance ($R^2 = .22$, $F_{4,84} = 5.775$, $p < .001$). Within this model, higher GABA concentration significantly predicted better score, even when accounting for demographic influences and CSF fraction ($B = 3.32$, $p < .01$). Cognitive performance was not independently related to age, years of education, and/or CSF fraction ($B = -.5$, $p = .12$; $B = .10$, $p = .24$; $B = 3.95$, $p = .37$, respectively). We additionally reran the analyses without the two high-GABA participants. The overall model remained significant ($R^2 = .16$, $F_{4,82} = 3.87$, $p < .01$). Higher GABA concentration continued to predict better MoCA scores ($B = 3.14$, $p < .01$), whereas the other covariates did not ($B = -.5$, $p = .12$; $B = .10$, $p = .27$; $B = 3.85$, $p = .39$, respectively). [Figure 2D](#) depicts the association between frontal GABA concentration and MoCA, controlling for age, education, and CSF fraction.

Posterior Voxel. The relation between posterior GABA concentration and MoCA score was investigated using a linear regression. The results of this regression indicated that posterior GABA concentration does not account for a significant amount of variance in performance ($R^2 = .04$, $F_{1,88} = 5.267$, $p = .07$). Next, the relation between posterior GABA concentration and MoCA score was queried, controlling for age, education, and CSF fraction. The results of this multiple regression demonstrated that the four predictors accounted for a significant amount of variance in cognitive performance ($R^2 = .12$, $F_{4,85} = 2.84$, $p < .05$). Among these variables, age was a significant predictor of cognitive performance ($B = -.08$, $p < .01$), such that greater age was associated with reduced performance. GABA concentration, years of education, and CSF fraction did not significantly predict cognitive performance ($B = 1.4$, $p = .34$; $B = .07$, $p > .44$; $B = 3.44$, $p = .48$, respectively). The addition of age, education, and CSF fraction reduced the contribution to the model of the posterior GABA concentration.

DISCUSSION

The primary findings of our study are that GABA concentrations in frontal and posterior regions decline with age and that decline in frontal, but not posterior, GABA concentration was associated with lower MoCA scores. The finding of reduced GABA in frontal and posterior cortices is consistent with previous work that has used $^1\text{H-MRS}$ to assess GABA in healthy adult populations (7), and it extends these findings by showing that this effect continues to occur with advanced age. Notably, the more aggressive rate of decline in frontal GABA concentrations is consistent with age-associated declines

observed in other neuroimaging methods, including cortical volume (40) and white matter integrity (41,42), as well as with cognitive measures (43–45). Because a decline in GABA in the frontal region was evident even after controlling for atrophy by including CSF voxel fraction, we conclude that this effect is not simply a function of cortical atrophy, but rather a reduction of GABA concentration in brain tissue.

The age-related decline in GABA concentrations we report here is consistent with those previously reported by Gao *et al.* (7) in both frontal and posterior regions; however, other factors could account for the relation between GABA concentrations and MoCA scores. As such, we controlled for what were likely the strongest contributors: age and CSF fraction. Years of education was also included in the model because higher educational attainment is associated with superior cognitive function in old age in general (46) and on the MoCA specifically (30). Lower GABA concentrations, controlling for age, CSF fraction, and education, corresponded with lower MoCA scores in the frontal but not posterior voxel. Importantly, a one-unit increase in GABA corresponded to more than three-point increase in the MoCA score ($B = 3.32$), which is greater than estimates of test-retest reliability in the measure (30). Although the data presented here do not represent within-subject change, they suggest that a one-unit decrease in GABA would correspond to clinically significant cognitive decline. Given the small effect size, however, it should be contextualized as merely one factor contributing to age-related cognitive change. Other considerations influencing this relation are targets for future study and are further elucidated below.

Previous work has identified several specific cognitive domains that are associated with GABA concentration, including memory and attention (9,10,47). This investigation extends work on those specific domains to identify a connection between GABA and higher-order cognitive performance. Notably, although the MoCA is not designed for subdomain analysis, it is composed of a number of subtests tapping frontoexecutive functions. Given the significant GABA-cognition relation in the frontal but not posterior voxel, our results demonstrate some regional specificity of the impact of GABA concentration on cognition. Mechanistically, this relation may be subserved by the effect of GABA on signal-to-noise ratios in the implicated cortical regions. By increasing signal to noise, GABA likely facilitates information extraction and retention, abilities that are reflected in the total MoCA.

A number of questions remain unresolved with respect to GABA and cognition in the context of aging. Although we controlled for age, education, and atrophy as measured by the percentage of CSF brain tissue, it is possible that other factors contribute to the observed associations. That is, although the present study demonstrates an overall association between age, GABA, and cognition, it lacks sufficient power to explore all possible covariates that contribute to this association. Potential mechanisms that may underlie our findings and that cannot be ruled out include changes in macromolecule concentrations and gray or white matter alterations beyond the sensitivity of the relatively coarse measure used here. For example, white matter integrity, as reflected by frontal scalar measures of anisotropy on diffusion MRI, may be a contributing factor. Indeed, age-related white matter changes may, to

some extent, account for both the relation between age and cognition and the relation between GABA and cognition. Although we may have obliquely been able to address these changes by controlling for medical and/or behavioral comorbidities, this question would be more accurately addressed with a specific and intentional quantification of white matter changes. Therefore, future studies examining the relation between other neuroimaging methods such as diffusion MRI and GABA MRS would be valuable.

In addition, the present study queried potential comorbidities, including drug and alcohol abuse, through an extensive medical interview. However, we did not conduct toxicology screens on the participants to verify their statements, which may affect the generalizability of the results. Further studies specifically investigating the relation between GABA, age, cognition, and drug and alcohol use should use such an objective measurement. The relation between other comorbid factors that affect cortical GABA concentrations, such as insomnia and depression (48,49) and their interaction with aging, should also be investigated because these may modulate the relation between GABA and cognition.

Finally, the mechanisms underlying the influence of GABA on cognitive performance need to be investigated in greater detail. Decreases in GABA concentrations may indicate alterations in interneuron population or function, and these facilitator systems for neuronal communication may be sensitive to sub-clinical variations in brain health and function (e.g., neuroinflammatory factors or baseline and reactive shifts in autonomic nervous system mobilization). Although beyond the scope of the present study, additional research exploring the mechanisms of decline in GABA concentrations during normal aging and associated cognitive consequences would be beneficial. Results of the present investigation may have important clinical implications. Given the relation between GABA concentrations and cognitive function, it may be fruitful to explore the longitudinal trajectory of GABA and cognitive decline in the context of mild cognitive impairment and Alzheimer's disease. Furthermore, decline in frontal GABA concentrations may serve as both a predictor of neurodegenerative disease and an opportunity for pharmacological intervention. Future work, then, should establish the reliability of the relation between GABA and cognitive function in healthy older adults and examine it in clinical populations as well.

In summary, we demonstrate that the previously reported age-related decrease in GABA (7) continues into later life. Furthermore, we introduce evidence that frontal concentrations of GABA are predictive of general cognitive function in an aging population, even when controlling for well-known predictors of cognitive function, such as age, education, and brain atrophy. Future research will be well served to investigate tissue-specific concentrations of GABA and their relation to cognitive function to facilitate pharmacological or other intervention approaches.

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ARTICLE INFORMATION

From the Center for Cognitive Aging and Memory (ECP, AJW, HC, AMG, TRS, DGL, JBW, RAC), Institute on Aging, McKnight Brain Institute, Department of Aging and Geriatric Research; Department of Neuroscience (AJW), University of Florida, Gainesville, Florida; FM Kirby Center for Functional Brain Imaging (RAEE, NAJP, ADH), Kennedy Krieger Institute; Russell H. Morgan Department of Radiology and Radiological Science (RAEE, NAJP, ADH), The Johns Hopkins University School of Medicine, Baltimore, Maryland; Department of Radiology (ADH), CAIR Program (ADH), Alberta Children's Hospital Research Institute, University of Calgary; Hotchkiss Brain Institute (ADH), University of Calgary, Calgary, Alberta, Canada; Department of Biostatistics (HC); Department of Clinical and Health Psychology (AMG, TRS), University of Florida; Brain Rehabilitation and Research Center (DGL, JBW), Malcolm Randall Veterans Affairs Medical Center; and Center for Neuropsychological Studies (JBW), Department of Neurology, University of Florida College of Medicine, Gainesville, Florida

Address correspondence to Eric C. Porges, Ph.D., University of Florida, 2004 Mowry Rd., Gainesville, FL 32610; E-mail: eporges@ufl.edu.

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Cognitively Engaging Activity Is Associated with Greater Cortical and Subcortical Volumes

Talia R. Seider^{1,2*}, Robert A. Fieo¹, Andrew O'Shea¹, Eric C. Porges¹, Adam J. Woods¹ and Ronald A. Cohen¹

¹ Center for Cognitive Aging and Memory, Department of Aging and Geriatric Research, Institute on Aging, University of Florida, Gainesville, FL, USA, ² Department of Clinical and Health Psychology, University of Florida, Gainesville, FL, USA

As the population ages and dementia becomes a growing healthcare concern, it is increasingly important to identify targets for intervention to delay or attenuate cognitive decline. Research has shown that the most successful interventions aim at altering lifestyle factors. Thus, this study examined how involvement in physical, cognitive, and social activity is related to brain structure in older adults. Sixty-five adults (mean age = 71.4 years, standard deviation = 8.9) received the Community Healthy Activities Model Program for Seniors (CHAMPS), a questionnaire that polls everyday activities in which older adults may be involved, and also underwent structural magnetic resonance imaging. Stepwise regression with backward selection was used to predict weekly time spent in either social, cognitive, light physical, or heavy physical activity from the volume of one of the cortical or subcortical regions of interest (corrected by intracranial volume) as well as age, education, and gender as control variables. Regressions revealed that more time spent in cognitive activity was associated with greater volumes of all brain regions studied: total cortex ($\beta = 0.289$, $p = 0.014$), frontal ($\beta = 0.276$, $p = 0.019$), parietal ($\beta = 0.305$, $p = 0.009$), temporal ($\beta = 0.275$, $p = 0.020$), and occipital ($\beta = 0.256$, $p = 0.030$) lobes, and thalamus ($\beta = 0.310$, $p = 0.010$), caudate ($\beta = 0.233$, $p = 0.049$), hippocampus ($\beta = 0.286$, $p = 0.017$), and amygdala ($\beta = 0.336$, $p = 0.004$). These effects remained even after accounting for the positive association between cognitive activity and education. No other activity variable was associated with brain volumes. Results indicate that time spent in cognitively engaging activity is associated with greater cortical and subcortical brain volume. Findings suggest that interventions aimed at increasing levels of cognitive activity may delay cognitive consequences of aging and decrease the risk of developing dementia.

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*Correspondence:

Talia R. Seider
tseider@php.ufl.edu

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INTRODUCTION

Older age is the primary risk factor for neurodegenerative diseases such as Alzheimer's disease (AD). As the size and proportion of the population over age 65 increases, the number of people with dementia is expected to increase substantially, raising healthcare costs and caregiver burden (Alzheimer's Association, 2015). Thus, it is becoming increasingly important to identify targets for intervention with the aims of delaying or attenuating cognitive decline.

While several pharmaceuticals exist to delay cognitive decline, research has shown that the best results come from interventions aimed at altering lifestyle factors (Williams et al., 2010; Imtiaz et al., 2014). Greater self-reported levels of engagement in cognitive, social, and physical activity have frequently been associated with higher cognitive functioning scores (Barnes et al., 2004; Newson and Kemps, 2005; McGue and Christensen, 2007; Vemuri et al., 2012; Opdebeeck et al., 2016). Furthermore, physical activity and cognitive engagement are among the only factors consistently associated with decreased risk for AD and cognitive decline (Fratiglioni et al., 2004; Kramer et al., 2004; Hertzog et al., 2009; Williams et al., 2010). Still, the mechanisms of such effects remain poorly understood.

Brain structure and function presumably mediate the link between an active lifestyle and reduced risk for cognitive decline. Observational studies have shown that increased levels of physical activity are associated with larger brain volumes, especially in frontal and hippocampal areas (Rovio et al., 2010; Bugg and Head, 2011; Doi et al., 2015), and interventional studies have shown that physical activity can increase hippocampal (Erickson et al., 2011) and frontal volumes (Colcombe et al., 2006).

Research on the association between cognitive or social engagement and brain volume is less extensive. Proxy measures of cognitive reserve, such as intellectual attainment, have been linked with greater brain volume (Stern, 2009). New learning has been shown to cause increased parietal and hippocampal size in young adults (Draganski et al., 2006), and cognitive training has been associated with increased hippocampal volume and preserved white matter integrity in older adults (Engvig et al., 2014). Greater frequency of cognitive leisure activities has been associated with larger gray matter volume in frontal and limbic regions (Schultz et al., 2015), while higher scores on measures combining cognitive and social activities have been related to more normal-appearing white matter (Gow et al., 2012a) and reduced hippocampal decline over time (Valenzuela et al., 2008). More self-reported social engagement has also been associated with greater temporal and occipital gray matter volume (James et al., 2012), and an intervention study reported that increase in social activity was associated with increased total brain volume (Mortimer et al., 2012). Still, other research has shown no relationship between cognitive or social activity and volumetric data (Foubert-Samier et al., 2012; Vaughan et al., 2014; Van der Vegt, 2015), and the link between these lifestyle factors and regional cerebral volumes remains understudied.

The purpose of this study was to examine how physical, cognitive, and social activity is related to brain structure. Levels of engagement in everyday activities was measured via self-report. Based on previous research, we generally expected higher self-reported levels of physical, social, and cognitive activity to be associated with greater volumes, especially in frontal and limbic regions for physical activity, temporal and occipital regions for social activity, and parietal, frontal, and limbic regions for cognitive activity.

MATERIALS AND METHODS

Participants

Sixty-five community dwelling individuals in the Gainesville and North Florida region were recruited to complete a magnetic resonance imaging (MRI) scan and a cognitive assessment, including the Montreal Cognitive Assessment (MoCA), a brief screen of cognitive functioning. Exclusion criteria included history of head injury with loss of consciousness greater than 20 min, neurologic condition such as dementia, epilepsy, or stroke, major psychiatric illness such as schizophrenia or bipolar disorder, inability to undergo MRI, and MoCA score less than 20. Sample demographics and characteristics are listed in **Table 1**. Participants had a mean age of 71, they were generally well educated with a mean education of 17 years, slightly more than half were females, they were mostly Caucasian, and they were generally cognitively intact with a mean MoCA score of 26. The study was approved by University of Florida Institutional Review Board and written informed consent was obtained from all study participants.

Activity Assessment

The Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire was developed as part of an intervention study aimed at increasing participation in physical activities in community dwelling elderly. It was designed to measure current levels of energy expenditure by taking a poll of everyday activities in which older adults may be involved (Stewart et al., 1997). Participants were asked whether or not they engaged in a particular activity during a typical week in the past month. If they had, they were asked to fill in the number of times they engaged in the activity per week and mark the total number of hours spent in the activity per week. Total hours were grouped into 6 integer values such that 1 indicated less than 1 h was spent engaged in that activity per week, 2 indicated 1 – 2½ h spent, 3 indicated 3 – 4½ h, 4 indicated 5 – 6½ h, 5 indicated 7 – 8½ h, and 6 indicated 9 or more hours (**Figure 1**). Participants are also allowed to fill in “other” and record an activity that was not listed in the questionnaire.

Physical activities were divided into light and moderate-heavy groups based on the ratio of work metabolic rate to resting metabolic rate (MET) adjusted for older adults (Ainsworth et al., 1993) as was done in the original CHAMPS research (Stewart et al., 2001). Light physical activities were those with a MET

TABLE 1 | Sample characteristics (N = 65).

	Mean (SD)	Range
Age (years)	71.4 (8.9)	48–85
Education (years)	16.8 (2.5)	12–20
% male	43.1	
% Caucasian	95.4	
MoCA	26.0 (2.7)	20–30

MoCA, Montreal Cognitive Assessment.

In a typical week during the past 4 weeks, did you...	If YES, How many TIMES a week?	How many TOTAL hours a week did you usually do it?					
		Less than 1 hour (1)	1-2½ hours (2)	3-4½ hours (3)	5-6½ hours (4)	7-8½ hours (5)	9 or more hours (6)
1. Visit with friends or family (other than those you live with)? ○ NO ● YES →	1 2 3 4 5 6 7 ○ ● ○ ○ ○ ○ ○ ○ Other: <input type="text"/>	1	2	3	4	5	6

FIGURE 1 | Sample Community Healthy Activities Model Program for Seniors (CHAMPS) test item.

score below 30 and included conditioning training, yoga or tai chi, leisurely walking, walking to do errands, light gardening, light house work, golfing using a cart, and aerobic dancing. Moderate-heavy physical activities were those with a MET score equal to or above 30 and included sports, light or heavy strength training, swimming gently or fast, water exercises, working on aerobic machines, bicycling, fast walking, uphill walking or hiking, jogging or running, working on machinery (car, lawn mower, etc.), heavy gardening, heavy housework, singles or doubles tennis, golfing without use of a cart, dancing (such as square, folk, line, or ballroom), and skating (ice, roller, or in-line). Social activities were any for which the majority of time spent likely involved interpersonal interaction. These included visiting with family or friends, going to a senior center, volunteering, church-related activities, participating in clubs or groups, playing cards or board games with others, and shooting pool or billiards. Cognitive activities were those for which the majority of time spent was likely cognitively engaging rather than interpersonal. These included using a computer, doing arts or crafts, attending a concert, movie, lecture, or sport event, playing a musical instrument, and reading. If the “other” option was filled in, the activity described was placed in the most appropriate group. The integer measures (1 through 6) representing amount of time spent weekly in each activity were summed to create totals for light and moderate-heavy physical activities, social activities, and cognitive activities.

Magnetic Resonance Imaging Acquisition

Magnetic resonance imaging data were acquired using a Philips Achieva 3.0 Tesla scanner (Achieva; Philips Electronics, Amsterdam, The Netherlands) at the McKnight Brain Institute (University of Florida, Gainesville, FL, USA) with a standard 32-channel receive-only head coil. High-resolution 3D T1-weighted MPRAGE scans were performed. Scans were acquired in a sagittal orientation with parameters as follows: voxel size = 1 mm isotropic; 1 mm slice thickness; TE = 3.2 ms; TR = 7.0 ms; FOV = 240 × 240; Number of slices = 170.

Analysis

T1-weighted MRIs were automatically segmented and volumes were calculated using FreeSurfer software, version 5.3.0, available at <http://surfer.nmr.mgh.harvard.edu/> (Fischl et al., 2002).

Following preprocessing, all results underwent quality control to confirm correct detection of gray and white matter. Any errors in segmentation were corrected manually and the T1 images were re-processed through FreeSurfer. Seventy one percent of cases had some form of manual edit. These consisted of adding control points to extend the white matter boundary (55%), removing voxels from the brain mask (29%), and editing the white matter mask (6%). Some subjects had edits from more than one category, such as both control points and brain mask edits. Previous research has shown that this semi-automated procedure yields accurate and reliable results when compared to manual segmentation (Fischl et al., 2002; Jovicich et al., 2009; Morey et al., 2009) and histological measures (Morey et al., 2009). Automatically parcellated FreeSurfer gray matter regions of interest (ROIs) were based on the Desikan-Killiany atlas. Intracranial volume (ICV) was calculated based on the talairach transform (Buckner et al., 2004). ROIs for the left and right hemisphere were summed and corrected for ICV (by dividing ROI volume by ICV and multiplying by 100) to create bilateral, normalized ROIs. Some of these were then summed to create volumes for the four major lobes of the brain. Specifically, the frontal ROI consisted of the caudal and rostral anterior cingulate cortices, the caudal and rostral middle frontal cortices, the lateral and medial orbitofrontal cortices, the pars orbitalis, the superior frontal cortex, and the frontal pole; the parietal ROI consisted of the precuneus, the inferior and superior parietal lobules, and the supramarginal gyrus; the temporal ROI consisted of the entorhinal cortex, the inferior, middle, and superior temporal regions, the transverse temporal region, and the temporal pole; and the occipital ROI consisted of the lateral occipital region, the lingual and fusiform gyri, the pericalcarine region, and the cuneus. All four lobes were added together to create a measure of total cortical gray matter volume. Subcortical ROIs were chosen based on their relevance to cognitive and behavioral functioning and included the thalamus, caudate, hippocampus, and amygdala.

Statistical Analysis

All statistical analyses were performed using SPSS. Stepwise regression with backward selection was used to predict weekly time spent in either light physical, heavy physical, social, or cognitive activity using a cortical or subcortical ROI (corrected

by total ICV) as well as age, education, and gender as covariates. Individual regression analyses were conducted for each ROI. In stepwise regression with backward selection, all independent variables (predictors and covariates) are entered into the equation and sequentially removed based on the probability of F . The criterion used was $p \geq 0.10$. The first model for which all independent variables included explained significant variance in the dependent variable was chosen as the best model. The benefits of using this analytic method is that it allows for all variables to be included, as it may be that a set of variables has better predictive validity than the subset, but it also removes those that may be falsely lowering the contribution of significant predictors by overlapping in variance explained. Thus, the final model efficiently explains the variance in the dependent variable and better identifies the unique contribution of the predictors.

RESULTS

Table 2 displays the percentage of ICV for cortical and subcortical regions in this sample. In regards to activity measures, scores do not refer directly to number of hours, but rather to ordinal measures reflecting roughly 1.5-h increments (**Figure 1**). Though it cannot be determined exactly how much time was spent in each of the activity categories, it appears that participants divided their time roughly evenly amongst light physical (mean = 8.7, SD = 5.1), heavy physical (mean = 8.4, SD = 5.7), social (mean = 10.5, SD = 6.0), and cognitive (mean = 11.3, SD = 4.5) activities.

Cognitive activity was the only outcome significantly associated with gray matter volume. **Table 3** lists these final, best-fitting models. Final models revealed a positive association with education ($p < 0.001$) and all cortical and subcortical ROIs examined ($ps < 0.05$). **Figure 2** depicts the relationships between cognitive activity and brain volumes, controlling for education. In each regression, variables excluded were age and sex.

In contrast, there were no significant associations observed between the volumes of these brain regions and engagement in light physical, heavy physical, or social activities as reported on the CHAMPS. More heavy physical activity was associated with younger age and male gender, whereas other activities were not

associated with age or sex. Supplemental material includes these results.

DISCUSSION

In this study, we found that greater time dedicated to cognitively engaging activities was associated with greater cortical and subcortical brain volume. We did not find significant associations with physical or social activity level and volumetric data. Cognitive engagement activities represent one of three potential proxies for the construct of cognitive reserve. Epidemiologic-based support for the association between leisure and cognitive status has been accumulating for nearly a quarter of a century (Christensen and Mackinnon, 1993). In an effort to clarify causal attributions or directionality, other epidemiologic work has used sophisticated dynamic change models to show that *change* in cognitive leisure participation can result in higher scores on cognitive ability measures (Mitchell et al., 2012). Such evidence has sparked experimental investigations, with clinical trials demonstrating improved cognition over short periods (3–6 months) for those participating in cognitive leisure activities compared to controls (Stine-Morrow et al., 2008).

More recently, investigators have begun to examine imaging and volumetric evidence, showing that one's level of cognitive reserve is associated with brain volume. For instance, it has been shown that a more active cognitive lifestyle is associated with greater frontal and parietal brain volume in healthy older adults (Stine-Morrow et al., 2008). Our finding that higher cognitive engagement is associated with greater hippocampal volume was also reported in a longitudinal study (Valenzuela et al., 2008). Valenzuela et al. (2008) used the Lifetime of Experiences Questionnaire (LEQ), in which sample activities included creative arts, reading, writing, and socializing. The investigators reported a significant association between total LEQ and average hippocampal volume, controlling for age, gender, hypertension, and ICV. High LEQ individuals experienced an average loss of 3.6% of hippocampal volume over a 3-year period, while low LEQ individuals exhibited more than twice this volumetric loss (8.3%). It is important to note that these studies used a composite measure to establish these associations, which, in addition to cognitive engagement activities, also included education and job complexity. Yet cognitive engagement or cognitive leisure activities show unique contributions to cognitive health, independent of the variance attributed to education and occupational status (Stern, 2009; Foubert-Samier et al., 2012). Indeed, in the current investigation, the relationship between more involvement in cognitively engaging leisure activities and greater brain volumes was not explained by education.

Findings from the current study are similar to those of another observational study in which greater frequency of cognitive leisure activities (playing games like cards, checkers, crosswords, or other puzzles) was related to better cognitive performance and reduced brain atrophy (Schultz et al., 2015). Schultz et al. (2015) reported that higher activity scores were associated with greater gray matter volumes in several ROIs including the hippocampus, posterior cingulate, anterior

TABLE 2 | Gray matter regions of interest presented as percentages of total intracranial volume.

Region of Interest	Mean (SD)	Range
Frontal lobe	7.83 (1.19)	6.18–11.52
Parietal lobe	5.77 (0.82)	4.34–8.35
Temporal lobe	4.75 (0.72)	3.48–6.94
Occipital lobe	4.43 (0.73)	3.43–6.33
Total cortex	22.78 (3.35)	18.14–32.43
Thalamus	0.91 (0.15)	0.71–1.35
Caudate	0.47 (0.08)	0.37–0.71
Hippocampus	0.54 (0.13)	0.30–1.01
Amygdala	0.22 (0.05)	0.13–0.37

TABLE 3 | Best-fitting models predicting cognitive activity from gray matter region of interest, age, sex, and education.

Model/ROI	Variable	β	p	R^2	Model p	Excluded Variables
Frontal lobe	Frontal lobe	0.276	0.019*	0.247	<0.001*	Age
	Education	0.493	<0.001*			Sex
Parietal lobe	Parietal lobe	0.305	0.009*	0.262	<0.001*	Age
	Education	0.505	<0.001*			Sex
Temporal lobe	Temporal lobe	0.275	0.020*	0.246	<0.001*	Age
	Education	0.501	<0.001*			Sex
Occipital lobe	Occipital lobe	0.256	0.030*	0.237	<0.001*	Age
	Education	0.490	<0.001*			Sex
Total cortex	Total cortex	0.289	0.014*	0.253	<0.001*	Age
	Education	0.502	<0.001*			Sex
Thalamus	Thalamus	0.310	0.010*	0.261	<0.001*	Age
	Education	0.526	<0.001*			Sex
Caudate	Caudate	0.233	0.049*	0.227	<0.001*	Age
	Education	0.482	<0.001*			Sex
Hippocampus	Hippocampus	0.286	0.017*	0.250	<0.001*	Age
	Education	0.513	<0.001*			Sex
Amygdala	Amygdala	0.336	0.004*	0.280	<0.001*	Age
	Education	0.519	<0.001*			Sex

ROI, region of interest. * $p < 0.05$.

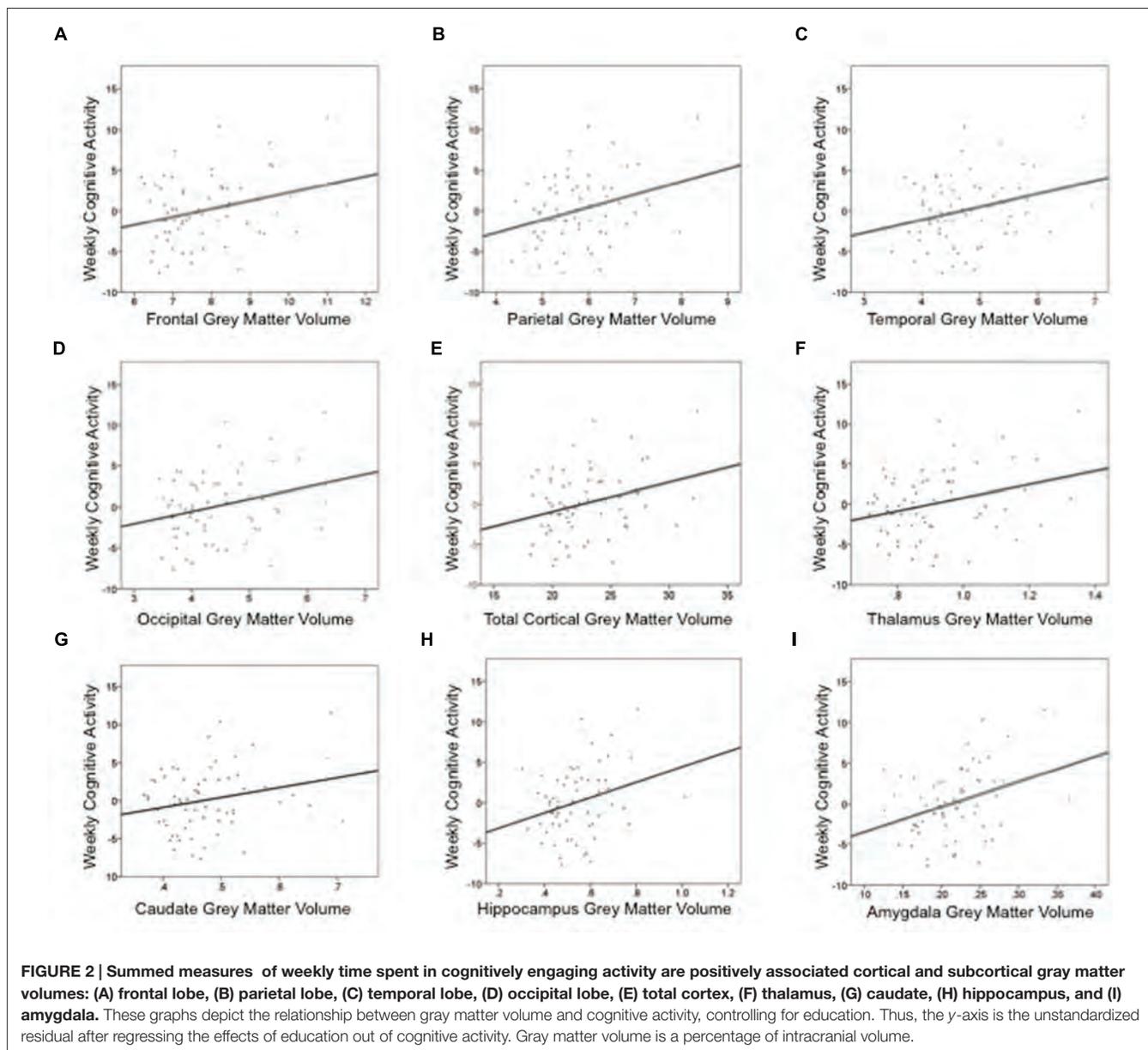
cingulate, and middle frontal gyrus. However, our findings differ from a longitudinal study that found no relationship between cognitive activity and MRI measures of whole brain volumes of gray and white matter (Vaughan et al., 2014). One potential reason for a lack of association in the Vaughan et al. (2014) study may be related to the items included in their 6-item measurement of cognitive activities. Like in the present study, they included reading and craft activities, but they also included group activities, social activities, and watching television. It is possible that group and social activities present with the local dependence, or relatedness; local independence, or unrelatedness, of items is a modern test theory assumption that, when violated, negatively impacts construct validity. Furthermore, television use has been shown to be negatively correlated with other cognitive activity items (Gow et al., 2012b; Fieo et al., 2014). Television time has also been associated with less frequent engagement in social and physical recreation (Hu et al., 2003) and increased risk for dementia (Rundek and Bennett, 2006). These factors may have reduced the power for detecting associations in the Vaughan et al. (2014) study. The current findings, combined with prior research in cognitive leisure activity, reinforce the relationship between hippocampal and cortical volumes and cognitive activity, consistent with our hypothesis.

It is somewhat surprising that heavy physical activity was not associated with volumetric data, as studies examining exercise effects on regional brain volume typically implicate frontal and hippocampal brain areas (Colcombe et al., 2006; Pereira et al., 2007; Erickson et al., 2011). Part of this discrepancy undoubtedly comes from the differences between intervention and observational studies. Intervention studies inherently involve introducing something novel in the lives of research participants. It may be that the amount of novelty an activity offers is

more important than the type of activity itself in impacting cognitive and neurological functioning (Bielak, 2010). Thus, when intervention studies implement a physical activity regimen in sedentary adults (Colcombe et al., 2006), the novelty of the activity and significant change in lifestyle may very well impact brain structure.

Part of the discrepancy between the current findings and results from previous physical activity studies may be due to differences in methodology for measuring physical activity. The CHAMPS questionnaire asked participants to retrospectively indicate their levels of physical activity in a variety of class categories for a typical week over the previous month. Given the number of parameters that had to be remembered, it is possible that participants gave unreliable reports of their own activity (LaPorte et al., 1985). Indeed, Buchman et al. (2008) showed that objectively measured physical activity was associated with cognitive function, whereas self-reported daily physical activity in the same group was not. However, an evaluation of the CHAMPS questionnaire revealed a moderately strong correlation between self-reported engagement in moderate physical activity and activity objectively measured by a waist monitor ($r = 0.48$) (Harada et al., 2001). Furthermore, other self-report studies have shown associations between physical activity levels and cortical volumes (Erickson et al., 2010; Bugg and Head, 2011; Gow et al., 2012a; Benedict et al., 2013). As such, it is unlikely that the lack of association is entirely explained by the self-report nature of the physical activity measurement.

Our findings are consistent with Gow et al. (2012a), who measured baseline physical activity via self-report at age 70 years and administered MRIs at age 73. They found physical activity to be associated with white matter volume, but not gray matter volume. Thus, lack of findings in the



present study may reflect the fact that only gray matter volume was measured. Given that the current findings are inconsistent with a large body of research on the impact of physical activity on brain volumes, we do not feel comfortable rejecting our hypothesis that greater physical activity is associated with greater volumes, particularly in frontal and limbic regions.

Social activity is more tenuously linked to brain volume than are physical and cognitive activity, and the relationship was not supported in the current investigation. Greater social involvement has been associated with greater normal-appearing white matter (Gow et al., 2012a), total brain (Mortimer et al., 2012), and gray matter (James et al., 2012) volumes. Yet methodological differences may explain discrepancies between prior results and our own.

Gow et al. (2012a) combined cognitive and social leisure activities when measuring the link between activity and brain structure. While greater cognitive and social activity was related to more normal-appearing white matter, these activities were not found to be associated with gray matter volume, consistent with the present data. Whether social activity has a stronger relationship to white matter than to gray matter volume deserves further investigation.

Mortimer et al. (2012) performed an intervention study and found that their social interaction groups showed significant increases in total brain volume over the study period. The fact that this study was an intervention may explain why their results differ from the current study, since, as previously discussed, the amount of novelty provided by an intervention may be a significant driver of results (Bielak, 2010). The intervention

group met three times per week at a neighborhood community center, the participants decided on their own to organize and select topics of conversation, and the discussions were described as extremely lively. Thus, lifestyle was significantly impacted in these participants.

James et al. (2012) demonstrated that higher social engagement was associated with greater total brain volume and total gray matter volume, as well as greater temporal and occipital gray matter lobar volumes. Yet methodological differences might also explain the discrepancies between their results and our own. The James et al. study included a question more related to physical than social activity in their eight-item measure of social engagement, as they asked participants how many times in the past week/month they had done any indoor or outdoor recreational activity like bowling, working out, fishing, hiking, boating, swimming, or golfing. Given that physical activity has a more robust association to gray matter volume than does social activity, responses to this question may have been a strong driver of results. Another important difference is in the study samples; the James sample was, for the most part, comprised of former lead workers who were recruited for a study of lead exposure and cognitive function. They were less socially engaged than population-based controls, who comprised 12% of the sample. Not only were the participants in the current study recruited from the same community population, but the sample size was significantly smaller compared to the James et al. sample of 348. Thus, differences in population and sample size may have given James et al. more statistical power to detect any relationship between the measured activities and gray matter volume.

Our null findings for social activity are similar to those of Foubert-Samier et al. (2012), who assessed activity in midlife and in retirement. They asked participants whether or not they engaged in a number of activities during midlife (when they worked) and currently (in retirement) and assigned one point for each activity. Social activity in both midlife and retirement was related to better semantic verbal fluency, but unrelated to total brain, gray matter, or white matter volumes.

Current findings are also consistent with Van der Vegt (2015), who found no association between social activity with family and friends and brain volume (Van der Vegt, 2015). In that study, participants were asked two questions: "In general, how often do you have contact with your family members (including telephone calls or letters)?" and, "In general, how often do you have contact with your friends or well-known acquaintances (including telephone calls or letters)?" Interestingly, factor analytic methods have shown that social activity can be presented as a bi-factor model: social-private and social-public (Jopp and Hertzog, 2010). Additionally, social-private was more highly correlated with cognitive functioning measures. As such, social-private may have a stronger relationship with brain volume than social private, and combining separable constructs may negatively impact predictive validity. The association between different types of social activity and volumetric data of cortical and subcortical regions warrants further study. Nevertheless, considering prior research and the results of the current study, we cannot support our hypothesis

that social activity is associated with greater gray matter volumes in temporal and occipital regions.

As mentioned previously, novelty may be an important driving factor in the relationship between leisure activities that are engaging, cognitively or otherwise, and larger brain volumes. Animal models have shown that, in aged animals, environmental enrichment attenuates the age-related changes in cortical thickness (Mora et al., 2007). A defining feature of enrichment in animals is often novelty. For instance, repeatedly substituting and replacing the objects in the home cages creates a wide range of opportunities for enhanced cognitive stimulation, formation of tuned spatial maps, and proficient detection of novelty (Petrosini et al., 2009). In humans, one theoretical explanation for how novelty exerts a protective effect in older adults relates to non-adaptive "routinization" (Tournier et al., 2012). That is, as older adults continue to accumulate age-related insults (e.g., medical comorbidities or muscle related fatigue), some individuals may seek out more controlled, stable, predictive environments, thus limiting exposure to novel environments or experiences. Evidence in support of this can be found in a Bergua et al. (2006) study, demonstrating that preferences for routine were positively correlated with cognitive decline over 3 years. Additional support can be observed in the finding that greater variety of participation in cognitively stimulating activities was associated a ~10% lower risk of cognitive impairment, regardless of how challenging these tasks were (Carlson et al., 2012). As such, though novel environments and situations may be more difficult to navigate as people age, they may be the most protective against neurological decline.

Limitations

The most common grouping scheme for investigating the efficacy of leisure activities has been the broad domains of physical, social activities, and other cognitive leisure activities, which is what we followed for this investigation. However, with such broad domains, many activities cannot be assigned unambiguously. Volunteering work, for instance, is likely to have a strong social component, but may in some instances entail more non-verbal cognitive or physical effort. Ideally, large item/activity pools would allow for the formation of more distinct, unidimensional activity constructs, for example, further delineating social activities into social-private and social-public (Jopp and Hertzog, 2010). Furthermore, historically, the evidence in observational studies, including the present study, has been limited to an examination of frequency of participation. However, contemporary conversations have moved beyond questioning the frequency of participation, placing greater emphasis on novelty/variety and cognitive challenge (Bielak, 2010; Chan et al., 2014). Finally, as previously mentioned, the accuracy of measurement in self-report questionnaires is questionable, as participants may have subjective biases and may, intentionally or unintentionally, misrepresent their objective activity levels (Erickson et al., 2012). Nevertheless, CHAMPS has several strengths, including robust psychometric properties (Harada et al., 2001; Wilcox et al., 2009), sensitivity to change

(Stewart et al., 1997), and correlations with objective measures of physical activity and functioning (King et al., 2000; Harada et al., 2001; Stewart et al., 2001).

CONCLUSION

Results of this study emphasize the relationship between volumetric data and the independent contribution of cognitively engaging activities. This process is of considerable value if we consider that cognitive leisure activities show unique contributions to cognitive health, independent of the variance attributed to other cognitive reserve proxies (e.g., education and occupational status) (Stern, 2009; Foubert-Samier et al., 2012). Results of the present study show that more time spent engaged in cognitive activity is associated with greater brain volume. Importantly, these lifestyle variables are modifiable, suggesting that interventions aimed at increasing levels of cognitive activity may delay onset or decrease risk of developing dementia. In fact, intervention studies have shown positive neurological effects of cognitive interventions. Still, the field of cognitive aging will benefit from more research investigating interventions aimed at increasing everyday cognitively engaging activities as well as the role of other biomarkers such as inflammatory agents and genetic factors in influencing brain structure and function.

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AUTHOR CONTRIBUTIONS

TS was involved in the conception and writing of the manuscript and conducted statistical analyses. RF was involved in the writing of the manuscript. AO conducted volumetric analyses that were used in the manuscript. EP, AW, and RC were involved in the conception of the study, and RC was involved in the conception of the manuscript and data analysis. All authors contributed intellectual content and approved the version to be published.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fnagi.2016.00094>

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Depressive symptoms modify age effects on hippocampal subfields in older adults

Sarah M Szymkowicz,¹ Molly E McLaren,¹ Andrew O'Shea,^{2,3} Adam J Woods,^{2,3,4} Stephen D Anton² and VONETTA M DOTSON^{1,4}

Departments of ¹Clinical & Health Psychology, ²Aging & Geriatric Research, ³Center for Cognitive Aging & Memory, and ⁴Neuroscience, University of Florida, Gainesville, Florida, USA

Aim: Major depression is associated with hippocampal volume changes, especially in late-life depression. These changes usually consist of volume reductions, but depression-related increases in hippocampal volume have also been reported. Subfield analysis has identified structural changes primarily in the cornu ammonis (CA) 1, CA2–3 and subiculum of the hippocampus in individuals with major depression; however, it is unclear whether lower levels of depressive symptoms are also associated volume reduction, or if depressive symptoms interact with age to impact hippocampal subfields. The current study addressed these questions.

Methods: A total of 43 community-dwelling older adults completed the Center for Epidemiologic Studies Depression Scale and underwent magnetic resonance imaging. Hippocampal subfield segmentation was carried out using an automated procedure, and left and right volumes from CA1, CA2–3, and the subiculum served as outcome measures. Multiple hierarchical regressions were carried out with age, Center for Epidemiologic Studies Depression Scale scores and their interaction as the independent variables, and sex and total intracranial volume as covariates.

Results: Higher Center for Epidemiologic Studies Depression Scale scores were associated with less age-related volumetric decreases in the right subiculum and right CA1.

Conclusions: Age-related atrophy in the hippocampus might be counteracted by depressive symptom-related enlargement of CA1 and the subiculum. More research is required to better understand the functional significance of this relationship. *Geriatr Gerontol Int* 2016; ●●: ●●–●●.

Keywords: aging, brain volume, depressive symptoms, hippocampus, magnetic resonance imaging.

Introduction

Major depression (MDD) is the most common psychiatric disorder seen in community-dwelling older adults.¹ Depression can be thought of as a continuum of symptoms that range from milder conditions, such as elevated depressive symptoms, to more severe forms of major depression. Elevated depressive symptoms are even more common than major depression in older adults, with an estimated prevalence of 7–15%.² These subthreshold depressive symptoms are of critical concern, as they are associated with similar cognitive and fronto-subcortical neural dysfunction, and adverse health outcomes as major

depression, but are often undiagnosed and therefore untreated.^{3,4}

For outcomes such as brain changes, the impact of sub-threshold depressive symptoms might be greater in older adults compared with young adults as a result of the cumulative effect of depressive symptoms and normal age-related changes. In particular, depression-related hippocampal alterations can be more pronounced in older adults compared with their younger counterparts because of the cumulative effect of depression⁵ and age-related hippocampal atrophy.⁶ Older age is associated with hippocampal volume reduction, but findings in major and subthreshold depression vary, with many studies reporting smaller hippocampal volume,^{7,8} but other studies reporting no differences^{9,10} or larger hippocampal volume for at least some subgroups of depressed individuals.¹¹

Inconsistencies in the depression literature might be due to heterogeneity within subregions of the hippocampus that is obscured when the hippocampus is examined globally. The hippocampus comprises histologically

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Correspondence: Ms Sarah M Szymkowicz MS, Department of Clinical & Health Psychology, University of Florida, P.O. Box 100165, Gainesville, FL, 32610-0165. Email: smszymkowicz@phhp.ufl.edu

distinct functional and structural subfields, including cornu ammonis (CA) 1–4, subiculum and dentate gyrus, that have different associations with memory and other functions, and might also be differentially related to both depressive disorders and non-pathological aging.¹² Findings for the relationships between hippocampal subfields, depression and aging are heterogeneous, with differing results for the subfield most affected. With respect to depression, some studies show smaller CA1, CA2–3 and subiculum volume in individuals with late-life depression,^{13,14} and less dentate gyrus volume as a function of multiple depressive episodes in young to middle-aged adults.¹⁵ In contrast, there is also evidence of larger volume of CA1 and portions of the subiculum bilaterally in unmedicated young to middle-aged depressed adults.¹⁶ Similarly, findings on the effect of age on hippocampal subfields vary with some studies showing age effects on volume in the subiculum and relative sparing of CA1 and other subfields,¹⁷ whereas others show age effects on volumes in CA2–3 and CA4–dentate gyrus.¹⁸ Less is known about the potentially interactive effect of age and elevated depressive symptoms on hippocampal subfield volume.

The purpose of the current study was to determine whether or not age effects on volume of hippocampal subfields are modified by elevated depressive symptoms in older adults. Based on CA1, CA2–3 and the subiculum being most consistently related to late-life depression, we focused on these regions.¹³ We predicted that older age would be associated with smaller volume in these hippocampal subfields, and that this association would be more pronounced at higher levels of depressive symptoms.

Methods

Participants

A total of 48 community-dwelling older adults (mean age 68.88 ± 7.21 years) were recruited for the present study. All participants were right-handed, native English speakers with at least 8 years of education. Participants were required to have a score of >30 on the Telephone Interview for Cognitive Status,¹⁹ and a score of >24 on the Mini-Mental State Examination,²⁰ which are the suggested cut-offs for cognitive impairment, respectively. Exclusionary criteria included self-reported history of major neurological or other medical illness, head trauma, learning disorders, current epileptic or antipsychotic medication use, language comprehension difficulties and magnetic resonance imaging (MRI) contraindications. Participants with MDD were not excluded in order to increase the range of depressive symptom severity in the sample. Two participants met the criteria for MDD per clinical interview. Both were taking antidepressant medication, as were five

additional individuals who did not meet the criteria for depression. Five individuals were excluded from analyses because of missing data, MRI evidence of past stroke, current substance abuse or a learning disorder diagnosis. Thus, our final sample comprised 43 individuals (9 young-old [aged 55–64 years], 24 middle-old [aged 65–74 years] and 10 old-old [aged ≥ 75 years]). Demographic data for this sample are presented in Table 1. All procedures were reviewed and approved by the University of Florida's institutional review board, and all participants provided verbal and written informed consent.

Measures

Participants completed the Center for Epidemiologic Studies Depression Scale (CES-D), which consists of 20 self-report questions assessing the frequency and severity of depressive symptoms over the previous week.²¹

MRI data acquisition

MRI data were collected within 1 week of completing the CES-D at the University of Florida's McKnight Brain Institute on the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility's Philips 3-Tesla scanner (Amsterdam, the Netherlands) using a Philips eight-channel radio-frequency coil. A high resolution, T₁-weighted turbo field echo anatomical scan was collected using the following parameters: TR = 81 ms, TE = 3.7 ms, 170 slices acquired in a sagittal orientation, flip angle = 8 degrees, 1 mm cubic resolution. To minimize noise while in the scanner, participants were given headphones and earplugs. Head movement was minimized by cushions positioned inside the head coil.

Table 1 Sample demographic characteristics

	Mean	SD	Range
Total sample (n = 43)			
Age (years)	68.79	7.00	55–81
Sex (% female)	69.76	–	–
Education (years)	15.07	2.53	10–20
MMSE total	28.91	1.25	25–30
CES-D total	7.84	8.90	0–45
Those using antidepressants (n = 7)			
Age (years)	62.57	6.78	56–72
Sex (% female)	71.46	–	–
Education (years)	15.57	2.64	12–19
MMSE total	29.42	0.79	28–30
CES-D total	17.29	16.09	1–45

CES-D, Center for Epidemiologic Studies Depression Scale; MMSE, Mini-Mental State Examination; SD, standard deviation.

Hippocampal subfield measurement

The Freesurfer image analysis suite (version 5.3, <http://surfer.nmr.mgh.harvard.edu>) was used to quantify brain volumes.²² Briefly, processing included motion correction, removal of non-brain tissue, automated Talairach transformation, segmentation of the gray and white matter tissue, and cortical surface inflation. Each image was also manually inspected for errors in the automatic processing by one of two raters. A two-way mixed effects model calculated the interclass correlation coefficient for manual volume adjustments. The interclass correlation coefficient between raters was extremely high (0.99), likely reflecting the minimal manual adjustments required after the automatic processing. Volumes of the bilateral hippocampi were obtained using an automated procedure for volumetric measurement of brain structure, which uses Bayesian inference and a probabilistic atlas of hippocampal formation based on manual delineations of subfields in ultra-high-T₁-weighted MRI scans from a number of participants.²³ The left and right hippocampi were segmented into seven subfields: CA1, CA2–3, CA4–dentate gyrus, subiculum, presubiculum, fimbria and hippocampal fissure. Average dice coefficients of approximately 0.7 for CA2-3 and subiculum were reported for overlap between manual and automated segmentation methods.²³ Regions of interest for the current study included left and right volumes from CA1, CA2–3 and the subiculum.

Statistical analysis

All analyses were carried out using SPSS 22.0 software (IBM, Armonk, NY, USA). Separate hierarchical regression analyses were carried out for the left and right CA1, CA2–3, and subiculum with age, CES-D scores and their interaction as the independent variables, and sex and total intracranial volume as covariates. Education and antidepressant use were initially entered as covariates, but were removed from final analyses due to a lack of statistical significance. CES-D scores were highly skewed; therefore, we applied a square root transformation to these data to ensure a more normal distribution. All variables besides sex were continuous measures in the models. Age and CES-D scores were mean-centered and multiplied to create the interaction terms. We used a statistical significance threshold of $\alpha \leq 0.05$. Because of the relatively small sample size, correcting for multiple comparisons would result in a highly stringent threshold for significance, and might increase the chance of type II error. We therefore present uncorrected results, but indicate when results met significance after Bonferroni multiple comparison correction.

Results

Results are summarized in Table 2 and Figure 1. With respect to the subiculum, there was a significant main effect of age, such that older age was associated with smaller

Table 2 Effects of age and Center for Epidemiologic Studies Depression Scale scores on hippocampal subfield volumes, adjusted for total intracranial volume and sex

	Total ICV			Sex			Age			CES-D			Age × CES-D			
	β	t	P	β	t	P	β	t	P	β	t	P	β	t	P	
Right																
CA1	0.361	2.514	0.016*	-0.204	-1.452	0.155	-0.214	-1.612	0.116	-0.024	-0.155	0.877	0.356	2.375	0.023*	
CA2-3	0.320	2.063	0.046*	-0.248	-1.632	0.111	-0.271	-1.893	0.066	-0.099	-0.594	0.556	0.113	0.698	0.490	
Subiculum	0.151	1.107	0.276	-0.246	-1.844	0.073	-0.408	-3.236	0.003**	0.209	1.418	0.165	0.497	3.489	0.001**	
Left																
CA1	0.456	2.875	0.007**	-0.096	-0.619	0.540	-0.206	-1.407	0.168	-0.081	-0.474	0.638	0.024	0.148	0.883	
CA2-3	0.269	1.739	0.090	-0.316	-2.089	0.044*	-0.209	-1.463	0.152	-0.156	-0.933	0.357	0.104	0.644	0.523	
Subiculum	0.360	2.467	0.018*	-0.177	-1.238	0.223	-0.376	-2.783	0.008**	0.166	1.050	0.301	0.277	1.818	0.077	

* $P < 0.05$. **Significant after Bonferroni correction ($P \leq 0.008$). Men were coded as 0; women were coded as 1. CA, cornu ammonis; CES-D, Center for Epidemiologic Studies Depression Scale; ICV, intracranial volume.

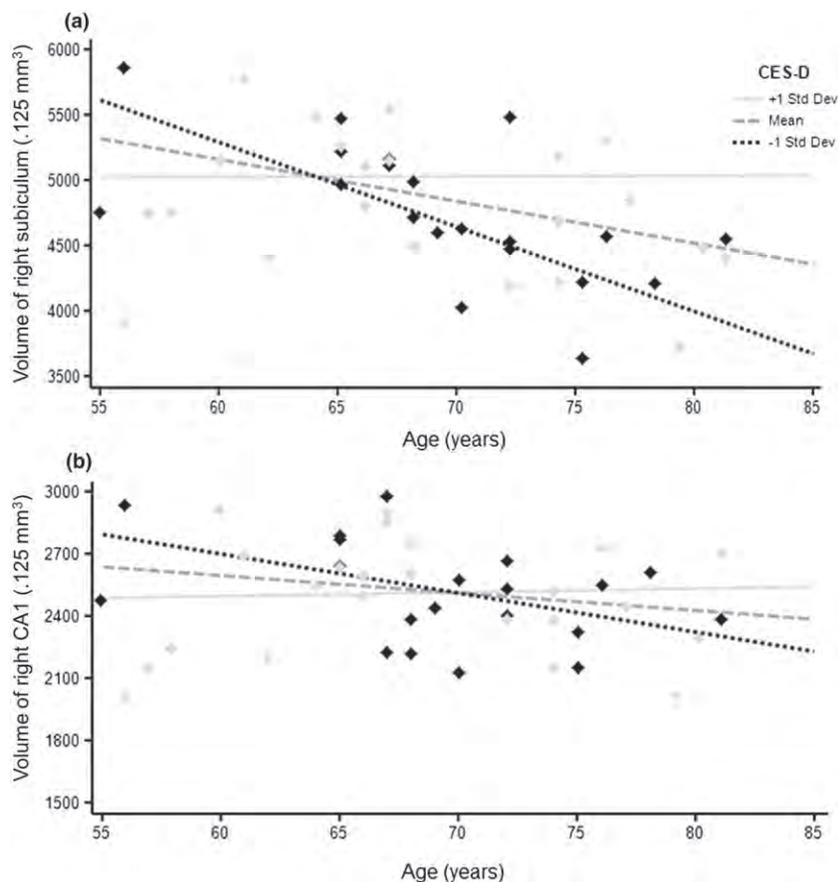


Figure 1 Significant results for the age \times Center for Epidemiologic Studies Depression Scale (CES-D) interactions on volumes in the (a) right subiculum and (b) right cornu ammonis (CA) 1. Raw scores are presented for ease of display, but age and CES-D scores were entered as continuous variables in the statistical models and were centered around the mean in all analyses.

volume of the subiculum bilaterally (right: $P=0.003$, left: $P=0.008$; both significant after Bonferroni correction). This was further qualified by a significant age \times CES-D interaction for the right subiculum ($P=0.001$; significant after Bonferroni correction), suggesting that age effects on volume were greater in individuals with lower CES-D scores, but minimized in individuals with higher CES-D scores. A similar age \times CES-D interaction was found for right CA1 subfield volume ($P=0.023$). There were no other significant main effects or age \times CES-D interactions for the other regions of interest. This pattern of results was unchanged when the two participants with MDD were excluded.

Discussion

The present study examined the interrelationships between depressive symptoms, age and hippocampal subfield volumes. Previous work has generally shown smaller volumes in the subiculum and CA1–3 subfields in both midlife and late-life depression, as well as smaller dentate gyrus volume in young depressed adults.^{13,15} We add to this limited literature by investigating the interaction of age and depressive symptom severity in older adults with mostly subthreshold symptoms. This focus is important considering the high prevalence of subthreshold

depressive symptoms in older adults² and the impact of non-pathological aging on hippocampal subfield volumes,¹⁸ which raises the possibility of a cumulative effect of aging and depressive symptoms on hippocampal structure.

Our finding of greater age effects on volume in individuals with lower depressive symptoms and less of an age effect at higher depressive symptom severity is contrary to our hypothesis. Nevertheless, the results are not completely unexpected in the context of previous reports of larger volumes in the hippocampus. At least one study found larger hippocampal regions analogous to CA1 and the subiculum bilaterally in patients with MDD,¹⁶ and depression-related enlargement of total hippocampal volume has also been reported.¹¹ In the present study, age effects on volume within the hippocampus might have been counteracted by depressive symptom-related enlargement of CA1 and the subiculum.

Although the functional significance of larger hippocampal volumes, particularly in CA1 and the subiculum, in individuals with elevated depressive symptoms remains unclear, it could be that CA1 and the subiculum are particularly vulnerable to the effects of depression, as the present study and others have found alterations in these subfields.^{13,16} Post-mortem studies of individuals with mood disorders have also provided

evidence of disproportionate structural changes in CA1 and the subiculum.²⁴ CA1 projects to the subiculum, which in turn provides the main output of the hippocampal formation to structures involved in mood regulation, including the entorhinal cortex, amygdala, ventromedial prefrontal cortex and striatum.²⁵ The subiculum is suggested to be integral to hippocampal interactions with the hypothalamic–pituitary–adrenal axis.²⁵ Hypothalamic–pituitary–adrenal axis dysfunction is thought to play a role in the pathophysiology of MDD, with persistent elevation of glucocorticoids leading to hippocampal atrophy.²⁶

The mechanisms underlying larger, rather than smaller, hippocampal volume in relation to elevated depressive symptoms are unclear. Some researchers have argued that the early stages of depression are marked by a compensatory inflammatory response, which might modulate neurogenesis in the hippocampus through activation of pro-inflammatory cytokines.²⁷ In addition to increased hippocampal volumes, increased blood flow to the hippocampus has been seen in acutely depressed patients, suggesting that these changes could reflect early or acute stages of depression.²⁸ It might only be through prolonged duration of depressive symptoms that hippocampal atrophy becomes evident.²⁹ Most of our participants had sub-threshold depressive symptoms, and results were unchanged when excluding two participants with MDD. Combined with evidence that subthreshold depressive symptoms are often a precursor to MDD, this suggests the present findings might reflect neurobiological changes that increase the risk for future clinical depression, which might subsequently lead to smaller hippocampal volumes if untreated.²

The impact of depression treatment on hippocampal volumes has been highlighted by other investigations. There is evidence that longer duration of untreated depression is related to hippocampal volume reduction,³⁰ whereas antidepressant treatment is associated with increased volume over time.³¹ Additional clinical variables might impact the relationship between depression and volume in the hippocampus. For example, morphological abnormalities were found in the left anterior subiculum and lateral CA1 in late-onset compared with early-onset depression in one study.¹³ Other studies have found differences in first-episode compared with recurrent depression, including evidence of a positive relationship between total and subfield hippocampal volumes, and severity of depression in first-episode MDD.^{15,32} Furthermore, comorbid symptoms of anxiety might also play a role in increased hippocampal volume, as research has suggested a positive relationship between increased anxiety and larger hippocampal volumes.³³ There is some suggestion from the pediatric depression literature that anxiety influences the ratio of hippocampal volumes to volumes in the amygdala.³⁴ The amygdala is a closely connected structure that is important for emotional expression and, together

with the hippocampus, has a role in the formation of emotion-related memories.^{35–37} Larger studies are required to investigate individual variability in anxiety and other clinical moderators, and their relationship to depression-related brain changes as possible methods for better understanding the underlying mechanisms of depression and improving intervention strategies.

The current findings should be interpreted in the context of limitations of the study, including the inherent limitations of the automated hippocampal segmentation program, as well as our relatively small sample size.³⁸ In addition, our sample included individuals taking antidepressants. Although we did not find any differences in subfield volumes between the two groups, it has been shown that antidepressant use can affect hippocampal volume, and that might have played a role in the present findings.³¹ Furthermore, although all participants in the present study had Telephone Interview for Cognitive Status scores >30 and Mini-Mental State Examination scores >24, we cannot rule out the possibility that individuals with mild cognitive impairment were included, which could have affected the hippocampal subfield results. Moreover, information regarding anxiety symptoms was not available for all participants in the present study; therefore, we were unable to determine the influence of anxiety on the present results. Nevertheless, the study adds to the literature by investigating depressive symptoms as a continuous measure and not as a dichotomous variable (MDD *vs* healthy controls), as many other studies have previously done. Gaining a better understanding of the longitudinal relationship between depressive symptoms and age-related hippocampal volume change might increase our understanding of the pathophysiology of depression in older adults, and provide potential targets for behavioral and pharmacological treatments.

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the study, supervised data collection, and supervised statistical analyses and manuscript writing.

Disclosure statement

The authors declare no conflict of interest.

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Current Heavy Alcohol Consumption is Associated with Greater Cognitive Impairment in Older Adults

Adam J. Woods, Eric C. Porges, Vaughn E. Bryant, Talia Seider, Assawin Gongvatana, Christopher W. Kahler, Suzanne de la Monte, Peter M. Monti, and Ronald A. Cohen

Background: The acute consumption of excessive quantities of alcohol causes well-recognized neurophysiological and cognitive alterations. As people reach advanced age, they are more prone to cognitive decline. To date, the interaction of current heavy alcohol (ethanol [EtOH]) consumption and aging remains unclear. This study tested the hypothesis that negative consequences of current heavy alcohol consumption on neurocognitive function are worse with advanced age. Further, we evaluated the relations between lifetime history of alcohol dependence and neurocognitive function

Methods: Sixty-six participants underwent a comprehensive neurocognitive battery. Current heavy EtOH drinkers were classified using National Institute on Alcohol Abuse and Alcoholism criteria (EtOH heavy, $n = 21$) based on the Timeline follow-back and a structured clinical interview and compared to nondrinkers, and moderate drinkers (EtOH low, $n = 45$). Of the total population, 53.3% had a lifetime history of alcohol dependence. Neurocognitive data were grouped and analyzed relative to global and domain scores assessing: global cognitive function, attention/executive function, learning, memory, motor function, verbal function, and speed of processing.

Results: Heavy current EtOH consumption in older adults was associated with poorer global cognitive function, learning, memory, and motor function ($ps < 0.05$). Furthermore, lifetime history of alcohol dependence was associated with poorer function in the same neurocognitive domains, in addition to the attention/executive domain, irrespective of age ($ps < 0.05$).

Conclusions: These data suggest that while heavy current alcohol consumption is associated with significant impairment in a number of neurocognitive domains, history of alcohol dependence, even in the absence of heavy current alcohol use, is associated with lasting negative consequences for neurocognitive function.

Key Words: Alcohol Consumption, Alcohol Dependence, Cognitive Aging, EtOH, Cognitive Impairment.

THE ACUTE CONSUMPTION of excessive quantities of alcohol causes well-recognized neurophysiological and cognitive alterations, including loss of consciousness, coma, or even death. Heavy alcohol consumption adversely affects the brain both directly and indirectly. Direct brain effects of alcohol include depression of central nervous system activity, alterations in cerebrovascular function, and

neurotoxicity (Alexander et al., 2004; Haorah et al., 2005; Shih et al., 2001; Vinod and Hungund, 2005; Webb et al., 1997; Wilhelm et al., 2015). Indirect effects include neurotoxicity tied to hepatic, renal, and gastrointestinal dysfunction, as well as sleep disturbance, anoxia, head injury, and other disturbances that may occur with chronic alcohol intoxication (Marksteiner et al., 2002; O'Dell et al., 2012; Schuckit, 2009; Solomon and Malloy, 1992; Spirduso et al., 1989; Wilde et al., 2004).

Despite a growing literature concerning the effects of acute and chronic heavy alcohol consumption, the neurocognitive manifestations of heavy alcohol consumption remain unresolved. Findings from past studies conducted to address this question have not been ubiquitous. While the neurocognitive effects of alcohol consumption appear to depend on the amount of alcohol consumed, the duration of use, and various other clinical factors, including age and comorbid neurological conditions, not all studies agree (Carey et al., 2004a, b; Draper et al., 2011; Friend et al., 2005; Green et al., 2010; Houston et al., 2014; Marksteiner et al., 2002; Molina et al., 1994; O'Dell et al., 2012; Solomon and Malloy, 1992; Squaglia et al., 2009, 2014; Sullivan et al., 2002, 2010).

For example, in the MATCH study of drinkers undergoing alcohol treatment for alcohol abuse–dependence, Friend

From the Department of Aging and Geriatric Research (AJW, ECP, VEB, TS, RAC), Center for Cognitive Aging and Memory (CAM), Institute on Aging, University of Florida, Gainesville, Florida; Department of Psychiatry (AG), University of California San Diego, San Diego, California; Department of Behavioral and Social Sciences (CWK, PMM), Center for Alcohol and Addiction Studies and the Alcohol Research Center on HIV (ARCH), Brown University School of Public Health, Providence, Rhode Island; and Department of Pathology and Laboratory Medicine (SM), Department of Neurosurgery, Brown University, Providence, Rhode Island.

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Reprint requests: Adam J. Woods, PhD, Cognitive Aging and Memory Clinical Translational Research Program, Institute on Aging, Department of Aging and Geriatric Research, University of Florida, 2004 Mowry Road, Gainesville, FL 32610; Tel.: 352-294-5842; Fax: 352-294-5836; E-mail: ajwoods@ufl.edu

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and colleagues (2005) found that while years of alcohol consumption was inversely associated with neuropsychological test scores, it did not account for much of the variance in these test scores. Yet, a recent study found in older adults that age of onset of alcohol dependence was not associated with greater cognitive deficits (Kist et al., 2014). However, this study also found that older adults had significantly poorer cognitive abilities when compared to nonalcohol-dependent controls. A recent study found age effects in 51 adults with alcohol dependence diagnoses who were abstinent from alcohol for 1 month (Durazzo et al., 2013). Mild deficits of learning, memory, cognitive efficiency, executive functions, processing speed, and fine motor skills were associated with alcohol dependence, although these deficits were greatest among people who also smoked cigarettes. In addition, another study found deficits in executive function in 560 heavy drinking men and women (Houston et al., 2014). In an earlier study, Drake and colleagues (1995) found that alcohol-dependent adults who abstained from alcohol after a 28-day treatment program showed recovery of cognitive functions. Another study found that moderate alcohol consumption was not associated with either the occurrence or exacerbation of dementia (Panza et al., 2009), and there have been reports that drinking 1 glass of wine a day may actually be associated with reduced rates of Alzheimer's disease (Solfrizzi et al., 2007). Yet, a recent study of brain morphometry and cognition reported that late life consumption of alcohol is associated with episodic memory difficulties and also reduced hippocampal volume in the Framingham cohort (Downer et al., 2015). These contrasting results demonstrate the need for further study of the influence of advanced age on possible heavy alcohol consumption effects on neurocognitive function. Further still, even less is known about the impact of advanced age on possible alcohol-related neurocognitive deficits.

In this study, we sought to understand the relationship between age, heavy alcohol consumption, and neurocognitive function. As people reach advanced age and are more prone to cognitive decline (Woods et al., 2012, 2013), the adverse effects of heavy alcohol use may be exacerbated (Riege et al., 1981). In fact, dementia secondary to alcoholism is commonly diagnosed in elderly adults whose cognitive and functional decline is inconsistent with progressive neurodegenerative disorders such as Alzheimer's disease and whose clinical history indicates chronic heavy alcohol consumption (Meyer et al., 1998; Tyas, 2001). As people reach more advanced age, they experience systemic physiological and neural alterations that may increase vulnerability to the effects of alcohol (Goldberg et al., 1994; Meyer et al., 1998; Snow et al., 2009; Tyas, 2001). Yet, relatively few studies have directly compared the neurocognitive performance of heavy drinkers with that of people who consume moderate quantities of alcohol or who are nondrinkers as a function of age. To address this question, this study was conducted to examine the association of heavy alcohol consumption with neurocognitive function at different ages. We hypothesized

that heavy alcohol consumption would be associated with significant cognitive impairments and that the adverse effects of heavy alcohol consumption would be greatest among older adults.

MATERIALS AND METHODS

Participants

Sixty-six participants in a National Institute on Alcohol Abuse and Alcoholism (NIAAA) sponsored study of the effects of heavy alcohol use and aging on neurocognitive and brain functioning were assessed. The mean age of the sample was 38.5 ± 11.7 years (range = 21 to 69 years). Mean educational attainment was 13.7 ± 2.75 years. The racial composition of the overall sample was 30.3% African-American and 69.7% Caucasian. Thirty-five (53%) participants were women. The sample consisted of adults recruited from the Brown University Center for Aids Research, who were at risk for HIV or hepatitis C virus (HCV) infection based on their association with HIV-infected friends or family, prior injection drug use, or sexual risk, but who were not infected with either HIV or HCV. Participants were recruited over 30 months using clinician referral, word of mouth, and flyers. All participants underwent a neurological examination and thorough medical history assessment. HIV infection was ruled out based on enzyme-linked immunosorbent assay (ELISA) and confirmed by Western blot, while active HCV infection was ruled out by negative anti-HCV ELISA and negative qualitative HCV RNA by polymerase chain reaction. Participants were also excluded for history of (1) head injury with loss of consciousness > 10 minutes; (2) history of severe anxiety, depression, or neurological disorders, including dementia, seizure disorder, stroke, and opportunistic brain infection; (3) severe psychiatric illness that might impact brain function (e.g., schizophrenia, bipolar illness); and (4) current (6-month) substance dependence or positive urine toxicology screen for cocaine, opiates, or illicit stimulants or sedatives. Inclusion/exclusion criteria were assessed using structured clinical interview by the study physician and self-reported medical history. The study was approved by the institutional review boards, and informed consent was obtained from each participant before enrollment.

Alcohol Consumption. Participants were recruited with the goal of obtaining relatively equal samples of nondrinkers, people who drink moderate quantities of alcohol, and heavy alcohol users (ethanol [EtOH] none; EtOH moderate, EtOH high) based on current use. Participants were categorized into alcohol groupings based on NIAAA criteria (see Alcoholism NIAAA: <http://rethinkingdrinking.niaaa.nih.gov/IsYourDrinkingPatternRisky/WhatsAtRiskOrHeavyDrinking.asp>) derived from Timeline follow-back (TLFB; Fals-Stewart et al., 2000) and a structured clinical interview by the study physician. The TLFB involves a self-report of drinking behavior over the past 90 days and was used to calculate the average number of drinks per week over the past 3 months. The EtOH heavy group consisted of people who reported drinking 5 or more drinks in a single day for men (or average more than 14 per week), and 4 or more in a single day (or average more than 7 in a week) for women. The EtOH moderate group consisted of people who reported consuming less than EtOH heavy quantities, while EtOH none reported no consumption of alcohol.

Given the study hypotheses of adverse neurocognitive effects among heavy drinkers, the EtOH none and EtOH moderate groups were pooled into a single group consisting of individuals who were currently drinking below the NIAAA threshold for "at-risk" alcohol consumption (EtOH low). About 31.8% ($n = 21$, 8 women) were heavy alcohol consumers compared to 68.2% ($n = 45$, 27 women) who were not. There were no significant differences by EtOH level

or age between EtOH none ($n = 11$) versus moderate ($n = 34$) participants on any cognitive domain examined, $F(1, 45) < 1.4$, $ps > 0.05$. Age and years of education were not significantly different between the EtOH heavy and EtOH low-risk groups ($ps > 0.05$). There was not a significant difference in racial composition between EtOH groups ($p > 0.05$). There were a greater percentage of women in the EtOH low group ($p > 0.05$, addressed below in the statistical section). Demographic characteristics by EtOH grouping are presented in Table 1.

Drug and Alcohol Dependence. No participants were currently using cocaine or opiates based on self-report and urinalysis, and no participants met criteria for current cocaine or opiate dependence based on the Kreek–McHugh–Schluger–Kellogg scale (KMSK scale; Kellogg et al., 2003). KMSK scale was also used to assess lifetime history of alcohol dependence. The KMSK quantifies self-reported exposure to opiates, cocaine, alcohol, and/or tobacco. Each section of the KMSK scale assesses the frequency, amount, and duration of use of a substance during the person's period of highest consumption. The scale also assesses the mode of use, whether the substance use is current or past, and whether each substance is the substance of choice. Six participants were excluded from alcohol dependence analyses because of incomplete KMSK scores. Thirteen women ($n = 32$, 53.3%) of the sample ($n = 60$) had a history of alcohol dependence, while 46.7% ($n = 28$, 18 women) did not have a lifetime history of alcohol dependence. About 21.6% ($n = 13$) of participants with past history of alcohol dependence were currently alcohol dependent. Thus, 13 of 32 persons with current alcohol dependence overlapped with the total number of people with a lifetime history. Age was not significantly different between lifetime alcohol dependence groups ($p > 0.05$), but education and sex were significantly different ($ps < 0.05$; addressed below in Statistical Analyses). There was not a significant difference in racial composition between dependence groups ($p > 0.05$). Demographic characteristics by lifetime alcohol dependence grouping are presented in Table 1. It is important to note that lifetime alcohol dependence was assessed based on positive or negative history of alcohol dependence, not a quantification of amount of alcohol consumed over the lifetime.

Neurocognitive Assessment. All participants completed a battery of standardized neuropsychological tests widely used in past studies by our group and others to assess the following cognitive domains: speed of information processing, attention/executive functioning, learning, recall memory, verbal fluency, and psychomotor speed. The battery was comprised of the following tests chosen for their sensitivity to HIV-associated neurocognitive deficit: Hopkins Verbal Learning Test—Revised (HVLTR; verbal learning and memory; Benedict et al., 1998; Brandt and Benedict, 1991); Brief Visuospatial Memory Test—Revised (BVMTR; visuospatial learning and

memory; Benedict, 1997); Controlled Oral Word Association Test (COWAT–FAS; verbal fluency [Benton et al., 1994]; category fluency [animals; categorical verbal fluency]); Stroop Color and Word Test (attention/executive function; Golden, 1978); Trails Making Test, Parts A and B (executive function; Reitan, 1992); Letter–Number Sequencing (working memory) from the Wechsler Adult Intelligence Scale—Third Edition (WAIS-III; Wechsler, 1997); Grooved Pegboard Test (fine motor speed; Kløve, 1963); and the Digit Symbol–Coding and Symbol Search (speed of processing measures) tests from the WAIS-III (Wechsler, 1997). *T*-scores from delayed recall on the HVLTR and BVMTR were averaged to calculate the delayed recall domain. Learning trial performance (*T*-scores) on these 2 tasks was averaged to create the learning domain. COWAT and animal naming *T*-scores were averaged for the verbal fluency domain. Stroop, Letter–Number Sequencing, and Trails A and B *T*-scores were averaged to compute the attention/working memory/executive functioning domain. Digit Symbol–Coding and Symbol Search were averaged to calculate the speed of processing domain. The Grooved Pegboard Test *T*-score was used for the psychomotor speed domain.

Demographically (age, education, gender, race) corrected *T*-scores were calculated using established norms. A global index of neurocognitive function was calculated by averaging all domain composite *T*-scores.

Statistical Analyses

Statistical analyses were performed using SPSS-22 software (IBM, Armonk, NY). Demographic and clinical characteristics of the overall sample were determined, and differences in these characteristics among the EtOH low and EtOH high groups examined using independent *t*-tests and χ^2 . Differences in neurocognitive performance as a function of alcohol grouping or lifetime alcohol dependence groups and age were examined using general linear modeling. The primary analyses consisted of 2-way analyses of variance (ANOVAs) (e.g., EtOH \times age or dependence \times age), in which the dependent measure was each of the domain scores and the global index. Age was dichotomized based on the median of the sample (median = 39 years) such that adults 40 years or older were compared to adults younger than 40 years. As age was corrected for using *T*-scores in the dependent measures, age was included in the models to specifically assess for abnormal change in the normal trajectory of age-related neurocognitive decline. Thus, the presence of an age effect denotes exacerbation of normal age-related decline in neurocognitive function. Interactions and simple effects were examined based on the results of the overall ANOVAs. Both EtOH groupings and lifetime dependence groupings had significant differences in the distribution of sex. Analyses including sex as a factor in each of the 2-way ANOVAs failed to show any significant interactions or main effects of sex on cognitive measures ($ps > 0.05$). Thus, sex was not included in the models presented below. Tables 2 and 3 provide mean values in *T*-scores used for analyses. Except for Fig. 1, *T*-scores were transformed into *z*-score format for ease of interpretation. Figure 1 depicts performance per domain by *T*-scores.

RESULTS

Current Alcohol Consumption

Descriptive statistics for EtOH high and low groups by age group are provided in Table 2 (*T*-scores) and 3 (raw scores). The interactions of age by EtOH for global cognitive performance, learning, memory, and motor function are shown in Fig. 2.

Table 1. Sample Demographics by Ethanol (EtOH) and Lifetime Alcohol Dependence History Groupings

	EtOH group	Mean	Std. dev.	Range
Age	EtOH– ($n = 45$)	39.82	12.21	21 to 69
	EtOH+ ($n = 21$)	35.38	10.20	22 to 54
	Dependence– ($n = 28$)	38.57	13.82	21 to 69
	Dependence+ ($n = 32$)	38.84	9.87	22 to 56
Education	EtOH– ($w = 27$)	13.84	2.80	8 to 20
	EtOH+ ($w = 8$)	13.05	2.94	7 to 18
	Dependence–* ($w = 18$)	14.61	2.47	11 to 20
	Dependence+* ($w = 13$)	12.53	2.92	7 to 18

EtOH+ = heavy EtOH consumption; EtOH– = nonheavy EtOH consumption; Dependence– = no history of alcohol dependence; Dependence+ = history of alcohol dependence; n = sample size, w = women.

* $p < 0.05$.

Table 2. Cognitive Performance by Ethanol (EtOH) and Age Groups (T-scores)

Domain score	EtOH group	Age group	Mean	Std. error	95% Confidence interval	
					Lower bound	Upper bound
Global cognition*	EtOH–	Younger	48.274	1.644	44.987	51.560
		Older	51.616	1.681	48.255	54.976
	EtOH+	Younger	51.666	2.107	47.453	55.878
		Older	45.489	2.980	39.532	51.446
Speed of processing	EtOH–	Younger	52.551	1.887	48.779	56.322
		Older	54.227	1.929	50.371	58.083
	EtOH+	Younger	54.381	2.418	49.547	59.215
		Older	48.643	3.420	41.807	55.479
Attention/executive	EtOH–	Younger	53.841	1.755	50.333	57.348
		Older	54.227	1.794	50.641	57.814
	EtOH+	Younger	54.381	2.249	49.885	58.877
		Older	49.714	3.181	43.356	56.073
Learning*	EtOH–	Younger	39.030	2.456	34.120	43.940
		Older	44.545	2.511	39.524	49.565
	EtOH+	Younger	45.417	3.148	39.123	51.710
		Older	34.662	4.452	25.762	43.562
Memory*	EtOH–	Younger	38.762	2.632	33.501	44.024
		Older	47.720	2.691	42.341	53.100
	EtOH+	Younger	45.072	3.374	38.328	51.816
		Older	38.097	4.771	28.560	47.634
Verbal	EtOH–	Younger	52.391	2.087	48.220	56.563
		Older	55.932	2.134	51.667	60.197
	EtOH+	Younger	54.571	2.675	49.225	59.918
		Older	55.500	3.783	47.938	63.062
Motor*	EtOH–	Younger	48.217	2.292	43.635	52.800
		Older	50.432	2.344	45.747	55.117
	EtOH+	Younger	53.857	2.938	47.984	59.730
		Older	43.643	4.155	35.337	51.949

EtOH+ = heavy EtOH consumption; EtOH– = nonheavy EtOH consumption; Std. = standard; Younger = years of age < 40 years; Older = years of age ≥ 40 years; Age range of sample = 21 to 69 years.

*Significant age × EtOH interaction at $p < 0.05$.

Global Cognitive Function. A significant age by EtOH interaction was found for global cognitive function, $F(1, 62) = 4.80$, $p < 0.05$, partial eta squared = 0.07. Overall cognitive performance varied as a function of level of alcohol consumption and age. Tests of simple effects revealed a significant effect of age on cognitive performance for the EtOH high group ($p < 0.05$). Heavy drinkers 40 years and older had lower global cognitive scores than younger heavy drinkers (Fig. 2). There was not an age effect on cognitive performance for the EtOH low group. Tests of simple effects conducted to examine EtOH high and EtOH low groups further demonstrate this relationship between age and alcohol use. Cognitive performance did not vary as a function of level of alcohol consumption for participants under the age of 40 years. In contrast, cognitive performance differed between the EtOH high and EtOH low groups for participants 40 years and older, with heavy drinkers showing lower cognitive scores ($p < 0.05$; Fig. 2A).

Learning and Memory. A significant age by EtOH interaction was found for composite learning performance, $F(1, 62) = 6.30$, $p < 0.05$, partial eta squared = 0.09 (Fig. 2B). Tests of simple effects revealed that among people

in the EtOH high group, a significant age effect existed ($p < 0.05$). Heavy drinkers 40 years and older had lower learning scores than younger heavy drinkers. Age group effects were not evident for the EtOH low group. Tests of simple effects conducted to compare EtOH high and EtOH low separately for the young and older age groups indicated similar effects. Among young drinkers, EtOH high and EtOH low did not differ significantly, whereas a significant EtOH effect existed among the older drinkers, with lower learning scores for the EtOH high group ($p < 0.05$).

A significant age by EtOH interaction was also found for composite memory performance, $F(1, 62) = 5.25$, $p < 0.05$, partial eta squared = 0.08. Heavy drinkers 40 years and older had lower memory recall score than younger heavy drinkers. An age effect was not evident for the EtOH low group. Tests of simple effects conducted to compare EtOH high and EtOH low separately for the young and older age groups indicated similar effects. Among young drinkers, EtOH high and EtOH low did not differ significantly, whereas a significant EtOH effect existed among the older drinkers with lower memory scores for EtOH high group ($p < 0.05$). The interaction of age by EtOH for learning and memory is shown in Figs 2B,C.

Motor Function

A significant age by EtOH interaction was also found for motor function, $F(1, 62) = 4.2$, $p < 0.05$, partial eta squared = 0.06 (Fig. 2D). Tests of simple effects comparing EtOH high and EtOH low separately indicated that differences between the age groups existed for the EtOH high group ($p < 0.05$), but not the EtOH low group. For the EtOH high group, older heavy drinkers had poorer fine motor function than younger heavy drinkers, whereas young and older adults in the EtOH low group did not differ in their motor function.

Other Cognitive Functions

There were not interactions of age by EtOH for the verbal, speed of processing, or attention/executive domains, $F(1, 62) < 2.2$, $ps > 0.05$. Accordingly for these cognitive domains, performance did not differ among young and older participants based on their level of current alcohol consumption. There were also not significant main effects for age or EtOH with respect to these cognitive domains, $F(1, 62) < 0.8$, $ps > 0.05$.

Alcohol Dependence

In subsequent analyses, the influence of lifetime alcohol dependence history was analyzed to determine whether dependence was also associated with reduced cognitive performance. Unlike current heavy alcohol use, lifetime history of alcohol dependence did not interact with age to adversely affect cognitive performance, $F(1, 60) < 1.5$, $ps > 0.22$.

Table 3. Cognitive Performance by Ethanol (EtOH) and Age Groups (Raw Scores)

Test name	EtOH group	Age group	Mean	Std. error	Test name	EtOH group	Age group	Mean	Std. error
HVLt-R Learning	EtOH-	Younger	23.65	1.23	Trails A (seconds)	EtOH-	Younger	28.04	1.84
		Older	23.91	0.93			EtOH-	Older	29.41
	EtOH+	Younger	25.50	1.48		EtOH+		Younger	28.00
		Older	18.86	1.72			EtOH+	Older	36.86
HVLt-R Delayed	EtOH-	Younger	7.91	0.59	Trails B (seconds)	EtOH-		Younger	64.83
		Older	9.00	0.44			EtOH-	Older	81.45
	EtOH+	Younger	8.93	0.69		EtOH+		Younger	82.79
		Older	7.00	0.78			EtOH+	Older	81.14
BVMt-R Learning	EtOH-	Younger	22.30	1.57	Letter Number Sequencing	EtOH-		Younger	10.74
		Older	23.41	1.24			EtOH-	Older	10.23
	EtOH+	Younger	26.14	1.81		EtOH+		Younger	11.64
		Older	20.43	2.74			EtOH+	Older	9.43
BVMt-R Delayed	EtOH-	Younger	8.61	0.57	Grooved Pegboard (D, seconds)	EtOH-		Younger	71.17
		Older	9.27	0.51			EtOH-	Older	74.14
	EtOH+	Younger	9.86	0.74		EtOH+		Younger	63.57
		Older	7.71	1.12			EtOH+	Older	84.71
COWAT	EtOH-	Younger	37.43	2.55	Grooved Pegboard (ND, seconds)	EtOH-		Younger	76.68
		Older	40.86	2.98			EtOH-	Older	79.36
	EtOH+	Younger	40.86	3.14		EtOH+		Younger	75.00
		Older	37.29	4.05			EtOH+	Older	100.29
Animal Naming	EtOH-	Younger	21.96	1.51	Digit Symbol-Coding	EtOH-		Younger	71.26
		Older	21.00	0.95			EtOH-	Older	66.55
	EtOH+	Younger	22.69	1.72		EtOH+		Younger	77.21
		Older	19.71	1.97			EtOH+	Older	63.00
Stroop Color and Word Test	EtOH-	Younger	83.48	1.61	Symbol Search	EtOH-		Younger	38.87
		Older	38.00	2.28			EtOH-	Older	32.55
	EtOH+	Younger	43.50	2.82		EtOH+		Younger	39.36
		Older	32.14	0.80			EtOH+	Older	28.71

HVLt-R = Hopkins Verbal Learning Test—Revised; BVMt-R = Brief Visual Memory Test—Revised; COWAT = Controlled Oral Word Association Test; D = dominant; ND = nondominant; EtOH+ = heavy EtOH consumption; EtOH- = nonheavy EtOH consumption; Std. = standard; Younger = years of age < 40 years; Older = years of age ≥ 40 years; Age range of sample = 21 to 69 years.

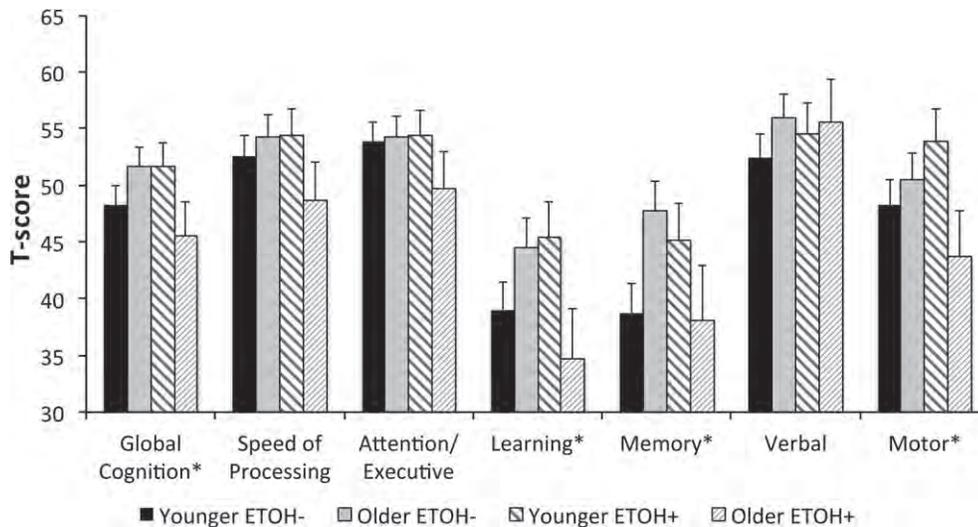


Fig. 1. Cognitive performance by ethanol (EtOH) and age groups. T-score data are presented with standard error bars for each age and EtOH group. Although visually different, younger EtOH- and younger EtOH+ were not significantly different on learning and memory domains, $F_s < 2.1$, $p_s > 0.15$.

There was also not a main effect of age, $F(1, 60) < 1.5$, $p_s > 0.22$. In contrast, the main effect of alcohol dependence was significant for global cognitive function, $F(1, 60) = 7.35$, $p = 0.001$, partial eta squared = 0.017 (Fig. 3A), learning, $F(1, 60) = 7.35$, $p = 0.001$, partial eta squared = 0.22 (Fig. 3B), memory, $F(1, 60) = 7.35$, $p = 0.001$, partial eta

squared = 0.32 (Fig. 3C), motor function, $F(1, 60) = 7.35$, $p = 0.001$, partial eta squared = 0.12 (Fig. 3D), and attention/executive function, $F(1, 60) = 7.35$, $p = 0.001$, partial eta squared = 0.08 (Fig. 3E), with cognitive performance lower in people with a history of lifetime alcohol dependence ($p_s < 0.05$; Table 4). Lifetime alcohol

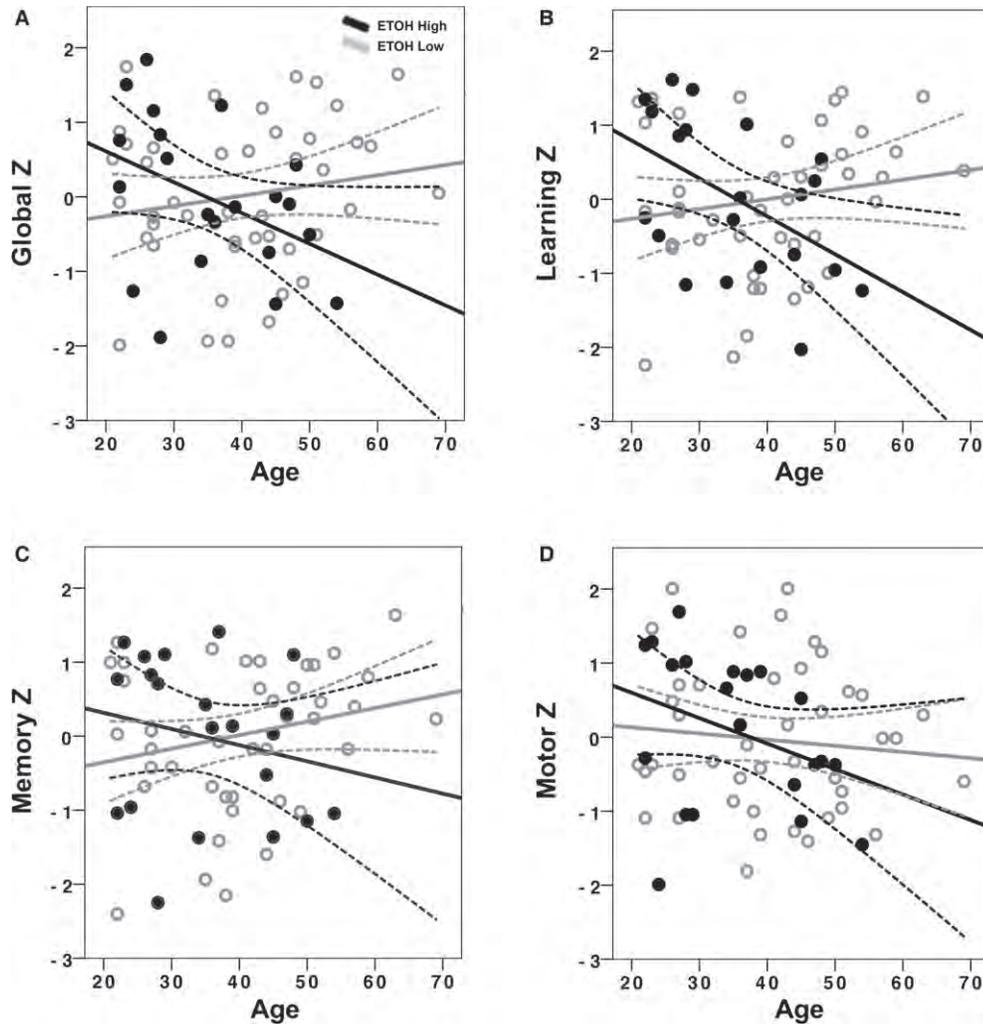


Fig. 2. Effects of age and current alcohol consumption on neurocognitive domains. *T*-scores were converted to *z*-scores for ease of interpretation. (A) Global cognitive function, (B) Learning, (C) Memory, (D) Motor. Ethanol (EtOH) high = heavy alcohol consumption, EtOH low: none/moderate alcohol consumption. Dashed lines represent 95% confidence limits.

dependence did not significantly affect verbal or speed of processing ($ps > 0.05$).

DISCUSSION

The results of this study indicate an interaction between quantity of current alcohol consumption and age with respect to global cognitive performance, as well as performance in the cognitive domains of learning, memory, and motor function. Current heavy drinkers who, by definition, consumed more alcohol on a weekly basis than the NIAAA threshold for “high-risk” drinking (EtOH high) exhibited greater cognitive deficits as a function of age compared to younger current heavy drinkers and compared to adults who were current nonheavy drinkers or abstainers (EtOH low). There was not an age association with cognitive performance for the EtOH low group. Adults who were not currently heavy drinkers tended to have average cognitive performance, relative to demographically corrected normative

values. The fact that people who did not drink alcohol at all did not differ significantly from people who consumed minimal to moderate quantities on any cognitive domain supports our original hypothesis that adverse cognitive effects would primarily be observed among current heavy drinkers.

That neurocognitive performance did not vary as a function of age in the EtOH low group is perhaps not surprising given that the mean age of the study cohort was only 39 years, with no participants over the age of 65. Age-associated cognitive decrements are not expected among healthy adults during midlife and are usually minimal until the seventh decade of life. The absence of aging associations in the EtOH low group, after normative correction for age, education, and socioeconomic status, demonstrates that neither abstaining from alcohol nor nonheavy drinking altered the normal trajectory of cognitive aging. The observed age findings among heavy drinkers are more the anomaly, suggesting that people who consume large quantities of alcohol may be prone to premature cognitive aging.

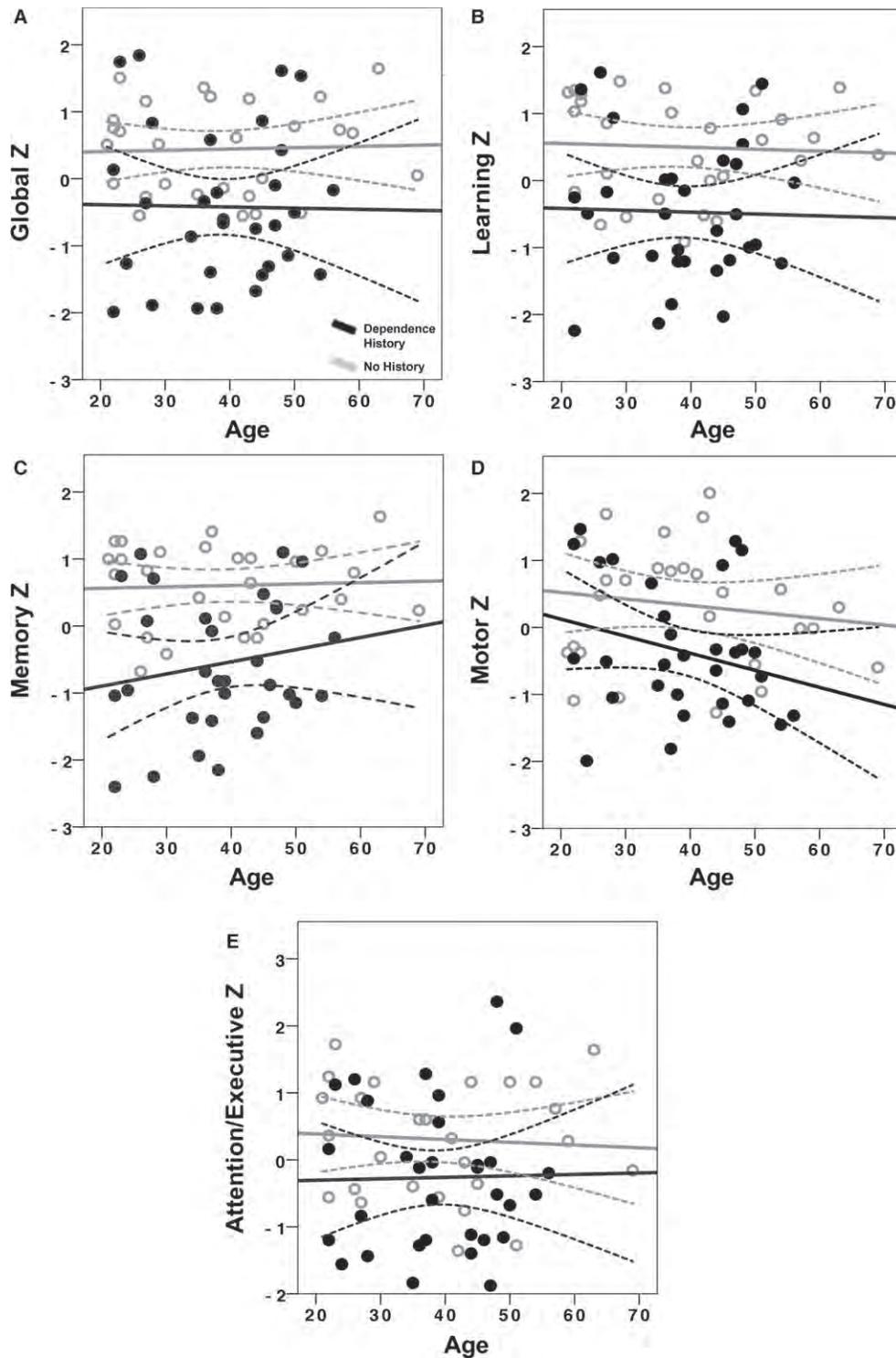


Fig. 3. Effects of lifetime history of alcohol dependence on neurocognitive domains. *T*-scores were converted to *z*-scores for ease of interpretation. (A) Global cognitive function, (B) Learning, (C) Memory, (D) Motor, (E) Attention/executive function. Dependence history = lifetime alcohol dependence history. Dashed lines represent 95% confidence limits.

Neurocognitive deficits in older current heavy drinking were not universal. Specifically, older current heavy drinkers had significantly lower performance on tasks related to learning, memory, and motor function. In contrast, attention/

executive functions, verbal fluency, and speed of processing did not differ as a function of age and current alcohol consumption. In terms of motor function, the measure used in the current study was designed to assess psychomotor speed

Table 4. Cognitive Performance by Lifetime Alcohol Dependence History (T-scores)

Composite cognitive measure	EtOH DH	Mean	Std. error	95% Confidence interval	
				Lower bound	Upper bound
Global cognition*	None	53.313	1.463	50.382	56.243
	DH	46.522	1.376	43.766	49.278
Speed of processing	None	54.244	1.799	50.640	57.847
	DH	51.990	1.692	48.601	55.379
Attention/executive*	None	56.163	1.597	52.964	59.362
	DH	51.390	1.502	48.382	54.399
Learning*	None	47.753	2.131	43.484	52.022
	DH	36.216	2.004	32.202	40.231
Memory*	None	50.849	2.103	46.636	55.063
	DH	36.226	1.978	32.263	40.188
Verbal	None	55.754	1.929	51.889	59.619
	DH	52.855	1.814	49.221	56.490
Motor*	None	53.359	2.016	49.319	57.398
	DH	45.629	1.896	41.830	49.428

EtOH DH = alcohol dependence history; DH = lifetime alcohol dependence history; None = no dependence history; Std. = standard.

*Main effect of lifetime alcohol dependence history at $p < 0.05$.

in a fine motor control task. Learning and memory composite scores were calculated using both visual and verbal learning and memory indices. These functionally specific results may provide insight into candidate neural structures for future investigations into the neural correlates of our findings, such as hippocampus, cerebellum, and primary and supplementary motor association cortices. As the functions of these brain regions are impacted acutely during heavy alcohol consumption (e.g., blackouts, loss of coordination, etc.), our data may suggest that these acute effects are more lasting in consequence.

In contrast to findings for current heavy alcohol consumption, lifetime history of alcohol dependence did not interact with age. Rather, neurocognitive deficits were evident in persons with a history of alcohol dependence irrespective of age. Global cognitive performance with specific deficits in learning, memory, motor function, and attention/executive function was associated with lifetime history of alcohol dependence. While neurocognitive effects of current heavy alcohol consumption appear to be exacerbated by age, long-term decline in cognitive function from lifetime history of alcohol dependence does not. Regardless, the same functions affected by current heavy consumption of alcohol were also affected in those with a lifetime history of alcohol consumption, in addition to attention/executive function. As with current heavy alcohol consumption, neurocognitive effects were not universal, with no evidence of change in speed of processing or verbal fluency. The consistency between current and lifetime history, as well as anecdotal reports of acute effects of heavy alcohol consumption, suggests that these patterns represent a consistent cascade of short- and long-term consequences from heavy alcohol consumption.

Our current findings provide evidence that the adverse effects of alcohol use on neurocognitive function may

interact with both age and quantity of alcohol consumed. Heavy alcohol consumption appears to have adverse cognitive effects, whereas drinking minimal to moderate amounts of alcohol does not produce these associations, even in older adults. The fact that heavy alcohol effects on cognition were associated with age in a cohort that was less than 70 years of age suggests that very advanced age is not a prerequisite for these adverse effects and that susceptibility may increase dramatically during midlife. Evidence for greater compromise of neurocognitive function in older adults with current heavy alcohol consumption may have significant implications for personal and public health, as these individuals are likely more susceptible to decline in driving performance, increased rates of injury, hospitalization and dependence on assisted living, poorer medical outcomes, increased mortality rates, and other factors commonly associated with cognitive decline in older adults (Woods et al., 2011, 2013). Evidence for long-term consequences of alcohol dependence is also potentially important. These data suggest that those with a lifetime history of alcohol dependence may suffer deleterious effects that compromise neurocognitive function throughout life, not merely during acute periods of heavy alcohol consumption. However, the alternative is also possible and cannot be discounted in the current study. That is to say, premorbid deficits in neurocognitive function may predispose people toward alcohol abuse and dependence. It is also important to note that these findings are specifically relevant to the presence or absence of lifetime history of alcohol dependence, not a direct quantification of the amount of alcohol consumed over the lifetime. Such data may be important for further exploring these effects.

Furthermore, prior studies found mixed results when investigating the consequences of heavy alcohol consumption on neurocognitive function. Our results provide evidence supporting recent studies on the interaction of age and heavy alcohol consumption and extend our understanding of their neurocognitive consequences. This study provides strong evidence that heavy alcohol consumption has both short- and long-term consequences for neurocognitive function and that these consequences increase with advancing age. Furthermore, our data suggest that heavy alcohol consumption is associated with accelerated cognitive aging.

Limitations and Future Directions

The population of noninfected but at-risk persons recruited from a larger EtOH-focused study on HIV may represent a significant sampling bias that could exaggerate the impact of EtOH on cognitive function. However, these data also represent realistic insight into a population with high rates of EtOH abuse and thus are, at the very least, representative of similar populations. Use of normative data between groups to assess age effects over different test measures might be viewed as a limitation versus a matched sample control across groups. Future study of persons not at risk

for contracting HIV and HCV will help to support the applicability of these data to the population at large. In addition, longitudinal studies would allow for better understanding of the long-term consequences of these effects. Use of self-report measures of alcohol consumption and lifetime history of dependence may have introduced an extra degree of variability over objective measurement. However, such objective measures are often impossible, especially when assessing past alcohol consumption. These self-report measures may actually underestimate the level of current consumption and presence of past dependence. While this study demonstrates the neurocognitive consequences of heavy alcohol consumption, the structural, metabolic, and functional brain changes underlying long-term consequences of heavy alcohol consumption remain unclear. Furthermore, the causal direction of the relationship between neurocognitive function and alcohol abuse–dependence requires further study. As such, future studies are needed to characterize the relationship between alcohol-associated cognitive impairments versus cognitive deficit-associated increase in alcohol consumption, metabolic and functional brain abnormalities that can be assessed using neuroimaging and other methods, and the amount of recovery of function versus persistent brain dysfunction that is likely to occur with reduced alcohol consumption as people age.

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Cognitive Aging and the Hippocampus in Older Adults

Andrew O'Shea¹, Ronald A. Cohen¹, Eric C. Porges¹, Nicole R. Nissim^{1,2} and Adam J. Woods^{1,2*}

¹Center for Cognitive Aging and Memory, McKnight Brain Institute, Department of Clinical and Health Psychology, University of Florida, Gainesville, FL, USA, ²Department of Neuroscience, University of Florida, Gainesville, FL, USA

The hippocampus is one of the most well studied structures in the human brain. While age-related decline in hippocampal volume is well documented, most of our knowledge about hippocampal structure-function relationships was discovered in the context of neurological and neurodegenerative diseases. The relationship between cognitive aging and hippocampal structure in the absence of disease remains relatively understudied. Furthermore, the few studies that have investigated the role of the hippocampus in cognitive aging have produced contradictory results. To address these issues, we assessed 93 older adults from the general community (mean age = 71.9 ± 9.3 years) on the Montreal Cognitive Assessment (MoCA), a brief cognitive screening measure for dementia, and the NIH Toolbox-Cognitive Battery (NIHTB-CB), a computerized neurocognitive battery. High-resolution structural magnetic resonance imaging (MRI) was used to estimate hippocampal volume. Lower MoCA Total ($p = 0.01$) and NIHTB-CB Fluid Cognition ($p < 0.001$) scores were associated with decreased hippocampal volume, even while controlling for sex and years of education. Decreased hippocampal volume was significantly associated with decline in multiple NIHTB-CB subdomains, including episodic memory, working memory, processing speed and executive function. This study provides important insight into the multifaceted role of the hippocampus in cognitive aging.

Keywords: cognitive aging, hippocampus, MoCA, NIH toolbox, structural magnetic resonance imaging

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Michael R. Foy,
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Australia

*Correspondence:

Adam J. Woods
ajwoods@ufl.phhp.edu

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INTRODUCTION

From the discovery of its role in episodic memory following bilateral resection in patient “HM” to the discovery of its role in symptoms of Alzheimer’s disease (AD), the hippocampus is considered a structure fundamental for human cognition (Scoville and Milner, 1957; Squire, 1992; Jack et al., 1999). In addition to its well-documented role in memory function, recent research demonstrates that the hippocampus also plays a role in executive function, processing speed, intelligence, path integration and spatial processing (Reuben et al., 2011; Papp et al., 2014; Yamamoto et al., 2014). Each of these cognitive processes is shown to decline in the context of cognitive aging, in the absence of neurodegenerative diseases or neurological injury (Salthouse, 2010). Thus, understanding how change in hippocampal structure impacts cognition in the context of aging may prove important for identifying: (a) critical neural underpinnings of the cognitive aging process; and (b) intervention targets for combating cognitive aging.

Most of our knowledge of hippocampal structure-function relationships in humans is based on the findings in various disease states or following resection of the medial temporal

lobes resulting in gross memory disturbance. Models of structure-function relationships in non-human animals have highlighted the hippocampus as a spatial map, crucial for navigation and spatial memory (O'Keefe and Dostrovsky, 1971; O'Keefe, 1979). In addition, recent functional magnetic resonance imaging (MRI) findings have provided insight into the functional role of the hippocampus in various cognitive abilities beyond episodic and spatial memory (Eldridge et al., 2000; Iaria et al., 2007; Woods et al., 2013). However, the impact of subtle changes in hippocampal structure in the context of normal aging, in the absence of neurodegenerative or other disease states, remains poorly understood.

Individuals with diagnoses of mild cognitive impairment (MCI) and dementia have smaller hippocampi than age-matched controls in numerous MRI studies (Shi et al., 2009). Hippocampal atrophy is considered a hallmark of Alzheimer's disease (AD) (Jack et al., 1999). Premorbid hippocampal volume in patients with MCI predicts future conversion to AD (Jack et al., 1999). Thus, change in the structure of the hippocampus appears to play an important role in dementia. However, hippocampal volume is also well-documented to decline in normal aging (Raz et al., 2005). Yet, the functional consequences of this age-related volumetric loss is not well characterized in the context of aging in the absence of neurodegenerative disease. While changes in cognitive scores on dementia screening and cognitive assessment measures in patients with MCI and AD are associated with smaller hippocampal volume, it is unclear whether these findings are unique to dementia/disease states or extend to more subtle variations in hippocampal structure from normal aging.

The few studies that have investigated cognitive aging and the hippocampus have produced results that contrast significantly with prior research in neurological and neurodegenerative disease (Van Petten, 2004; Paul et al., 2011; Colom et al., 2013). For example, Van Petten (2004), in a meta-analysis, reported that the relationship between hippocampal size and episodic memory were weak. These inconsistencies between aging and disease-related findings highlight the need for further investigation of the role of the hippocampus in cognitive aging. Understanding the relationship between hippocampal structure and function in cognitive aging may have predictive value for identifying persons at higher risk for future cognitive decline, cognitive frailty and conversion to MCI (Woods et al., 2013). The prevalence of older adults is expected to accelerate over the coming decades. With this shift in the age of the world population comes an increase in the number of people that will suffer from MCI and other neurodegenerative disorders. Thus, there is a pressing need to identify predictive markers of decline. However, pursuit of such markers is difficult, if not impossible, without first understanding the normal variation present in the aging brain, as well as the overall structure-function relationship between the hippocampus and different components of cognitive function.

In the current study, we sought to examine the relationship between hippocampal volume and a commonly administered dementia-screening tool and a comprehensive cognitive battery in a cohort of 93 older adults without neurological injury,

neurodegenerative disease or major psychiatric illness to: (1) better understand the structure-function relationship between the hippocampus and cognitive aging; and (2) to providing a foundation for development of predictive biomarkers by characterizing the sensitivity of commonly administered MCI screening and cognitive assessment tools to age-related structural changes in the hippocampus. We specifically examined the relationship between the Montreal Cognitive Assessment (MoCA) and the NIH Toolbox Cognitive Battery (NIHTB-CB). The MoCA is a brief (10 min) screening tool for MCI (Nasreddine et al., 2005), whereas the NIH toolbox cognitive assessment is a brief comprehensive computerized cognitive battery (~60 min) consisting of tests to assess executive function, attention, episodic memory, language, processing speed and working memory. These measures are sub-divided into two composite cognitive scores comprising cognitive abilities that change with age (fluid cognitive function) or remain stable over time (crystalized cognitive functions). The delineation of a two-factor model (a fluid factor and a crystalized factor) instead of a single general intelligence factor is valuable when studying cognitive aging due to differences in the age curves of fluid and crystalized abilities (Cattell, 1987). Mungas et al. (2014) found a two-factor solution fit the NIHTB-CB validation data better than a single general intelligence factor; however, the best fitting model was a five-factor solution comprised of the following: reading, vocabulary, episodic memory, working memory and executive function/processing speed. An extension of the two-factor, fluid and crystalized model, the Cattell-Horn-Carroll (CHC) theory of cognition extends the factors of general intelligence to nine broad abilities (fluid reasoning, comprehension-knowledge, short-term memory, visual processing, auditory processing, long-term storage and retrieval, cognitive processing speed, quantitative knowledge and reading and writing; McGrew, 2009). The CHC taxonomy may provide a more thorough description of individual domains of the NIHTB-CB. However, a single NIHTB-CB task would likely incorporate multiple factors of the CHC model, rather than representing distinct entities.

We hypothesized that older adults with smaller hippocampal volumes would evidence lower performance on both the MoCA and NIHTB fluid cognition scores. In contrast, language-based cognitive abilities (i.e., crystalized cognition) would not change as a function of hippocampal volume. Furthermore, we hypothesized that hippocampal volume would be most strongly associated with performance on the memory domain of the MoCA and NIHTB. In addition, we also predicted that smaller hippocampal volume would be associated with slower processing speed and poorer executive functions. These data would not only support the role of the hippocampus in cognition as shown in prior research on neurodegenerative disease and neurological disease states, but also extend these findings to cognitive aging. Furthermore, these data would provide a strong foundation for development of predictive hippocampal biomarkers for future decline in longitudinal cohorts by characterizing cognitive aging in the hippocampus

in the absence of neurological and neurodegenerative disease.

MATERIALS AND METHODS

Participants

Ninety-three older adults (60% female) were recruited from the north-central Florida community through newspaper advertising, fliers and community outreach. Participants had a mean age of 71.7 years ($SD = 9.8$ years) and an average of 16.26 years education ($SD = 2.61$, see **Table 1** for detailed demographics). All participants provided written informed consent prior to enrollment. All study procedures were approved by the University of Florida Institutional Review Board prior to the start of the study. Participants had the opportunity to ask the researchers any questions about study procedures prior to the start of the study. No vulnerable populations were studied. Exclusionary criteria included pre-existing neurological or psychiatric brain disorders, MRI contraindications (such as metal or medical devices inside the body not approved to be scanned at 3T), reported diagnosis of a neurodegenerative brain disease (i.e., dementia or Alzheimer's) or self-reported difficulty with thinking and memory.

Study Procedures

Participants completed a neuropsychological battery (see "Measures" Section for more details) that included the NIHTB-CB and MoCA. Neuropsychological tasks were administered at an onsite clinical research facility by trained study staff. The neuropsychological battery was completed as a single visit. Neuroimaging scanning was completed at a subsequent MRI visit.

Measures

NIH Toolbox

In this study, NIH Toolbox was used as a brief, comprehensive assessment to examine neurological and behavioral function, allowing for the study of functional changes across the lifespan. The cognitive domain measure was used which covered subdomains of: executive function and attention, episodic memory, language, processing speed and working memory. Executive function and attention was measured by NIH-Toolbox Flanker Inhibitory Control and Attention test and

the Dimensional Change Card Sort test. Flanker measures the ability to inhibit visual attention to irrelevant task dimensions. The Dimensional Card Sort test was used to assess the set-shifting component of executive function. Working memory was tested by the List Sorting test. Episodic Memory was assessed by Picture Sequence memory test and the Auditory Verbal Learning (Rey) test. To test language, the Oral Reading Recognition test and the Picture Vocabulary test were used. Processing speed was assessed by the Pattern Comparison test and the Oral Symbol Digit test. The fluid cognition composite is composed of the following tasks: Dimensional Change Card Sort, Flanker, Picture Sequence Memory, List Sorting and Pattern Comparison. The crystallized cognition composite is composed of the Picture Vocabulary Test and the Oral Reading Recognition Test. The NIH toolbox cognitive battery has been shown to have high test-retest reliability, as well as high convergent validity with "gold standard" measures of crystallized and fluid cognition (Heaton et al., 2014).

MoCA

The MoCA is a 10-min, 30-point clinical assessment of multiple cognitive functions, including orientation (6 points), attention (6 points), short-term memory recall (5 points), abstract thinking (2 points), visuospatial executive function assessed by a clock-drawing task, trails task and reproducing a geometrical figure (5 points), naming task (3 points) and language function assessed by verbal fluency test (3 points). An additional one point was added for subjects with less than/equal to 12 years in education (per guidelines of MoCA administration (Nasreddine et al., 2005)). The suggested cut-off point on the MoCA is below 26 for MCI.

Neuroimaging Acquisition

All participants were imaged in a Philips 3.0 Tesla (3T) scanner (Achieva; Philips Electronics, Amsterdam, Netherlands) at the McKnight Brain Institute (University of Florida, Gainesville, FL, USA) with a 32-channel receive-only head coil. A pillow was placed under the head to limit motion during the scan. A high-resolution 3D T1 weighted MPRAGE scan was performed. Scanning parameters consisted of: voxel size = 1 mm isotropic; 1 mm slice thickness; TE = 3.2 ms; TR = 7.0 ms; FOV = 240 × 240; Number of slices = 170; acquired in a sagittal orientation.

Neuroimaging Processing

T_1 -weighted MRI scans were processed with the software FreeSurfer version 5.3. To measure hippocampal volume, the automated subcortical segmentation stream in FreeSurfer was used. The software uses Bayesian inference methods relying on prior anatomical probabilities in a labeled data set, along with *a priori* known T_1 intensity characteristics of subcortical regions, as well as T_1 intensity information from the scan being processed, in order to label discrete regions (Fischl et al., 2002). Previous research has shown this automated procedure produces accurate and reliable results, while taking a fraction of the time of the gold standard of manual segmentation (Fischl et al., 2002;

TABLE 1 | Sample demographics.

	Mean	SD	Range
Age	71.69	9.45	43–85
Education	16.26	2.61	12–20
Sex distribution			
	Number	% of sample	
Male	37	40	
Female	56	60	

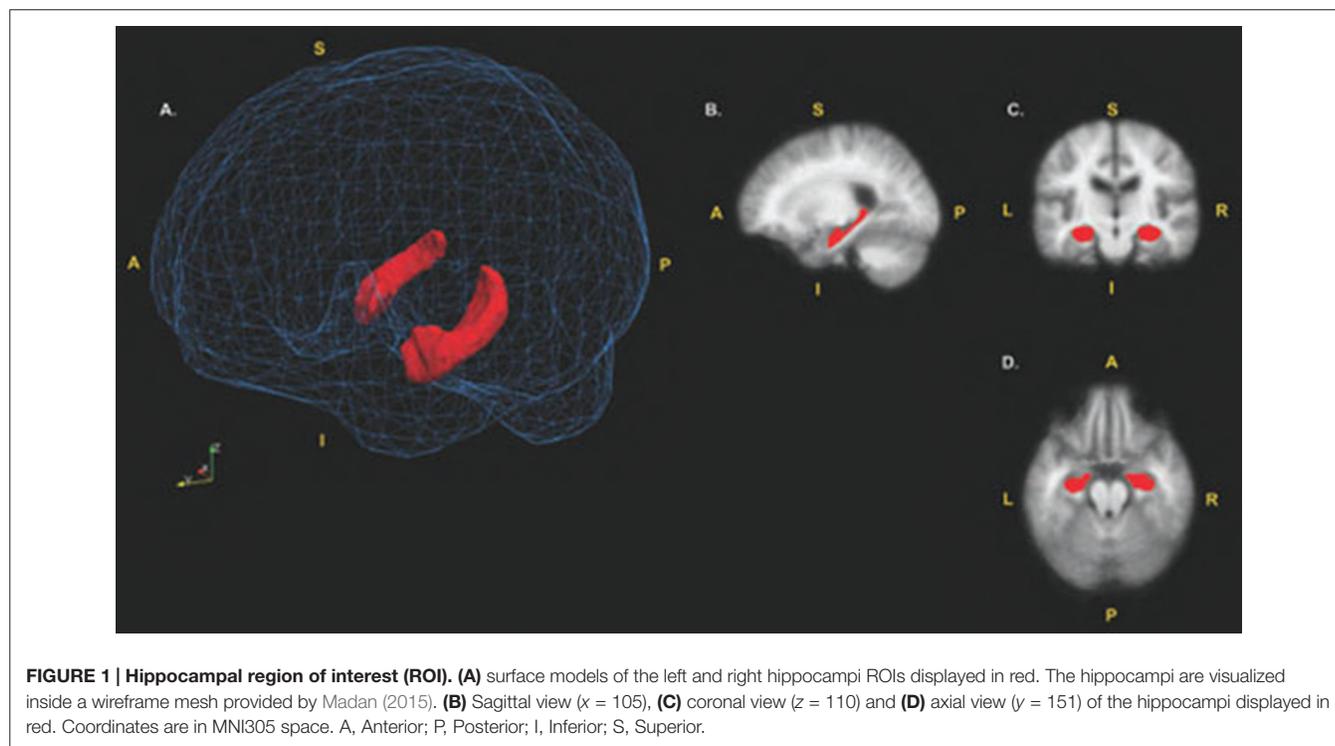


FIGURE 1 | Hippocampal region of interest (ROI). (A) surface models of the left and right hippocampi ROIs displayed in red. The hippocampi are visualized inside a wireframe mesh provided by Madan (2015). (B) Sagittal view ($x = 105$), (C) coronal view ($z = 110$) and (D) axial view ($y = 151$) of the hippocampi displayed in red. Coordinates are in MNI305 space. A, Anterior; P, Posterior; I, Inferior; S, Superior.

Jovicich et al., 2009). This makes automated segmentation well suited for large samples. Any errors in segmentation were fixed manually, and were re-processed through FreeSurfer, producing results that have been validated against manual segmentation (Morey et al., 2009) and histological measures (Cardinale et al., 2014). Whole hippocampal volume was computed as a sum of left and right hemisphere measures; this measure was then normalized in respect to total intracranial volume. All subsequent uses of the term “hippocampal volume” refer to the normalized value. See **Figure 1** for a visual depiction of the hippocampal region of interest (ROI; mesh provided by Madan, 2015).

Statistical Analyses

Neuroimaging data was analyzed using a ROI approach predicting hippocampal volume. MoCA and NIHTB-CB composite scores were used as predictor variables. Descriptive statistics and inter-measure correlations can be found in **Tables 2, 3**. Hippocampal volume was normalized using estimated total intracranial volume, to control for differences in head size. Covariates of sex and education years were included in all models. A secondary set of analyses was aimed

TABLE 2 | Descriptive statistics.

Measure	Mean	SD	Range
NIH Toolbox crystallized cognition	127.54	11.05	100–154
NIH Toolbox fluid cognition	96.21	9.72	80–133
MoCA	25.74	2.53	20–30

SD, Standard Deviation.

TABLE 3 | Correlation matrix.

	MoCA	Crystal	Fluid
MoCA	1	0.37**	0.42**
Crystal	0.37**	1	0.28**
Fluid	0.42**	0.28**	1

Crystal, NIH Toolbox crystallized cognition; Fluid, NIH Toolbox fluid cognition. ** $p < 0.01$.

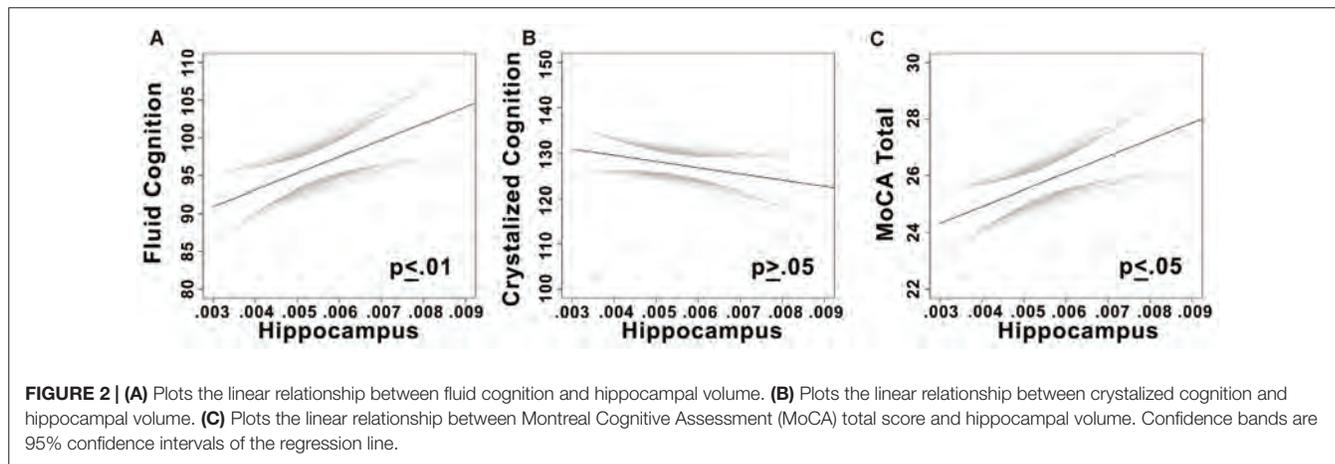
at examining the sub-scales of MoCA and NIH toolbox fluid cognition composite to determine whether a sub-scale was driving the relationship in the total score. Due to the characteristics of MoCA, certain sub-scales did not lend themselves to further analyses. Naming, Language, Abstraction and Orientation sections were excluded due to a restriction of range in observation (i.e., a 1 point scale) and/or a lack of variability. Two subjects were excluded as outliers because they had values greater than 2.5 the standard deviation from the mean (1 hippocampal volume outlier; 1 crystallized cognition outlier).

RESULTS

NIH Toolbox

Relationship Between NIH Toolbox Fluid Cognition and Neuroimaging Measures

There was a significant positive linear relationship between hippocampal volume and fluid cognition composite score ($t = 3.3$, $p = 0.001$, partial $\eta^2 = 0.11$; see **Figure 2**) while controlling for sex and years of education (full model ($F_{(3,89)} = 3.81$, $p = 0.013$, $r^2 = 0.11$).



Relationship Between NIH Toolbox Crystallized Cognition and Neuroimaging Measures

As expected, no relationship was observed between hippocampal volume and crystallized cognition while controlling for covariates ($t = -0.21, p = 0.84$). Univariate models were nonsignificant as well; neither composite nor individual sub-scales of crystallized cognition were significantly related to hippocampal volume (p 's > 0.05).

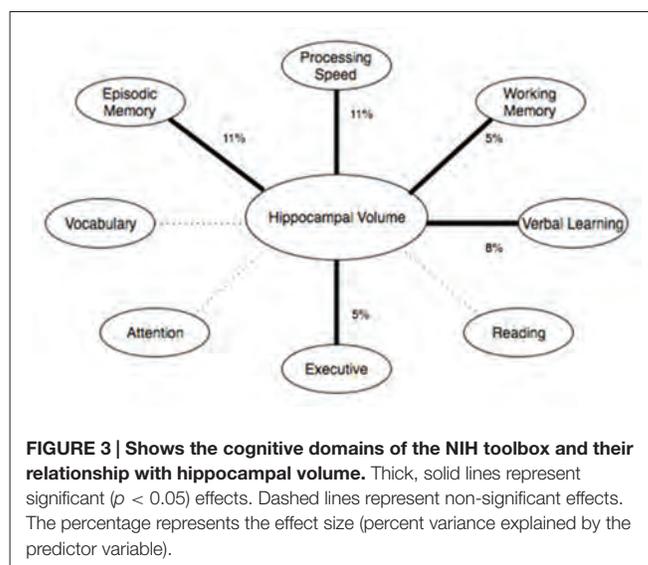
Relationship Between NIH Toolbox Sub-Scales and Neuroimaging Measures

Five linear models were analyzed using each sub-scale of the NIH toolbox fluid cognition composite while controlling for sex and years of education. Dimensional Change Card Sorting ($p = 0.027$, partial $\eta^2 = 0.05$, observed power = 0.60), Picture sequence memory ($p = 0.001$, partial $\eta^2 = 0.11$, observed power = 0.90), List Sorting ($p = 0.04$, partial $\eta^2 = 0.05$, observed power = 0.54) and Pattern comparison ($p = 0.002$, partial $\eta^2 = 0.11$, observed power = 0.89) were predicted by hippocampal volume. The attention domain flanker task was not significantly ($p > 0.25$) related to hippocampal volume. The strongest predictor of hippocampal volume was the pattern comparison task, which is in the processing speed domain. The episodic memory (picture sequence memory task) domain and the working memory domain (list sorting task) were both significantly related to hippocampal volume. Two supplemental tasks, the symbol digit search and Rey verbal learning were also analyzed (these tasks do not factor into the fluid cognition composite score, but were including in our NIH-Toolbox cognitive module). Rey verbal learning ($p = 0.008$, partial $\eta^2 = 0.08$, observed power = 0.77) and symbol digit search ($p = 0.073$, partial $\eta^2 = 0.04$, observed power = 0.44) scores showed a positive relationship with hippocampal volume. See **Figure 3** for a summary.

MoCA

Relationship Between MoCA and Neuroimaging Measures

There was a significant positive linear relationship between hippocampal volume and total MoCA score while controlling for sex and years of education ($t = 2.36, p = 0.02$, partial $\eta^2 = 0.06$).



Relationship Between MoCA Subscales and Neuroimaging Measures

Three subscales of the MoCA were analyzed individually: delayed recall, attention and visual-spatial/executive. As hypothesized, delayed recall was associated with hippocampal volume ($t = 1.96, p = 0.052$). No associations were found between attention ($t = 0.75, p = 0.454$) or visual-spatial/executive ($t = 0.88, p = 0.382$).

DISCUSSION

Hippocampal volume predicted cognitive performance on both the MoCA and NIH toolbox fluid cognition composite score in a community sample of 93 older adults without clinical history of MCI, neurodegenerative disease, neurological injury or self-reported memory problems. This finding supports the study hypothesis that smaller hippocampal volume is associated with poorer cognitive performance in older adults, particularly with respect to memory-related functions. A relationship in hippocampal volume was found only for fluid abilities, and not crystallized abilities such as vocabulary or reading. This

disassociation has been described in patient studies such as HM, where bilateral hippocampal resection caused profound memory disturbances while general knowledge remained intact (Scoville and Milner, 1957).

Prior literature demonstrates a strong relationship between the hippocampus and learning, memory and other fluid cognitive functions in both animals and humans (Raz et al., 1998; Petersen et al., 2000), with smaller volumes associated with poorer performance (Persson et al., 2006). However, these results have not been universal in older adults (Van Petten, 2004). Our data not only demonstrate a strong relationship between hippocampal volume and episodic memory, but also relationships with executive function, working memory and speed of processing.

Cognitive Subdomains and Hippocampal Volume

Within the MoCA and NIHTB, subtests that targeted the memory domain were significantly related to hippocampal volume. This replicates previous research showing a positive relationship between hippocampal atrophy and memory measures in non-demented subjects (Golomb et al., 1996; Persson et al., 2006). However, as mentioned, not all studies have replicated this finding. A meta-analysis by Van Petten (Van Petten, 2004) suggested that overall evidence in the literature for a positive relationship between hippocampal size and episodic memory in older adults was “surprisingly weak.” In addition, a prior study investigating the relationship between hippocampal volume and MoCA failed to find such a relationship (Paul et al., 2011). While the current study and Paul et al. (2011) were similar in statistical power, imaging methods, and study inclusion/exclusion criteria, our sample was approximately 10 years older. As the relationship between hippocampal volume and memory is non-linear with age, this difference in our cohort's average age may account for the difference in our findings (Chen et al., 2016).

Regardless, this study is the first to report a significant positive relationship between hippocampal volume, MoCA and NIHTB-CB memory measures. However, caution in the interpretation of these findings is warranted. “Bigger is better” is certainly an oversimplification; smaller hippocampi have been associated with better memory in children and adolescents (Sowell et al., 2001). Furthermore, in pathological conditions, such as Fragile X syndrome, enlarged hippocampi are associated with poorer memory performance (Molnár and Kéri, 2014). The biological change associated with increased or decreased brain volume could be the result of multiple processes, which we are unable to elucidate with T1 structural MRI techniques. For example, increased volume could be the result of increased neuronal cell bodies, increases in glia or astrocytes, neuroinflammation or insufficient neuronal pruning. Nonetheless, our results demonstrate that smaller hippocampal volume is associated with decreased performance on two well validated and commonly administered measures of cognitive function in older adults, with particular sensitivity to memory function across both tasks.

Hippocampal volume was associated with performance in other cognitive domains besides memory on the NIHTB Cognitive Battery, specifically speed of processing, working memory and executive function (see **Figure 3**). In fact, the association between hippocampal volume and processing speed on the NIHTB was slightly stronger than for episodic memory. As delayed recall is not assessed by the NIH-Toolbox, it is possible that the relationship with delayed recall observed on the MoCA was not detectable from the NIH-Toolbox Cognitive Battery. Regardless, our results highlight the multifaceted role of the hippocampus in cognitive aging. The hippocampus contributes to other cognitive functions besides memory, and optimal learning and memory depends on other cognitive functions, such as working memory, processing speed and executive functioning, in addition to encoding and storage. A relationship between speed of processing and hippocampal volume has been shown in some (Tisserand et al., 2000), but not all past studies (Colom et al., 2013). An association between hippocampal volume and executive functioning was only evident on the NIH toolbox (dimension change card sorting), not for the MoCA executive-visual spatial sub-scale. Dimensional Change Card Sorting has greater cognitive demand and requires higher-order executive processes compared to the MoCA executive tasks. For example, the Dimensional Change Card Sorting task would require effort from multiple CHC factors, such as fluid reasoning, short-term memory, visual processing and reaction speed. Even though the executive tasks in NIH Toolbox and MoCA are classified as part of the same domain, performance on these tasks was not correlated ($r = 0.07$, $p > 0.05$). This supports the conclusion that these tests measure different elements of executive functioning. While the relationship between fluid cognition and hippocampal volume may seem surprising due to the traditional association of fluid abilities (particularly executive function and processing speed) and the pre-frontal cortex, previous studies have implicated hippocampal volume as a predictor of fluid ability in older adults (while no such association was found in younger adults; Reuben et al., 2011). A potential mechanism of the hippocampal association with fluid ability in older adults may relate to compensatory processes in the hippocampus as a result of the pre-frontal atrophy observed with age. Further research, particularly longitudinal studies, are needed to clarify whether the relationship between hippocampal volume and fluid ability changes throughout the lifespan, and which potential mechanisms may account for such change.

CONCLUSION

Prior research has produced controversy over the role of the hippocampus in cognitive aging, casting doubt on its role in episodic memory, as well as other domains (e.g., speed of processing, Van Petten, 2004; Colom et al., 2013). Our findings demonstrate that the hippocampus is a critical structure in cognitive aging, playing a role not only in episodic memory, but also processing speed, working memory and executive function. Whether effects of the hippocampus on domains outside of episodic memory are direct or mediational in nature remains to be seen. Our findings also demonstrate that performance on

a commonly used bedside dementia screening (MoCA) and a comprehensive cognitive battery (NIH Toolbox) are significantly related to hippocampal volume. Whereas, a prior study failed to find a relationship between MoCA and hippocampal volume in an older adult population (Paul et al., 2011), we found that our cohort, approximately 10 years senior in average age, evidenced a significant relationship. These data suggest a foundation for longitudinal research investigating hippocampal volume in older adults as a possible predictor of future decline or MCI conversion. Such data would help to elucidate issues of acute vs. progressive atrophy in the study, and further clarify potential implications for pathologies like MCI and AD. More importantly, our data provide strong evidence in support of the multifaceted role of the hippocampus in cognitive aging.

AUTHOR CONTRIBUTIONS

AO, RAC, ECP, NRN and AJW contributed text to the manuscript. AO and AJW performed data analysis. All

authors provided edits and approved the final version of the manuscript.

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Evelyn F. and William L. McKnight Brain Institute of the University of Florida
P.O. Box 100015 • Gainesville, FL 32610-0015
352.273.8500