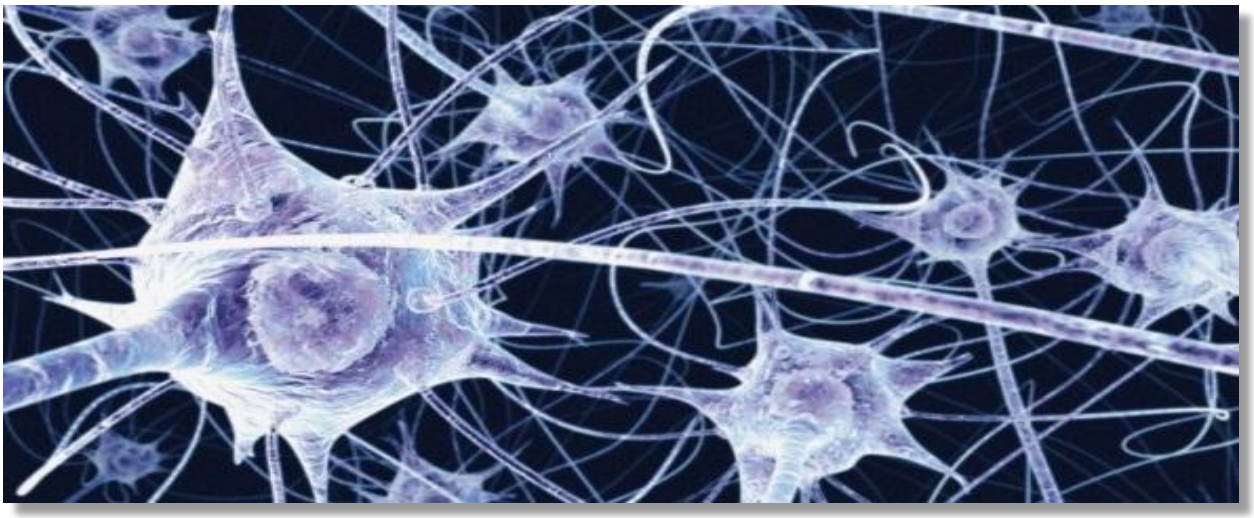
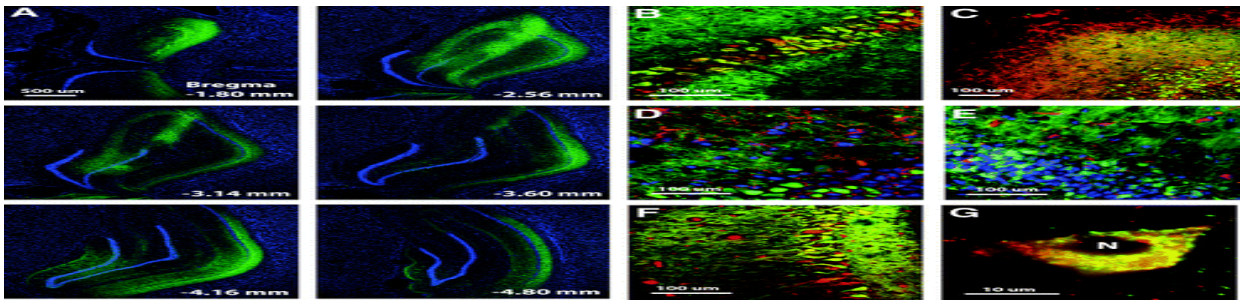


2012 Annual Report

Age Related Memory Loss (ARML) Program & Cognitive Aging and Memory Clinical Translational Research Program (CAM-CTRP)



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

UF | UNIVERSITY of FLORIDA
The Foundation for The Gator Nation

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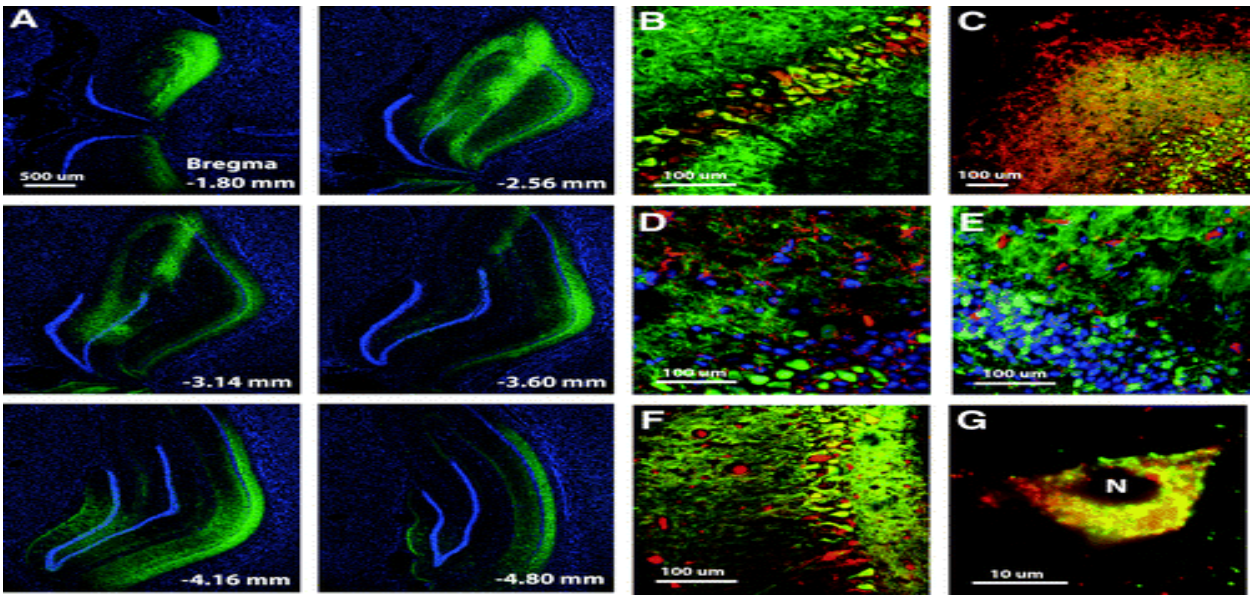

UF | UNIVERSITY of FLORIDA

Office of the Senior Vice President, Health Affairs
President, UF&Shands Health System

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Gainesville, FL 32610-0014
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352-733-1701 Fax

January 9, 2013

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

Dear Trustees:

I would like to express my sincere gratitude to the McKnight Brain Research Foundation (MBRF) for its ongoing generous support of the University of Florida McKnight Brain Institute (MBI). The enclosed report provides information on the latest research developments at the MBI.

Under the skillful leadership of Dr. Tetsuo Ashizawa, Executive Director of the MBI, 2012 has been a year of transition. An increasing number of collaborations between the Age-Related Memory Loss Program (ARML) and the Cognitive Aging and Memory Clinical Translational Research Program (CAM-CTRP) at the Institute of Aging, has laid the groundwork for the implementation of effective translational research. Dr. Ronald Cohen, recruited in 2012 as Director of the CAM-CTRP, and Dr. Thomas Foster, McKnight Chair for Research on Cognitive Aging and Memory and Chair for the ARML Program oversight committee, are working to build close collaborations for integrated translational efforts. Their combined leadership is generating great momentum towards our pursuit of understanding and treating age-related memory loss. In 2012, Dr. Foster was honored with a prestigious MERIT award by the NIA National Advisory Council on Aging for his research achievements in the field.

The ongoing generosity of the MBRF continues to provide critical resources that make a vital difference in our efforts to understand the mechanism of age-related memory loss and to aid in its prevention. We look forward to continuing our partnership with the MBRF and thank you again for your support.

Sincerely,

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

UF UNIVERSITY of FLORIDA

Dean, College of Medicine
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January 10, 2013

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

Dear Trustees:

On behalf of the UF College of Medicine, please accept my sincere appreciation for the ongoing support and partnership of the McKnight Brain Research Foundation. The past year has been extremely productive for both the Age-Related Memory Loss Program (ARML) and the Cognitive Aging and Memory Clinical Translational Research Program (CAM-CTRP). Our faculty have made significant progress on several key studies, leading enhanced collaboration across the University and successful submission of several manuscripts to scientific journals.

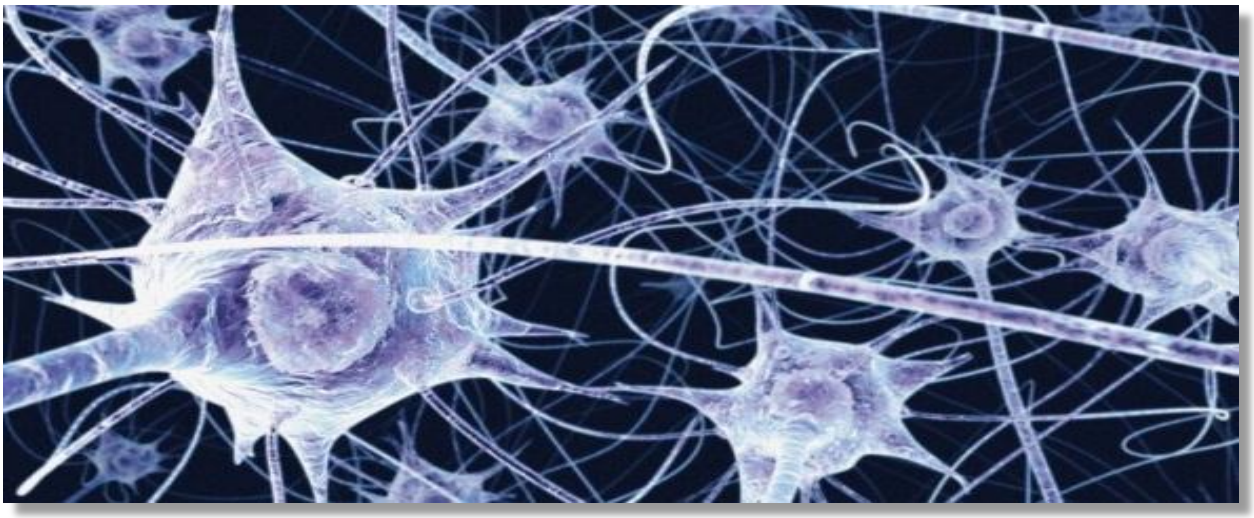
The support of the MBRF fueled studies by more than a dozen leading researchers in 2012, and we continue to aggressively recruit emerging leaders in neuro-medicine to join this impressive team. The research resources provided by the MBRF are critical to ensuring that the University of Florida continues on its journey to becoming a national leader in brain research.

I hope you enjoy reviewing the enclosed report and learning more about this year's work and results. Thank you again for all that you make possible in neuro-medicine through your support. We look forward to another year of strong collaboration and discovery.

Sincerely,

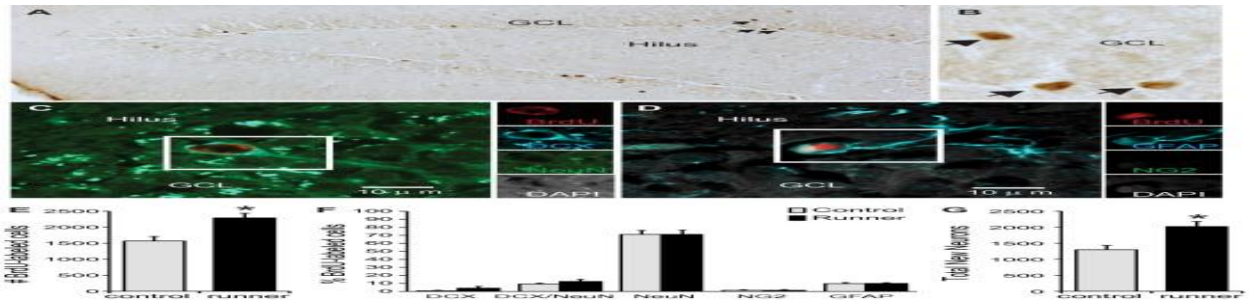
M L Good, MD

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



2012 Annual Report

Age Related Memory Loss (ARML) Program



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

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The Foundation for The Gator Nation

UF UNIVERSITY of FLORIDA
Evelyn F. & William L.
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January 2, 2013

McKnight Brain Research Foundation
Foundations & Endowments Specialty Practice
Sun Trust Banks, Inc.
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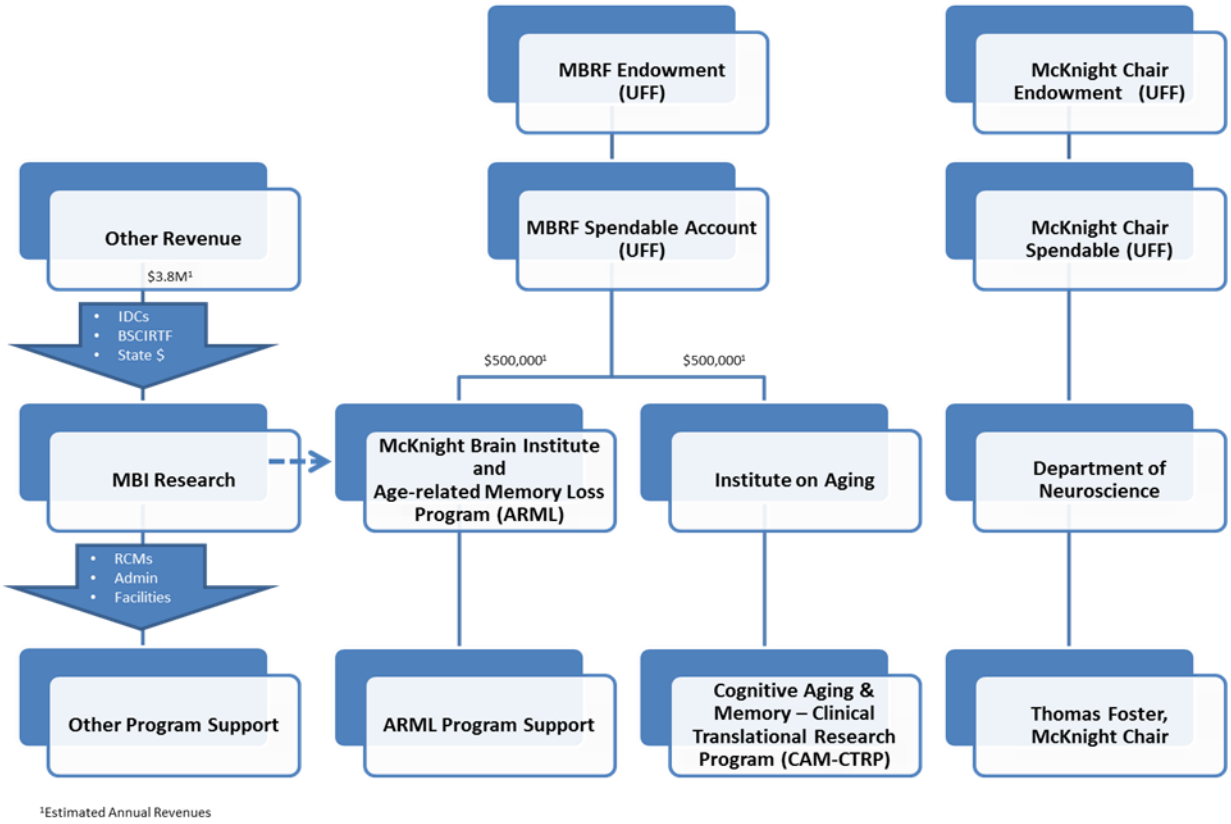
300 South Orange Avenue, Suite 1600
Orlando, FL 32801

Dear McKnight Brain Research Foundation Trustees:

In 1997, at the inception of the McKnight Brain Institute (MBI) at the University of Florida, the McKnight Brain Research Foundation (MBRF) provided a generous gift of $15 million to the University of Florida (UF) to investigate age-related cognitive loss. The gift was matched by the UF to establish a $30-million-dollar endowment at the McKnight Brain Institute (MBI). Since then this endowed fund has provided approximately $1 million dollars per year of spendable funds for research on cognitive aging. The MBRF donated an additional $2 million endowment to the UF College of Medicine, with a $2 million state match, to establish the McKnight Chair for Research on Cognitive Aging and Memory (Dr. Thomas Foster, Chair holder) in the Department of Neuroscience in 2000. During the first decade of the MBI history, spendable funds generated by the MBRF fund were primarily used to support basic science research to investigate mechanisms of age-related memory loss (ARML) in cellular and animal models. These studies significantly contributed to the current knowledge of effects of aging on memory and learning. The NIA National Advisory Council on Aging recognized Dr. Foster's achievements and awarded him a prestigious MERIT award in 2012. Furthermore, the MBRF endowed the UF with $300,000 to establish a permanent annual lectureship in memory of the late William G. "Bill" Luttge, Ph.D., the founding director of the MBI. Meanwhile, an increasing recognition of the feasibility of translational research in the field led to the decision to split the fund to support two programs at UF in 2009. A half of the fund (approximately $500,000 per year) was allocated to support the newly established Cognitive Aging and Memory Clinical and Translational Program (CAM-CTRP) at the Institute on Aging (IOA, Dr. Marco Pahor, Director) while the other half remained to support the ARML Program at the MBI (Figure).

McKnight Brain Research Foundation Grant Funding Flow



The UF IOA and MBI are grateful for these funds generously provided by the MBRF. The MBI is committed to support the ARML research. Using non-MBRF funds, the MBI provides an outstanding research environment to the ARML and CAM-CTRP researchers. Key core facilities are available at the Cell and Tissue Analysis Core, the Flow Cytometry Core and the Animal Care Services Core, including Rodent Behavioral Research Facilities. In collaboration with the UF Clinical and Translational Science Institute (CTSI), the MBI has recruited Dr. Song Lai as Director of the new Human Imaging Core, which allows for a turnkey operation of 3T human research protocols. This core will be essential for CAM-CTRP projects.

The major advance in the MBRF-supported research at the UF in the past year was the recruitment of Dr. Ronald Cohen as Director of the CAM-CTRP at the Institute on Aging. Since his arrival at UF in July 2012, he has been busy developing the interdisciplinary team for the CAM-CTRP across many Departments, Colleges, Centers and Institutes within UF. He closely collaborates with Dr. Foster to have seamless translational efforts from bench to bedside and from bedside to bench. To further ensure researchers in the CAM-CTRP and the ARML Program to be on the same page, Dr. Cohen has been added to the MBI Executive Committee, which I chair to oversee the entire MBI operation. Likewise, Dr. Foster and I have been members of the IOA Executive Committee. Together, we have successfully established a highly collaborative research environment at the UF.

The collaborative research environment also extends extramurally. The MBRF has organized inter-institutional meetings involving the UF MBI and three other newer McKnight institutes at the University of Alabama Birmingham (UAB), University of Arizona and University of Miami. Under the strong leadership of Dr. Lee Dockery and MBRF Trustees, these four institutions are working together as inter-

institutional working teams focusing on specific strategic areas, including clinical, imaging and molecular/epigenetic studies. These interactions have created enthusiasm among participating researchers, and cooperative studies are gaining a strong momentum. Investigators at the UF MBI and IOA are working together to participate and lead in these study groups. Dr. Cohen has been actively involved in clinical and imaging studies. He also established a collaborative arrangement with Dr. Ranjan Duara at Mount Sinai Medical Center, Miami to acquire well-studied cohort of elderly subjects for the clinical studies. The LIFE study at the IOA has become an integral part of the CAM-CTRP. Dr. Cohen has also initiated recruitment of new CAM-CTRP faculty. Meanwhile, Dr. Foster has also been working on his own award-winning research on ARML, which included collaborations with many UF investigators. He also took a leadership role in the molecular/epigenetic inter-institutional project on cognitive aging. With Dr. David Sweat (UAB) and Ds. Leonid Moroz (UF), he has decided to purchase the Ion Torrent new generation sequencer. The MBI expended a state fund to cover a significant part of the cost of this instrument. The instrument is capable of investigating epigenetic signatures of aging within a single neuron from a human brain, and is expected to provide important translational data. Dr. Foster and his ARML Program Oversight Committee have decided to recruit an additional ARML faculty, who can bridge the gap between research on animal/cellular models and clinical human research on cognitive aging. Dr. Foster, along with Dr. Jennifer Bizon, is also organizing UF researchers who have received seed funds from the MBRF fund to work together toward establishment of program projects on ARML. The list of these researchers includes federally funded investigators, such as Drs. Dawn Bowers, Vonetta Dotson, Leonid Moroz, Charles Frazier, Jake Streit, Brandi Ormerod, Michael Marsiske, and Todd Manini.

In summary, 2012 has been a year of a transition toward intramural and extramural collaborative research. The arrival of Dr. Cohen has already produced a visible impact on the UF effort on cognitive aging research. Increasing collaborations between the IOA CAM-CTRP and the MBI ARML Program has laid a foundation for the initiation of effective translational research. Working together with other McKnight institutions has made exciting progresses. I look forward to working toward the success of these collaborative research programs to decipher mechanisms of age-related cognitive loss and to discover efficacious interventions.

Best regards,

Tetsuo Ashizawa, M.D.
Executive Director, McKnight Brain Institute
Melvin Greer Professor of Neurology
Chair, Department of Neurology

Age Related Memory Loss - Annual Institute Report

McKnight Brain Research Foundation
Sponsored Institutes and Research Programs
Report Period: 2012

Summary of scientific achievements since last report:

OVERVIEW OF THE ORGANIZATION OF UNIVERSITY OF FLORIDA PROGRAMS SPONSORED BY THE MCKINGHT BRAIN RESEARCH FOUNDATION

Two interacting programs at the University of Florida; the Age-Related Memory Loss Program (ARML) and the Cognitive Aging and Memory-Clinical Trials Research Program (CAM-CTRP) are sponsored be the McKnight Brain Research Foundation. The ARML Program is formally aligned under the McKnight Brain Institute (MBI) at the University of Florida (UF). Dr. Thomas Foster serves as The Evelyn F. McKnight Chair for Research on Cognitive Aging and Memory also serves as the Chair for the ARML Program oversight committee which includes the Chair of the Department of Neuroscience, Dr. Lucia Notterpek, The Chair of Neurology and Director of the McKnight Brain Institute, Dr. Tetsuo Ashizawa, and the Director of the Biochemistry of Aging Laboratory at the Institute of Aging, Dr. Christiaan Leeuwenburg. Housing of the ARML Program under MBI provides a pre-existing organizational structure for administration of the grant, and for promoting scientific interactions with the other research centers and institutes at UF with allied research interests on age-related memory loss. The ARML Program interacts with the CAM-CTRP, which is formally aligned with the Institute of Aging and directed by Dr Ronald Cohen, who in turns reports Director of the Institute on Aging, Dr. Marco Pahor. This alignment of ARML and CAM-CTRP provides direct access to the highest levels of the University administration, including the Director of the McKnight Brain Institute Dr. Tetsuo Ashizawa, Director of the Institute on Aging, Dr. Marco Pahor, the Dean of the College of Medicine Dr. Good and the Vice President of the Health Science Center Dr. Guzick, who in turn reports to the President of the University.

The ARML Program and CAM-CTRP are providing leadership in generating interest, support, and a commitment to scientific research in the understanding and alleviation of age-related memory loss. The Chair of the ARML Program Committee (Foster) and the CAM-CTRP Director (Cohen) regularly meet with the Director of the Biochemistry of Aging Laboratory (Leeuwenburgh), the Director of the McKnight Brain Institute (Ashizawa), and the Director of the Institute on Aging (Pahor) for discussions of the direction of aging research at the University of Florida. Interactive projects concerning behavioral, anti-inflammation, and hormone treatments or biomarkers including brain imaging, bio-plex arrays, and genomics are currently being discussed. Finally, the ARML program is interacting with the CAM-CTRP and the Pepper Center to nurture new researchers through funds for pilot projects (Dotson) and mentoring (Ebner).

Jennifer Bizon, Ph.D.

In the past year, my laboratory has maintained a high degree of productivity associated with our research program related to understanding the neural and cognitive mechanisms of cognitive dysfunction in aging. In total, we have contributed 12 new publications in 2012, including 7 directly related to the topic of age-related cognitive decline. Among these publications are two large studies funded by our NIA R01 on the topic of basal forebrain, GABAergic signaling and cognitive aging, both of which are published in *Neurobiology of Aging*. One manuscript (McQuail et al., 2012) describes regionally specific changes in GABA(B) receptor expression and signaling in behaviorally characterized

aged rats. A second manuscript (Banuelos et al., 2012) provides the most comprehensive study to date regarding age-related alterations in basal forebrain of aged rats and the relationship of such changes to memory dysfunction. A third manuscript, published in a special issue of Frontiers in Neuroscience represents the first investigation to date of risk-based decision making in aged rodents and identifies unique cognitive and affective mechanisms that contribute to individual choice behavior of aged rats under conditions in which there is a risk of adverse consequences.

Four other manuscripts were published in conjunction with my participation in the McKnight-sponsored Cognitive Test Battery working group and appeared in a recent special issue of Frontiers in Aging. This working group met three times and was focused on identifying core cognitive assays that are optimal for assessment of the cognitive deficits associated with normal aging and that translate well across species. In particular, I, in collaboration with my McKnight colleagues, Tom Foster, Betty Glisky and Gene Alexander, authored a manuscript focused on executive functions which are supported by prefrontal cortex and represent critical control and planning mechanisms that mediate and guide goal-directed behavior. While executive dysfunction is among the earliest and most severely impaired cognitive abilities in normal aging, animal models of executive dysfunction are less well established than those available for hippocampal-supported memory. Consequently, relatively little is known regarding the neural mechanisms that mediate executive impairments. Relevant to this issue, my laboratory will submit one additional experimental paper on the topic of aging and executive function to Neurobiology of Aging later this month. In this paper, we describe the novel finding that normal aging can produce diverging manifestations of executive dysfunction. This study was designed to build upon an emerging literature from human cognitive neuroscience which indicates that two primary executive functions, working memory and cognitive flexibility, represent functionally opposing processes. These processes are normally seamlessly integrated but can become dysregulated, disrupting an individual's ability to carry out complex cognitive operations and behaviors. Based on this literature, we have used the F344 rat model to determine that cognitive flexibility and working memory abilities are *inversely* related among aged rats, with some rats showing impaired working memory and others showing impaired cognitive flexibility. These results are significant both with respect to understanding the underlying mechanisms that contribute to age-related executive dysfunction and to the development of tailored intervention strategies targeting distinct forms of decline. Indeed, optimizing executive function in aged individuals should positively impact behavioral strategies, decision making, and maintenance of independence at advanced ages.

In the past year, we have submitted three new grant applications related to age-related cognitive decline. One proposal (R21) is focused on identifying alterations in prefrontal-striatal circuitry that contribute to a loss of cognitive control and impaired executive processes in aging. The second proposal is a NSF application which is focused on investigating the cognitive and neural mechanisms that contribute to maladaptive decision making in normal aging. While these two proposals were ultimately not funded, we have received significant positive feedback on both, and have been encouraged by program officials at NIH and NSF to resubmit in upcoming funding cycles. The third proposal is the competitive renewal of our R01 which is focused on GABAergic signaling alterations in prefrontal cortex and their role in different forms of age-related executive dysfunction. This proposal is currently under review at NIH.

Our work has been presented in numerous local, national and international venues in the past year, including the Federation of European Neuroscience meeting and the Society for Neuroscience meeting. I have also presented my work related to cognitive aging in several venues locally and in meetings supported by the McKnight Brain Research Foundation (including the annual meeting in Tucson, AZ and the site visit at UF). Recently, I was invited to serve on a scientific panel focused on basal forebrain function and cognition at the International Behavioral Neuroscience Society meeting in Dublin, Ireland

which will be held this upcoming June. I have also participated in several grant panels in the past year, including the CDIN study section for NIH in October and for Alzheimer's Drug Discovery Foundation in December. I continue to serve on the editorial board for *Neurobiology of Aging*.

Dawn Bowers, Ph.D. (& Michael Marsiske, Ph.D.)

Over the past year, we have continued the VITAL pilot clinical trial with older community-residing adults through funding provided by the Age-Related Memory Loss program of the McKnight Brain Institute. The innovative aspects of this project include: a) examining the effects of exercise pre-dosing on the cognitive training outcomes of older adults; b) enrollment of a much older cohort of elders (mean age = 82) that is typical of most studies, and c) developing a community based partnership with The Village and its owner, Santa Fe Healthcare corporation. The pre-dosing question is innovative because many studies have shown benefits of physical and cognitive training on mental outcomes for older adults in isolation, but few human studies have asked whether improved aerobic fitness might actually boost cognitive plasticity. The very old nature of our sample means that we have a higher proportion of adults in the "high decline" phase of lifespan, including a sample much more likely to experience negative cognitive changes within a very narrow time window. In February 2012 we presented results from an interim analysis of ½ of our sample at the annual McKnight Trustees meeting in Gainesville. Our preliminary findings were highly positive, namely improved performance on delayed memory tasks that were specific to one of the intervention arms. Since that time, we have continued to recruit, "intervene" and collect data and have recently completed recruitment for the last cohort of participants, resulting in a total N of 60 (our target goal). Based on the positive preliminary findings, we are now obtaining pre- and post-intervention brain scans (functional MRI, resting state, DTI) on a very small subset of participants in the final cohort. The scanning effort has been supported by resources from the McKnight Foundation (via Dr. Ron Cohen) and by colleagues in Biomedical Engineering (Mingzhou Ding, Ph.D.). Future plans include two NIH grant submissions during early 2013, one focusing on mild cognitive impairment and the other a full clinical trial. The funding provided by the ARML for this project has been important in terms of providing essential data for establishment of effect sizes for these applications and has facilitated an extensive set of community-university partnerships that well serves future ARML studies.

Vonetta Dotson, Ph.D.

Title: "*Effect of Exercise on Memory in Geriatric Depression: An fMRI Pilot Study*"

The relationship between depression and age-related memory deficits is well established, and there is increasing evidence to suggest that the underlying neurobiological changes in depression may be the basis of this relationship. Consequently, interventions that reverse or minimize depression-related changes in the brain may have the potential to simultaneously treat memory deficits secondary to depression. The overall aims of this proposal are 1) to examine whether aerobic exercise (AE) improves memory functioning and alters memory-related brain functioning in depressed older adults, and 2) gather preliminary data for an R01 application examining potential mechanisms for the antidepressant and cognitive-enhancing effect of AE in older adults.

Funding for her award began in March 2011. The longitudinal nature of the study and initial difficulty in recruitment and retention of subjects have extended the timeline for completing the study, addressing the aims of the original proposal, and submitting an R01. Dr. Dotson had anticipated completing post-treatment scans in the spring of this year, but difficulty in retaining subjects has delayed that goal. Data collection is ongoing, and she anticipates that all participants will have completed the exercise intervention by February 2013, with follow-up fMRI scans thereafter. In the past year, the project has become a UF Claude D. Pepper Center-supported study. Although the Pepper Center is not currently providing additional funds, support has included assistance with recruitment and access to the indoor track at the Health Promotions Center, which have been critical to the study's progress.

In the interim, the funds from this award have been essential to Dr. Dotson maintaining her laboratory and gathering data for scientific presentations and publications that are directly related to the mission of the MBRF. Data from the baseline fMRI scans have resulted in presentations at the Society for Neuroscience on sex differences in brain activity during episodic and working memory in older adults, age differences in encoding-related brain activity, and the impact of depressive symptom dimensions on memory-related brain activity. These subprojects are leading to manuscript submissions as well, with one manuscript under review and one in preparation thus far. Highlights of current findings include the following:

- Despite similar error rates and response times in older men and women, sex differences were observed in patterns of fMRI activity during the N-Back working memory task, characterized primarily by greater activity in men in numerous task-relevant brain regions including the cingulate gyrus, dorsolateral prefrontal cortex, and temporal regions. Women recalled more words than men on a list learning task, but there were no significant sex differences in brain activation during memory encoding. Findings provide further support of sex differences in cognitive and brain functioning in older adults. Increased functional activity in men compared to women in the context of similar working memory task performance suggests that men may have required additional neural resources to perform at the same level as women. These results emphasize the importance of considering sex in studies of cognitive and brain aging.
- fMRI data revealed a number of regions activated during memory encoding, including hippocampal and temporal regions. Word-encoding activity within the left hipppocampus negatively correlated with participants' age in an older sample (age 65-81). The greatest encoding-related signal change from baseline was seen in young-old adults. These results are consistent with previous studies suggesting that encoding-related brain activity in hippocampal and other temporal areas differs as a function of age. Moreover, findings suggest that this age effect can also be observed within an elderly sample, as middle-old participants showed the greatest reduction in hippocampal activity. Results have implications for memory decline and risk for Alzheimer's disease in late life.
- Increasing working memory load was associated with significant activation of the left dorsal-lateral prefrontal cortex (dlPFC) and right anterior cingulate cortex (ACC) in older adults. dlPFC activation significantly correlated with scores on the depressed mood subscale of the Center for Epidemiologic Studies Depression Scale, but not with the somatic symptoms, lack of well-being, or interpersonal problems subscales. Results suggest the presence of altered neural functioning in older adults with subthreshold symptoms of depression, and a possible clinical correlate between working memory load and depressed mood in older adults.

Funding has been instrumental in Dr. Dotson developing additional grant funding related to exercise interventions and late-life depression. Work on this project initiated her collaboration with other researchers at UF who are conducting exercise intervention studies, including Dr. Marco Pahor, PI of the Lifestyle Interventions and Independence for Elders (LIFE) Study. These collaborations, combined with her readings on exercise and depression for this award, led to the development of a diversity supplement to the LIFE study, which began in February 2012, to study the impact of genetic markers on the antidepressant effect of exercise in older adults**.** Data from that project, combined with the data from the MBRF award, will be used for an R01 submission in June 2013 focusing on an imaging genetics study of the impact of exercise on memory and depression in older adults. In addition, preliminary results from the current project, which demonstrated distinct brain correlates associated with different symptoms dimensions of depression in older adults, were used as preliminary data for an R21 application focused on components of depression in Parkinson's disease, which was submitted in October 2012.

Charles Jason Frazier, Ph.D.

At the time of our last report we had made significant progress in refining techniques to allow for whole cell patch clamp recording in hippocampal slices taken from aged animals. Our success in this area helps mitigate a long standing technical problem that has limited the ability to perform quantitative mechanistic studies on individual cells and synapses in aged preparations. Further, at the time of the last progress report, we demonstrated initial success in leveraging two-photon based laser scanning epifluorescence microscopy to measure calcium influx through NMDA receptors expressed on a single dendritic spine of an aged neuron. This work represented development of a sophisticated technical ability that to date has been employed by very few other labs interested in revealing specific mechanistic deficits in synaptic transmission that occur with aging. In the time since that progress report (over the last 8 months) we have begun to leverage these new tools to test our core hypothesis that age related increases in activation of calcium activated potassium channels ultimately impairs synaptic plasticity by reducing NMDA receptor currents at individual spines. Our preliminary data has to date been consistent with this hypothesis, and was used in support of an R01 grant application submitted in collaboration with Dr. Tom Foster (Foster PI, Frazier, Co-I) in the Summer of 2012. The application was scored but not funded and we anticipate resubmission in Spring of 2013. During this time period we have also begun to use non-optical (but single cell) techniques in both young and aged slices to further asses changes in release probability of glutamate from presynaptic axon terminals, postsynaptic response to vesicular glutamate release, and effect of SK channels on each of these processes. These data have also served to further support and develop our initial hypothesis and we are expecting to use this in combination with the imaging data to support an application of the American Federation of Aging Research in December, 2012. Finally, in terms of additional scientific accomplishments since the last progress report it should be noted that we have begun a new local collaboration with Dr. Jennifer Bizon. This project is outside the scope of our original ARML award, but still very much in keeping with the ARML mission. Specifically, we are working with Dr. Bizon on a new project designed to provide a better mechanistic explanation for their observation that age-related decline in performance on prefrontal cortex dependent working memory tasks can be largely reversed by administration of a GABAB receptor antagonist. Our initial mechanistic hypothesis at this point is that impaired performance on PFC dependent tasks is associated with an age-related increase in ambient GABA. Our initial experiments have indicated there is minimal if any tonic GABAergic tone in PFC of very young animals, and current work is seeking to specifically expose a hypothesized increase in inhibitory tone in older animals. If we are successful in this regard, we expect the data to be able to support an additional extramural grant application from Dr. Bizon and myself during the first half of 2013 that develops a more detailed mechanistic model of how aging impairs cellular and synaptic function in the prefrontal cortex.

Leonid Moroz, Ph.D.

Title: *Epigenomic Bases of Age-related Memory Loss: Single-Neuron Approach*

All kinds of *long-term* associative and non-associative memories require neuron-specific changes in transcription and translation. Yet, how our memories are formed, and more importantly, how they are stored for an entire human lifetime (i.e. far beyond a few days or few weeks – the maximal half-life of proteins in our body) is one of the greatest enigmas in modern science. How memories are lost as a result of disease, or normal aging, is an opposing set of questions to conceptually the same fundamental problem. Recent evidence indicates that formation and maintenance of *long-term* memories are in fact epigenetic processes. These processes, which do not change the order of DNA sequences, directly modify access of >1500 transcription factors and other regulators to the core regions in the nuclear genome to activate, suppress, or even change the dynamics of transcription. It is recognized that at least several thousand genes dynamically and persistently change their expression in virtually any neuron in memory-forming circuits to maintain long-term plasticity. However, the scope of these processes at the level of any specific neuronal circuit is completely unknown.

Dr. Moroz proposes to investigate two specific aims: 1) to obtain integrated single-neuron transcriptome/methylome genome-wide profiling in age-sensitive and age resistant neurons, and 2) to elucidate the dynamics of targets of DNA methylation in individual neurons as a function of aging. As the critical step to investigating these specific aims, Dr. Moroz has developed three novel methodologies that allow his lab to:

> **(i)** perform direct RNA-seq (transcriptome) profiling from single identified neurons,
> **(ii)** characterize even a synaptic transcriptome with picogram amounts of starting material, and
> **(iii)** capture nascent RNAs following natural learning and memory processes both in invertebrate and mammalian brains.

All of these approaches have been outlined in three papers that are in press now (Moroz, Kohn, 2013; Kohn et al., 2013; Puthanventill et al., 2013).

The first characterization of a synaptic transcriptome (PNAS, 2013) led to identification of up to 5,000 unique coding and non-coding RNAs (including antisense RNAs responsible for learning and memory). Importantly, Dr. Moroz identified novel targets that directly link neuronal aging to epigenomic cell machinery (such as the 6th base in DNA -5-hydroxymethylcytosine and its synthetic enzyme TET), responsible for memory formation and decline.

Next, he identified elementary components of molecular machinery that couple cellular excitability with localized protein synthesis (*J. Neuroscience*, 2012). They consist of novel slack-type channels and RNA-Binding proteins including FMRP. Surprisingly, the most dramatic coupling between cell excitability and translation occurs in presynaptic cells of the simple memory-forming circuit. Apparently this coupling negatively declines in aging, providing a completely new mechanism of age-related memory loss that will be investigated in 2013.

Consequently, Moroz's lab initiated the transfer of newly developed technologies and protocols to mammalian and human neurons. To meet the enormous challenges of cellular heterogeneity in human and other mammalian brains, Dr. Moroz started to collaborate with Dr. Foster to establish at the McKnight Brain Institute a neurogenomic laboratory, with cost-efficient RNA-seq analysis of single neurons and ultra-small human tissue samples. Specifically, he adapted a novel semiconductor sequencing platform (Ion Torrent) for these purposes and this innovative instrumentation will be installed by the end of December at the MBI. This cost-efficient sequencing system should reduce the price of individual RNA-seq experiments from the human brain down to $50-$100. Finally, the Moroz lab has developed a new software package (to be tested early next year) that will perform automatic assembly, annotation and visualization of neuronal transcriptome datasets within days. Combined, these advantages open unprecedented opportunities for the genomic dissection of complete memory-forming circuits as specific neurons learn, remember and age. Using these tools of genomics integrated with powerful bioinformatics workflow, his goal is to determine whether there is a subset of epigenomic "master regulators" and non-coding RNAs responsible for ARML. Current targets include a set of non-coding RNA and regulators of chromatin remodeling. While he uses a range of species, including non-vertebrates, the novel concept should be applicable to humans due to remarkable conservation of chromatin remodeling machinery, and his intent is to extensively validate the developed approaches in a clinically relevant environment (neurosurgery) as well as test it for diagnostic purposes.

The investigation of the novel molecular mechanism by this exceptional investigator may revolutionize our understanding of the biology of age related memory loss. This work has evolved into novel collaborations between Drs. Moroz and Foster: they were recipients of a 2012 UF Research Opportunity Seed Fund competition. In addition, preliminary data obtained from McKnight Funding were used in his

applications to NSF, NIH and NASA. As a result, Dr. Moroz has received NSF funding, and his NIH R01 application targeting the most elusive synaptic transcriptomes was also awarded. Finally, his NASA application was successfully reviewed and should be awarded in 2013.

Brandi Ormerod, Ph.D.

Title: *Neurogenesis and Cognition Across Age*

Over the past 12 months, we have published a number of exciting manuscripts related to aging research made possible by McKnight Brain Research Foundation ARML funds that we were awarded. In collaboration with Dr. Tom Foster's laboratory, we linked impaired hippocampal neurogenesis and an impaired ability to learn and remember a hidden water maze platform in aged rats and then showed that we could rejuvenate both hippocampal neurogenesis and memory for the hidden platform location in aged rats by exposing them to an enriched environment (Speisman et al., 2013, ***Neurobiology of Aging***). This finding is important because neurogenesis is now acknowledged to be a robust ongoing physiological process that occurs during young adult life but that diminishes across age, along with changes in extrahippocampal regions that we explored with Dr. Bizon (Bañuelos et al., 2012, ***Neurobiology of Aging***). Understanding how to maintain hippocampal neurogenesis through age may therefore, preserve hippocampal integrity and therefore forms of memory that the hippocampus mediates cognition through life. Given this notion, and the knowledge that daily exercise increases but that neuroinflammation compromises hippocampal neurogenesis in young adult rodents, we tested whether daily exercise could potentiate both neurogenesis and water maze learning and memory in aged rats and explored whether an age-related change in inflammatory and neuroinflammatory signaling (by screening 32 cytokines in each blood and brain sample obtained) may underlie either deficit. We found that daily exercise could both modulate inflammatory and neuroinflammatory signaling in aged rats and potentiate neurogenesis and learning and memory in a coordinated fashion. We developed a novel analysis that revealed relationships between circulating and brain factors that were associated with our measures of neurogenesis and/or learning and memory (Speisman et al., 2012, ***Brain, Behavior and Immunity***). These data contributed to the pursuit of identifying biomarkers of successful and unsuccessful aging that is the basis of a strong ongoing collaborative venture between my laboratory and Dr. Foster's laboratory. In that collaboration, we are exploring the possibility that specific anti-inflammatory strategies that we have identified as protective to neurogenesis and cognition during an inflammatory challenge in young rodents (Ormerod et al., 2012, ***Brain, Behavior and Immunity***) in aged rodents. To this end, we are currently completing the inflammatory and neuroinflammatory analyses on brain and blood samples of aged rodents treated with non-steroidal anti-inflammatory drugs that have been characterized behaviorally and electrophysiologically and expect several manuscripts to emerge from this study. We have discussed many of the concepts derived from these experimental studies in a review paper that we were invited to submit earlier this year (Ogle et al., 2012, ***Gerontology***). We hope that the McKnight Brain Research Foundation is as excited as we are about the groundbreaking work that we were to complete in 2012 because of the generous MBRF ARML funding that we were awarded.

Matthew Sarkisian, Ph.D.

Title: *Role of Cilia in Brain Plasticity*

The potential of brain cells to renew themselves varies across the lifespan and can be significantly altered by disease and aging. My laboratory has made significant progress toward understanding how neuronal cilia influence neuronal development and plasticity in developing, aging and diseased brain tissues, research that has been generously supported by the McKnight Brain Research Institute. Cilia are tiny hair-like organelles found on virtually every neuron in the brain. Changes in neural cilia function can disrupt learning and memory, food intake, generation of new neurons in the hippocampus, and neuronal plasticity. These observations suggest that age-related anatomical or physiological changes in cilia could contribute to diminishing neural plasticity and memory decline during aging. In our quest to

understand the contribution of cilia to brain function, and in particular, the aging brain, we have examined the morphologies of neuronal cilia across brain regions and over the lifespan. Earlier this year, we published the findings of this study that included an extensive characterization of the first appearance of neuronal cilia in the cerebral cortex, the growth patterns of these cilia over the lifespan of an animal, and the identification of neuronal subtypes possessing cilia. Remarkably, our most recent studies show that when the growth or signaling capacity of neuronal cilia is impaired neurons fail to extend and develop normal dendritic processes, an effect that is reversible (manuscript under minor revisions). These results show that cilia play an active role in supporting neuronal function, a role that may be increasingly critical in maintaining cortical plasticity and function in aging brain. The results of collaborative studies with the Foster lab that are currently underway suggest that the normal molecular machinery present in the neuronal cilia of neurons present in younger brains, including those that support learning and memory, is altered in aged rodent and human brain (manuscript in preparation). Our results provide a solid foundation for new investigations of markers of brain aging and suggest potential mechanisms for cognitive changes during aging. The data above have been presented at several meetings including local and Annual Society for Neuroscience.

Publications in peer reviewed journals:

Jennifer Bizon, Ph.D.

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Dawn Bowers, Ph.D. (& Michael Marsiske, Ph.D.)

Alexander, G., Ryan L., Bowers, D, Foster, T., Bizon, T., Geldmacher D.S., Glisky, E. *Charactering cognitive aging in humans with links to animal models*. (2012) Frontiers in Aging Neuroscience. 4, 21. doi: 10.3389/fnagi.2012.00021

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Vonetta Dotson, Ph.D.

Under review:

Kirton, J.W., Sozda, C.N., Perlstein, W.M., Manini, T.M., Anton, S.D., & Dotson, V.M. *Older Men and Women Differ in Brain Activity during Working Memory but Not Memory Encoding*. Cortex.

In preparation:

Memory Encoding as a Function of Age in an Elderly Sample: A Functional Magnetic Resonance Imaging (fMRI) Study.

Charles Jason Frazier, Ph.D.

Our ARML supported work has not yet produced publications in peer reviewed journals.

Leonid Moroz, Ph.D.

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Brandi Ormerod, Ph.D.

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Matthew Sarkisian, Ph.D.

Sarkisian MR, Siebzehnrubl D. *Abnormal levels of Gadd45alpha in developing neocortex impair neurite outgrowth*. PLoS ONE 2012; 7(9): e44207.

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Publications (other):

Dawn Bowers, Ph.D. (& Michael Marsiske, Ph.D.)

Bowers, D., Jones, J., Dietz, J. (2012, in press). *Assessment of Emotion*. In J. Synder, Nussbaum, and Parsons, M. (eds). *Pocket Handbook of Neuropsychological Assessment*.

Bauer, R.M., Bowers, D. (2012, in press). *Intellectual Antecedents to the Boston Process Approach to Neuropsychological Assessment*. In D. Libon (Ed.), Boston Process Approach to Neuropsychological Assessment. New York: Oxford University Press.

Matthew Sarkisian, Ph.D.

Sarkisian MR, Arellano JI, Breunig JJ. *Primary cilia in cerebral cortex: growth and functions on neuronal and non-neuronal cells. In: Cilia and Nervous System Development and Function*. Caspary T, Tucker KL (Eds). Springer, (in press)

Presentations at scientific meetings:

Jennifer Bizon, Ph.D.

Invited

April 2012: *Executive Function and Working Memory in Animal Models*, McKnight Inter- Institutional Meeting, Tucson, AZ

April 2012: *Preclinical assessment of interventions for cognitive decline in aging*, Discussion Leader, McKnight Inter-Institutional Meeting, Tucson, AZ

Feburary 2012: *Aging Across Multiple Cognitive Domains*, McKnight Brain Research Foundation Site Visit, University of Florida College of Medicine, Gainesville, Fl.

(Poster Presentations)

Bizon, J.L, Beas, B.S., & Setlow, B. *Dissociation Between Attentional Set-Shifting and Working Memory in Aging*. Federation of European Neuroscience.

Beas, BS, Setlow B, & Bizon JL. (2012). *Attentional set-shifting and working memory are inversely related in aged rats*. Society for Neuroscience. New Orleans, LA.

Mitchell, MR, Weiss, VC, Morgan, D, Bizon, JL., & Setlow, B. (2012). *Adolescent risk-taking, dopamine signaling, and cocaine self-administration; a vicious cycle*. Annual Meeting of the Society for Neuroscience, New Orleans, LA.

Montgomery, K.S., Kumar, A., Demars, K., Foster, T., Bizon, J.L. (2012). *Synaptic dysfunction and early cognitive impairment in a mouse model of AD*. Society for Neuroscience Abstracts, Louisiana.

Banuelos, C., Beas, BS, McQuail, JA, Gilbert, RJ, Setlow, S., Bizon, JL. *GABA(B) receptor blockade enhances delayed match-to-sample working memory performance in aged but not young rats*. Society for Neuroscience Abstracts, Louisiana.

Dawn Bowers, Ph.D. (& Michael Marsiske, Ph.D.)

Invited

August 2012:. *Startling facts about emotion in Parkinson's disease.* Invited address. Presented at the annual meeting of the American Psychological Association, Orlando, FL

August 2012: Kretzmer, T., Pavalla, S. *Professional Dilemmas: Managing inappropriate exchanges in the workplace. A conversation sponsored by Women in Neuropsychology WIN).* Discussants: D. Bowers, R. Nakase-Richardson, Z. Proctor-Weber, R. Ready, M. Rivera-Mindt, P.K. Shear. Presented at the annual meeting of the American Psychological Association, Orlando, FL.

February 2012: Marsiske, M. *Cognitive training with older adults: Intervention, rehabilitation, and engagement approaches*. Invited workshop, International Neuropsychological Society, Montreal, QC, Canada.

Peer Reviewed

Bowers, D., Sapienza, C., Springer, U., MIkos, A., Nisenzon, A., Clark, A., Rodriguez, R., Fernandez, H., Okun, M.S. (2012, February*). Unmasking the face of Parkinson's patients: Results from a randomized double-blind sham controlled behavioral intervention*. Paper presented at 40th meeting of the International Neuropsychological Society, Montreal, Canada.

Bowers, D. (2012, February). Symposium Organizer and Chair: *Current Controversies in Parkinson Disease*. Speakers: A. Troster: Mild cognitive impairment (MCI) in Parkinson disease: new criteria and controversies; L. Zahodne: *Cognitive decline following deep brain stimulation: debates regarding clinical relevance and potential mechanism*; D. Bowers: *The apathy-depression conundrum in Parkinson disease- does it matter?; C. Price: PD as a disconnection syndrome versus a fractionation disorder*. Discussant: Don Stuss; Oral presentation at annual meeting of International Neuropsychological Society, Montreal, CA.

Butterfield, L., Song, W., Kirsch-Darrow, L., Okun, M.S., and Bowers, D. (2012, March). *Apathy and dysphoric mood as independent predictors of quality of life in Parkinson's Disease.* To be presented at annual meeting of American Neuropsychiatric Association, New Orleans LA.

Cunningham, H., Penney, D., Davis, R., Tanner, J., Nguyen, P., Schwab, N., Malaty, I., Okun, M.,Bowers, D., Libon, D. J., Price, C. C. (February, 2012) *Clock Drawing in PD: What makes the clock drawing test tick?* Poster presentation at 40th annual meeting of the International Neuropsychological Society, Montreal, Canada

Dietz, J., Jones, J., Perlstein, W.M., Okun, M.S., Bowers, D. (2012, February). *Detecting emotional significance: the Late positive potential in Parkinson disease*. Poster presented at 40th meeting of the International Neuropsychological Society, Montreal, Canada.

Freedland, A., Glass Umfleet, L., Schwab, N., Ward, J., Leninger, S., Coronado, N., Tanner, J., Nguyen, P., Okun, M., Bowers, D., Price, C.C. (2012, February). *Test-retest reliability on the Rey-Osterreith Complex figure test in a sample of Parkinson's disease patients compared to normal controls*. Poster presentation at 40th annual meeting of the International Neuropsychological Society, Montreal, Canada

Glass Umfleet, L., Bowers, D., Price, C., Bauer, R., Keiski, M., Dede, D., Kay, D., Jones, J., Jacobson, C., Foote, K., Okun, M.S. (2012, February). *Parkinson's disease normative study: Normative data for commonly used clinical neuropsychological data in 379 PD patients.* Poster presentation at 40th annual meeting of the International Neuropsychological Society, Montreal, Canada.

Jones, J., Malaty, I., Okun, M., Bowers, D. (2012, February) *Health comorbidities, cognition, and quality of life in Parkinson disease: Results from the National Parkinson Foundation Quality Initiative with 1935 patients.* Poster presentation at 40thmeeting of the International Neuropsychological Society, Montreal, Canada.

Jones, J., Skoblar, B., Kirsch-Darrow, Okun, M.S., Bowers, D. (2012, February). *Contribution of apathy and depression to global cognitive status in 209 nondemented Parkinson patients.* Poster presentation at 40th annual meeting of the International Neuropsychological Society, Montreal, Canada.

Leninger, A., Freedland, A., Umfleet, L., Schwab, N., Ward, J., Coronado, N., Tanner, J., Nguyen, P., Bowers, D., Libon, D., and Price, C. (2012, February). *Rey-Osterrieth Complex Figure Flowchart Organizational Approach as a Measure of Executive Functioning in Parkinson's Disease Patients.* Poster presentation at 40th annual meeting of the International Neuropsychological Society, Montreal, Canada.

Tanner, J.J., Price, C.C., Malaty, I., Okun, M.S., Libon, D., Bowers, D. (2012, February) *Verbal learning profiles and entorhinal cortex volume in Parkinson's Disease.* Poster presentation at 40th annual meeting of the International Neuropsychological Society, Montreal, Canada.

Umfleet, L., Jones, J., Price, C., Bauer, R., Okun, M.S., Bowers, D. (2012, February). *Neuropsychological test performance: Effects of age of onset in a non-demented Parkinson's Disease patient population.* Poster presentation at 40th annual meeting of the International Neuropsychological Society, Montreal, Canada.

Belchior, P., & Marsiske, M. (2012, February) *The association of a multidimensional construct of useful field of view with standardized visuospatial and non-visuospatial measures.* Poster presented at the International Neuropsychological Society, Montreal, QC.

Thomas, K. R., & Marsiske, M. (2012, February). *Verbal Prompting as a Method for Improving Everyday Cognition in MCI and Unimpaired Older Adults.* Poster presented at the International Neuropsychological Society, Montreal, QC.

Zahodne, L., and Bowers, D. (2012, March). *Depression in Parkinson's disease: Relation to startle eyeblink psychophysiology and affective chronometry*. To be presented at annual meeting of American Neuropsychiatric Association, New Orleans LA.

Gravano, J., Sozda, C., Kaufman, D., Bowers, D. Okun, M.S., Perlstein, W. (2012, April). *A behavioral and electrophysgiologyal examination of attentional networks in Parkinson disease.* Presented at annual meeting of the Cognitive Aging Conference. Atlanta, GA.

Altmann, L., Stegemoller, E., Hazamy, A., Wilson, J.P., Bowers, D. Sapienza, C., Okun, M.S., Hass, CJ (2012, June). *Graded dual task benefits of cognitive tasks on cycling in Parkinson's Disease: Effects of kinesia paradoxical*. Presented at the Movement Disorders Society 16th International Congress of Parkinson's disease and Movement Disorders, Dublin, Ireland. Abstract: Movement Disorders 2012;27 Suppl 1 :31.

Hazamy, A.A., Altmann, L.J.P., Wilson, J.P., Stegemoller, E., Bowers, D., Sapienza, C.M., et al; *Dual task dissociations in cognitive performance in Parkinson's disease* . Presented at Movement Disorders Soceity 16th International Congress of Parkinson's disease and Movement Disorders, Dublin Ireland. [abstract]. Movement Disorders 2012;27 Suppl 1 :56.

Belchior, P, & Marsiske, M. (2012, June) *Older adults' engagement with a video game training program.* Paper presented at the Canadian Association of Occupational Therapists, Montreal, QC

Kirsch-Darrow, L., Okun, M.S., & Bowers, D. (2012, June). *Characteristics of apathy and depression in Parkinson's Disease patients with dementia*. Presented at the annual meeting of the American Association of Clinical Neuropsychology, Chicago ILL.

Jones, J., Jacobson, C., Murphy, M.C., Okun, M.S. Bowers, D. (2012, August). *Health comorbidities and cognition in a clinic sample of 403 Parkinson's Disease patients.* Presented at the annual meeting of the American Psychological Association, Orlando, FL. (Award Winner)

Thomas, K., Marsiske, M., Jones, J., Marra, D., Bowers, D. (2012, November). *Age as a moderator of fatigue severity on a task of cognitive Switching.* Submitted for presentation at 65th annual meeting of the Gerontologic Society of America. San Diego, CA.

Belchior, P, & Marsiske, M. (2012, November) *Older Adults' Subjective Engagement with a Home-Based Video Game Training Program.* Paper presented at the 65th Annual Scientific Meeting of the Gerontological Society of America, San Diego, CA.

Thomas, K.R., Marsiske, M., Marra, D., Jones, J., & Bowers, D. (2012, November) *Age as a moderator of fatigue severity on a task of cognitive switching.* Poster presented at the 65th Annual Scientific Meeting of the Gerontological Society of America, San Diego, CA.

Vonetta Dotson, Ph.D.

Green, M.L., Sozda, C.N., Kirton, J.W., Manini, T., Anton, S., Perlstein, W.M., & Dotson, V.M. (2012). *Memory encoding as a function of age in an elderly sample: An fMRI study*. Poster presented at the 2012 Society for Neuroscience conference in New Orleans, LA.

Sozda, C.N., Perlstein, W.M., Manini, T.M., Anton, S.D., & Dotson, V.M. (2011). *Effect of symptom dimensions of depression on the neural correlates of working memory load in a geriatric population*. Poster presented at the 2011 Society for Neuroscience conference in Washington, D.C.

Leonid Moroz, Ph.D.

Epigenomic Analysis of Single Neurons in Memory Circuits, Simpler Nervous Systems, Institute Higher Nervous Activity, Moscow, Russia, Sept. 2012

Cephalopod Genomics, Cephalopod Biology Meeting, San Paulo, Brazil, Oct 2012

Capture of Nascent RNAs During Learning and Memory, Advances in Genomic Biology and Technology, Marco Island, International Conference, Feb 16, 2012

Semiconductor Sequencing of Single Cells, Advances in Genomic Biology and Technology, Marco Island, International Conference, Feb 15, 2012

Genome-wide sequencing for diagnostics and treatments of Neurological Diseases, Neurobiology of Epilepsy, GeorgiaTech, Atlanta, Sept 7, 2012

Cephalopod Genomics, Genome Biology of Cephalopods, International Conference, Duke University, May 24, 2012

*The genome of Aplysia californica and Genomic dissection of Memory Circuit*s, Molluscan Neurobiology, Scripts Florida, May 16, 2012

The genome of the ctenophore Pleurobrachia bachei: Insights into Independent Origin of Nervous Systems, Invited, Cold Spring Harbor Lab, May 3, 2012

Genomics of Memory, Mechanisms of Memory, University of California, Los Angeles, March 5, 2012

Synaptic Transcriptome, Invited, Columbia University, March 1, 2012

The Ctenophore Genome: Insights into Independent Origins of Nervous Systems, Society for Integrative and Comparative Biology, January 5, 2012

Citarella, M. R., Girardo, D. O., Kohn, A. B., Moroz, L.L. (2012). *Global Discovery and Validation of Signaling Molecules in the Ctenophore*. Integrative & Comparative Biology. Volume. 52 Suppl: 1; Pages E226-E226

Girardo, D. O., Citarella, M. R., Kohn, A. B., Moroz, L.L. (2012). *Automatic Transcriptome Analysis and Quest for Signaling Molecules In Basal Metazoans*. Integrative & Comparative Biology. V. 52 Suppl.: 1 Pages: E252-E252

Moroz, L. L., Citarella, M., Kohn, A. (2012). *Genome-wide analysis of plasticity-induced active DNA demethylation in memory-forming circuits: Insights from the Aplysia genome*. Society for Neuroscience Meeting, Abstracts, New Orleans, #294.08/CCC33

Kohn, A., Citarella, M., Moroz, L. L. (2012). *Single-neuron semiconductor sequencing: From methodology to memory mechanisms*. Society for Neuroscience Meeting, Abstracts, New Orleans, #294.09/CCC34

Kohn, A. B., Citarella, M. R., Gillette, R., Romanova, E.V., Rubakhin, S.S., Sweedler, J.V., Moroz, L.L. (2012). *Genome-wide Characterization of Signaling Peptides in Molluscs: Insights into Neuronal Evolution*. Integrative & Comparative Biology. V: 52 Suppl: 1 Pages: E95-E95

Moroz, L.L.; Kohn, A.; Citarella, M.; et al. (2012). *The Genome of the Ctenophore Pleurobrachia bachei: Molecular Insights into Independent Origins of Nervous Systems*. Integrative & Comparative Biology. V. 52 Supplement: 1 Pages: E125-E125

Swore, J. J., Kohn, A. B., Citarella, M. R., Moroz, L.L. (2012). *Molecular Mapping of Ctenophore Neurons and Glutamate Signaling*. Integrative & Comparative Biology.Volume: 52 Supplement: 1 Pages: E336-E336

Brandi Ormerod, Ph.D.

Speisman, R.B, Kumar, A., Rani, A., Asokan, A., Foster T.C., Ormerod. B.K. (2012). *Multiplex Approach for Designing a Biomarker Assay for Age-related Cognitive Decline*. Annual Meeting of the Biomedical Engineering Society, Atlanta, GA.

Speisman, R.B, Kumar, A., Rani, A., Asokan, A., Foster T.C., Ormerod. B.K. (2012). *Age related changes in central and circulating cytokines and their relationship to learning and memory*. Annual Meeting of the Society for Neuroscience, New Orleans, LA. Soc Neurosci Abstr Vol 37. 243.07.

Matthew Sarkisian, Ph.D.

Guadiana SM, Semple-Rowland S, Madorsky I, Daroszewski D, Mykytyn K, Sarkisian MR. *Differentiation of developing neocortical neurons is linked to primary cilia structure*. Soc Neurosci Abstr 2012.

Sarkisian MR, Siebzehnrubl D, Deleyrolle L, Silver DJ, Siebzehnrubl FA, Guadiana SM, Steindler DA, Reynolds BA. *Detection of primary cilia in human glioblastoma*. 8th Annual Florida Genetics 2012 Symposium. Univ. of Florida Nov. 28-29, 2012.

Siebzehnrubl F, Silver D, Tugertimur B, Deleyrolle L, Suslov O, Siebzehnrubl D, Sarkisian M, Kuepper M, Yachnis A, Brabletz S, Brabletz T, Reynolds B, Steindler D. *ZEB1 mediates invasion and chemoresistance of glioblastoma*. In: Proceedings of the 103rd Annual Meeting of the American Association for Cancer Research; 2012 Mar 31-Apr 4; Chicago, IL. Philadelphia (PA): AACR; *Cancer Res* 2012;72(8 Suppl):Abstract nr 4308.

Presentations at public (non-scientific) meetings or events:

Dawn Bowers, Ph.D.
Invited educational presentations were given by Ms. Kim Foli, the project manager for the VITAL project for the following organizations:

University of Florida Retired Faculty
Village Gator Club
Oak Hammock
Kiwani's Club

Leonid Moroz, Ph.D.
Personalized Genomics and Biodiversity – West Palm Beach Public Lecture, Oct, 2012

Genomic Bases of Multiple Origins of Nervous Systems, Seattle, University of Washington, April 4, 2012

Next Generation Sequencing for Ocean and Human Health, Public Lecture, Friday Harbor, Washington, May 2, 2012

Matthew Sarkisian, Ph.D.
Guadiana SM, Semple-Rowland S, Madorsky I, Daroszewski D, Breunig JJ, Mykytyn K, Sarkisian MR. *Arborization of dendrites by developing neocortical neurons is dependent on primary cilia and type 3 adenylyl cyclase*. Evelyn F. McKnight Brain Research Foundation Poster Reception at SFN, New Orleans 2012.

Awards (other):

Dawn Bowers, Ph.D. (& Michael Marsiske, Ph.D.)

Source: NIH/NINDS - R21NS079767 (2012-2014) $275,000
Emotion Regulation, Executive Function, and Parkinson Disease.
Role: Bowers PI
(this grant received a perfect score)

Awards: Dr. Bowers was awarded Fellow status in Division 40, American Psychological Association, Dr. Bowers was elected to the Board of Governors of the International Neuropsychological Society
Laura Zahodne (Bowers graduate student) was awarded T32 Post-doctoral position in cognitive aging (5T32 AG0026114; Neuropsychology and Cognition in Aging) at Columbia University Taub Center. She is being mentored by Yaakov Stern, Jennifer Manley, and Adam Brickman

Jenna Dietz (Bowers graduate student) is continuing her NINDS funded NRSA on Emotion Psychophysiology and Parkinson Disease. Ms. Dietz also received two national awards as well as two local awards for her research 2012 Walter McMillen Memorial

Award for Parkinson Disease Research, Division 20 (Aging) of the American Psychology Association

2012 Benton Meier Award for Excellence in Neuropsychology, Division 40 of American Psychology Association

2012 Robert Levitt Award for Excellence in Neuropsychology Dept CHP, University of Florida

2012 Research Award, Dept. CHP, University of Florida
Jacob Jones (Bowers graduate student) received 2012 Best Student Research Award from Division 40 (Neuropsychology) of the American Psychological Association for his poster "Health comorbidities and cognition in a clinic sample of 403 Parkinson's Disease patients.

Kim Foli, project manager for the Mcknight funded VITAL study, received Employee of the Year Award from the College of Public Health and Health Professions (PHHP) for her efforts on the VITAL study

Source: NIH/NIA - PI: Levy (01/2013-12/2016) $824,839
Virtual Environments for Therapeutic Solutions (VETS) mTBI/PTSD Phase II
Role: Co-I

Awards: Dr. Marsiske received a University of Florida Research Foundation Professorship (2012-2015)
Dr. Marsiske received the 2012 Classroom Teaching Award from the Department of Clinical & Health Psychology

Honor: Dr. Marsiske was named Chair-Elect of the Behavioral and Social Sciences Membership Committee, Gerontological Society of America

Dr. Leonid Moroz

New NSF grant "Quest for the earliest transmitters"
Funding Agency: NSF# 1146575
PI: L.L. Moroz
Period: 03/01/12-28/02/2016

New R01 NIH "Genomic Approaches to Deciphering Memory Circuits"
Funding Agency: NIH/NIMH 1R01MH097062-01
Period: 01/01/2013-08/31/2017

Dr. Matthew Sarkisian

Source: University of Florida 2011 Research Opportunity Seed Fund Award from
Title: "Mechanisms of Abnormal Brain Development in the VPA Model of Autism"
Award: $84,000
Funding Period: 05/01/11-04/30/13
Role: Co-PI (with Dr. Mark Lewis, Psychiatry)

Source: University of Florida McKnight Brain Institute Agency: Brain & Spinal Cord Injury Research Trust Fund (BSCIRTF
Title: "A Comparison of Pathogenic Processes in Acute Spinal Cord Injury and ALS"

Award: $50,000
Funding Period: 07/01/11-06/30/12
Role: Co-PI (with Dr. David Borchelt, Neuroscience)

Source: American Cancer Society (Research Scholar Grant)
Title: Identifying and Targeting Therapy Resistant Cells in Glioblastoma
Award: $720,000
Funding Period: 01/01/13-12/31/16
Role: PI

Source: Epilepsy Foundation of America Pre-doctoral Training Fellowship
Role: Mentor (for Sarah Guadiana (my graduate student))
Award: $20,000
Award period: 01/01/13-12/31/13

Faculty. Please include abbreviated CV with publications for previous 12 months:

See page 85

Trainees:

Post-Doctoral:

Jennifer Bizon, Ph.D.
Karienn Montgomery (Graduated summer 2012- now a postdoc at Baylor College of Medicine)
Cristina Banuelos (graduate student)
Blanca Sofia Beas (graduate student)
Caitlin Orsini (postdoc)

Dawn Bowers, Ph.D.
Caleb Peck, Psy.D. (clinical post-doc, 2011-ongoing)

Charles Jason Frazier, Ph.D.
Anatoli Kabakov (left the lab in October, 2012)

Leonid Moroz, Ph.D.
Dr. Andrea Kohn
Dr. Mat Roshchin

Pre-Doctoral:

Dr. Dawn Bowers (& Dr. Michael Marsiske)
Daniel Kay, M.S. (on internship)
Jenna Deitz, M.S. (5th year) - funded by F31 NRS
Jacob Jones, M.S. (3rd year) - funded by Graduate Fellowship (minority)
Paul Mangal, B.S. (1st year) – funded by department
Jacob Lafo, B.S. (1st year) – funded by NIH grant
Anna Yam, M.S. – funded by T32
Kelsey Thomas, M.S. – funded by T32
Jackie Maye, B.S. – funded by Graduate Fellowship

Charles Jason Frazier, Ph.D.
Haley Carpenter

Leonid Moroz, Ph.D.
Gabriella Winter, 2nd year Grad Student, Neuroscience
Emily Dabe, 2nd year Grad Student, Neuroscience
Caleb Botchwick, 2nd year Grad Student, Neuroscience

Matthew Sarkisian, Ph.D.
Sarah M. Guadiana

Other:

Dawn Bowers, Ph.D.
(Undergraduate Honors Theses) Kerry Hyman, Jessica Hunter, Catherine Labrie, Natasha Diemunsch

Leonid Moroz, Ph.D.
Yelena Bobkova – histochemistry, Alexander Fodor – epigenomics, Rebecca Brusers -epigenomics, neurodevelopment, Joshua Swore – evolution of Glu signaling, David Girado – bioinformatics, epigenomics, Jim Netherton – Behavior Dr. Peter Williams – Bioinformatics

Matthew Sarkisian, Ph.D.
Dorit Siebzehnrubl, Kathleen Park

Clinical/translational programs:

New Programs:

Leonid Moroz, Ph.D.
Developmental protocols for genomic analysis of human neurons after neurosurgery.

Update on existing clinical studies

Dawn Bowers, Ph.D. (& Michael Marsiske, Ph.D.)
See introductory paragraph. In brief, we are now collecting data on the final cohort of VITAL participants. Due to the positive findings that emerged during the interim analysis (i.e., improved memory), we are obtaining functional scans on a small subset of participants, pre and post intervention. Resources for the scans (task specific fMRI, resting state fMRI, DTI) and other assistance are being provided by Dr. Ron Cohen via McKnight funds, along with technical and conceptual support from Dr. Mingzhou Ding and his graduate student. Imaging pilot data is almost requisite for an NIH submission. Plans are to submit two NIH grants in early 2013. One will focus on mild cognitive impairment and the second will be a carefully crafted clinical trial modeled on the Vital study. In order to assess feasibility for the MCI proposal, we were provided de-identified cognitive screening data (i.e., Montreal Cognitive Assessment, MOCA) that had been collected by the Village health director and her staff. The VITAL team also assisted with this project (i.e., Foli, Marsiske, Bowers, students). We were able to examine over 400 MOCA's and determined , based on the range and distribution of scores, that there were sufficient numbers of individuals with MCI living at the Village to make this subgroup a specific target for intervention using a a combined exercise-cognitive training trial.

Technology Transfer:

a. Patents applications : N/A
b. Revenue generated from technology : N/A

Budget update:

a. Status of matching funds, if applicable : N/A
b. Projected budget for coming year: Financial summaries start on page 63
c. Extramural funding

Vonetta Dotson, Ph.D.
Obtained a diversity supplement to the Lifestyle Interventions and Independence for Elders (LIFE) Study.

Charles Jason Frazier, Ph.D.
Completed and planned submissions of extramural grant applications are covered in other sections. We have not yet received new funding on an extramural award.

Leonid Moroz, Ph.D.
Two R01s, R21, NSF

Educational programs focusing on age related memory loss:

Scientific:

Dawn Bowers, Ph.D. (& Michael Marsiske, Ph.D.)
Marsiske, M. "Cognitive training with older adults: Intervention, rehabilitation, and engagement approaches". Workshop given at the International Neuropsychological Society Annual Meeting, Montreal, QC, Canada

Marsiske serves as training director (2003-2013) of an National Institute on Aging funded predoctoral T32 training program in aging, "Physical, cognitive and mental health in social context"

Leonid Moroz, Ph.D.
Genomic bases of memory and epigenomic of age related memory related

Public:

Dawn Bowers, Ph.D. (& Michael Marsiske, Ph.D.)
D. Bowers, M. Marsiske. (February 28, 2012). Cognitive Interventions in the Very Old:
Update on the Vital Study. Presented at annual McKnight Research Foundation Meeting, Gainesville, FL

Marsiske directs two montly community speaker series (Primetime Institute at the Alachua County Senior Center; Institute on Aging Speaker Series at the Institute for Learning in Retirement at Oak Hammock) which focus on aging topics in general, and specifically on cognitive aging several times a year

Marsiske and Bowers direct/contribute to an online certificate program, "Precertification Training in Psychometry", which provides psychological testing information for non-licensed neuropsychological

aides. One of four courses is entirely devoted to adulthood, aging, and cognitive disorders of later life (e.g., dementia, Parkinsons Disease).

Marsiske participates in a committee to establish an online Masters Degree in Aging and Geriatrics, as part of the University of Florida Department of Aging and Geriatrics Research.

Leonid Moroz, Ph.D.
Two Open houses (spring/summer) to introduce innovative genomic technologies for brain research and mental health

Collaborative programs with other McKnight Institutes, institutions and research programs:

Dawn Bowers, Ph.D.
Dr. Bowers is part of a cross center McKnight workgroup examining elements of a core neuropsychological battery. This workgroup met on 2 occasions during 2012 (in April and again in August), and will convene again as part of the INS meeting in February 2013. Results from their initial meetings were presented at the April 2012 meeting of the McKnight Centers.

Leonid Moroz, Ph.D.
Establishing of the epigenomic initiative together with McKnight Institutes at the University of Arizona (Tucson) and University of Alabama (Birmingham)

Collaborative program with non-McKnight Institutes, institutions and research programs:

Leonid Moroz, Ph.D.

- Columbia University (Kandel, Hawkins and Ju laboratories) – genomic bases of neuronal plasticity
- University of Illinois Urbana/Champaign (Sweedler laboratory) – single cell microchemical analysis
- University of Washington (Swalla laboratory) – Origin of Nervous Systems
- Yale University (Kaczmarek Laboratory) – control of excitability and translation by RNA-binding proteins
- University College of London (Tedford laboratory) – phylogenomics
- Scripts Florida (Puthaventill lab) – genomic bases of aging in nervous system

Briefly describe plans for future research and/or clinical initiatives:

Dawn Bowers, Ph.D.
See Clinical/Translational Programs (b); Two NIH submissions. Ideally, we would like to see the infrastructure that has been developed at the Village continue to be used for other ARML research projects by other investigators. At this point the Village would embrace this with open arms. Practically speaking, this would require support for a project manager, such as Ms. Kim Foli, who could coordinate trials at least until external support could be obtained. Ms. Foli is an exceptionally talented individual with a perfect skill set for this type of enterprise. She is an ideal "face" for the VITAL project, for the McKnight sponsored projects at the Village and for the University at large.

Charles Jason Frazier, Ph.D.

We plan to continue our ARML funded project on NMDA receptor hypofunction in single spines of aged pyramidal cells. We plan to further invest in a new collaborative effort with Dr. Bizon to study cellular and synaptic mechanisms that underlie age related impairment in memory tasks that depend on the prefrontal cortex. Progress we have made to date in these areas are expected to support several additional grant applications in the upcoming year. Specifically, we plan to resubmit an R01 application with Dr. Foster in Spring of 2013 that was scored but not funded in Summer/Fall of 2012. We plan to submit a new R series grant with Dr. Bizon in the first half of 2013. We plan to submit an American Federation of Aging Research grant in December 2012.

Leonid Moroz, Ph.D.

- Transfer the developed technologies for single-neuron genomic and epigenomic profiling to mammalian and human preparations'
- Reconstruct the entire genomic machinery in key neurons of memory-forming circuit
- Identity early genes associated to first phases of learning and memory at the genomic level
- Identify key epigenomic events associated to age-related memory loss focusing on components of active DNA demethylation machinery and 5hmc dynamics within simpler (three synaptic) memory circuit

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

Please describe any negative events (loss of personnel, space, budget, etc.) that occurred during the report period and the possible impact on carrying out the Gift Agreement:

Charles Jason Frazier, Ph.D.

Dr. Kabakov left the lab in October of 2012. Ms. Haley Carpenter remains assigned to our ARML funded project. Loss of Dr. Kabakov is a negative in that this may temporarily slow experimental work (although Ms. Carpenter is still quite productive). On the other hand, this change saves money on salary support for Dr. Kabakov and lengthens the amount of time we can support the project with existing funds. We will seek to replace Dr. Kabakov with a second person working on ARML related goals, but at a lower salary level. Several graduate students have rotated through the lab this Fall, and recruitment of one student towards our ARML goals in the Spring remains a possibility, but the decision will not be made till all rotations are complete.

Please provide any general comments or thoughts not covered elsewhere – a response is not required. Please respond only if you would like to add something not otherwise covered:

Leonid Moroz, Ph.D.

I am confident that McKnight Institute in particular, and University of Florida in general should implement a proactive program to upgrade genomic infrastructure including bioinformatics to support growing demands of biomedical community, promote personalized medicine and secure a competitive position of UF/McKnight Institute at the national and international level. All details and suggestions can be provided upon request.

Signature, date and title of person submitting the report,

January 9, 2013

Tetsuo Ashizawa M.D.
Executive Director, McKnight Brain Institute
Melvin Greer Professor
Chairman, Department of Neurology

McKnight Endowed Chair - Annual Report

McKnight Brain Research Foundation
Sponsored Institutes and Research Programs
Report Period: 2012

Summary of scientific achievements since last report:

This year we have 11 manuscripts either published or accepted for publication in peer reviewed scientific journals and 3 invited chapters, reviews or commentaries. In addition, in collaboration with Drs Moroz and Frazier we have received a UF Opportunity Grant Genomic Bases of Differential Aging in Hippocampal Circuits: Single Cell Approaches. Two NIH Grants have been submitted, one in collaboration with Dr. Frazier and another in collaboration with Dr. Bizon.

Decline in NMDAR function mediates impaired working memory. Alternative splicing of Mbnl2 resulted in a decrease in NMDAR synaptic transmission, impaired hippocampal synaptic plasticity, and memory deficits (Charizanis et al., 2012). A viral mediated increase in the expression of the GluN2B subunit of the NMDAR increased NMDAR synaptic transmission and improved the rapid acquisition of novel spatial information (i.e. working memory) in aging mice (Brim et al., 2012). The results indicate that the age-related decline in NMDAR function we have described contributes to one of the earliest cognitive deficits during aging, impaired working memory. These ideas were outlined and expanded in recent review papers (Foster, 2012; Foster et al., 2012).

Role of estrogen receptor alpha and beta in preserving hippocampal function during aging. The expression of the ERα and ERβ estrogen receptors in the hippocampus may be important in the etiology of age-related cognitive decline. To examine the role of ERα and ERβ in regulating transcription and learning, we employed wild-type (WT) and ERα and ERβ knockout (KO) mice to examine learning and memory as well as gene transcription (Han et al., in press). Briefly, our results suggest that one function of ERβ is to regulate ERα-mediated transcription in the hippocampus. This model is supported by our observations that knockout of ERβ under conditions of low estradiol allowed ERα-mediated transcription. As estradiol levels increased in the absence of ERα, we observed that other mechanisms, likely including ERβ, regulated transcription and maintained hippocampal-dependent memory. In the absence of estradiol treatment, young and middle-age ERβKO mice exhibited preserved learning on the water maze. The preserved memory performance of middle-age ERβKO mice could be reversed by lentiviral delivery of ERβ to the hippocampus. Similarly, our work indicates that lentiviral mediated upregulation of ERα in the hippocampus protects working memory during aging (Wittya et al., 2013). Thus, our results indicate that ERα and ERβ interact with hormone levels to regulate transcription involved in maintaining hippocampal function during aging. Thus, an increase in the ratio of ERα/ERβ preserves memory function in the absence of estradiol treatment. As this ratio declines with advanced age, higher levels of estrogen are required to maintain cognitive function.

Other biological markers of cognitive decline. In collaboration with Dr Ormerod's lab (Speisman et al., 2013a,b) we found that a decline in rapid flexible spatial memory (e.g. working memory) was associated with a decline in neurogenesis and both could be revered by environmental enrichment. In addition, we found that memory, neurogenesis, and cytokine inflammatory markers in the brain and blood serum could be regulated by exercise. It is hoped that we will be able to identify serum cytokine markers of brain function.

Publications in peer reviewed journals:

Foster, T.C. (2012) *Dissecting age-related cognitive decline in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca2+ channels in senescent synaptic plasticity*. Progress in Neurobiology, 96:283-303, PMID: 22307057

Han, X., Aenlle, Bean, L.A., Rani, A., Semple-Rowland, S.L., K., Kumar, A., and **Foster**, T.C. *Role of estrogen receptor alpha and beta in preserving hippocampal function during aging*. Journal of Neuroscience, in press.

Charizanis, K., Lee, K-Y., Batra, R., Goodwin, M., Zhang, C., Yuan, Y., Shiue, L., Cline, M., Scotti, M.M., Xia, G., Kumar, A., Ashizawa, T., Clark, H.B., Kimura, T., Takahashi, M.P., Fujimura, H., Jinnai, K., Yoshikawa, H., Gomes-Pereira, M., Gourdon, M. Sakai, N., Nishino, S., **Foster**, T.C., Ares Jr, M., Darnell, R.B., and Swanson, M. (2012) *Muscleblind-Like 2 Mediated Alternative Splicing in the Developing Brain and Dysregulation in Myotonic Dystrophy*, Neuron, 75:437-450, PMID: 22884328.

Speisman, R.B., Kumar, A., Rani, A., Pastoriza, J.M. Severance, J.E. **Foster**, T.C., and Ormerod, B.K. (2013) *Environmental enrichment protects neurogenesis and performance in a short water maze task from the effects of age*. Neurobiology of Aging, 34, 263-274.

Speisman, R.B., Kumar, A., Rani, **Foster**, T.C., and Ormerod, B.K. (2013) *Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats*. Brain, Behavior and Immunity, in press, PMID: 23078985.

Brim, B., Haskell, R., Awedikian, R., Elinwood, N.M., Jin, L., Kumar, A., **Foster**, T.C., and Magnusson, K. (2013) *Memory in aged mice is rescued by enhanced expression of the GluN2B subunit of the NMDA receptor*. Behavioural Brain Research, in press, PMID: 23103326.

Wittya, C.F., **Foster**, T.C., Semple-Rowland , S.L., and Daniel, J.M. (2012) *Increasing hippocampal estrogen receptor alpha levels via viral vectors increases MAP kinase activation and enhances memory in aging rats in the absence of ovarian estrogens*. PLOS ONE, in press.

Boye1, S.L., Peshenko, I.V., Huang, W.C., Min, S.H., McDoom, I., Liu, X., Dyka, F.M., **Foster**, T.C., Umino, Y., Karan, S., Jacobson ,S.G., Baehr, W., Dizhoor, A., Hauswirth, W.W., and Boye, S.E.. (2013) *AAV-mediated gene therapy in the guanylate cyclase (RetGC1/RetGC2) double knockout mouse model of Leber congenital amaurosis*. Human Gene Therapy, in press.

Roberson, E.D, DeFazio, R.A., Geldmacher, D.S, Alexander, G.E., Barnes, C.A., **Foster**, T.C., Bizon, J.L., Glisky, E.L., Ryan, L., Levei, B.E., Wright, C.B., and Bowers, D. (2012) *Challenges and Opportunities in Characterizing Cognitive Aging Across Species*. Frontiers in Aging Neuroscience, 4:6 doi: 10.3389/fnagi.2012.0006, Epub 9/12/2012.

Foster, T.C., DeFazio, R.A., and Bizon, J.L. (2012) *Characterizing cognitive aging of spatial and contextual memory in animal models*. Frontiers in Aging Neuroscience, 4:12 doi: 10.3389/fnagi.2012.00012, Epub 9/12/2012, PMCID: PMC3439636.

Bizon, J.L., **Foster**, T.C., Glisky, E.L., Alexander, G.E., (2012) *Characterizing Cognitive Aging of Working Memory and Executive Function in Animal Models*. Frontiers in Aging Neuroscience, 4:19 doi: 10.3389/fnagi.2012.00019, Epub 9/12/2012.

Publications (other):

Guidi, M. and **Foster**, T.C. (2012) *Animal model of memory and cognitive disorders*. In F.H. Kobeissy (Ed.) Psychiatric disorders: Methods and Protocols, Volume 829: 145-153. Methods in Molecular Biology Springer, USA (Humana Press, Inc), PMID: 22231811.

Craft, S., **Foster**, T.C., Landfield, P.W., Maier, S.F., Resnick, S.M. and Yaffe, K. (2012) *Session III: Mechanisms of Age-Related Cognitive Change and Targets for Intervention: Inflammatory, Oxidative, and Metabolic Processes*. Journal of Gerontology: Biological Sciences, in press PMID: 22570133.

Foster, T.C. (2012) *Challenges and opportunities in characterizing cognitive aging across species.* Frontiers in Aging Neuroscience, doi: 10.3389/fnagi.2012.00033, Epub 11/07/2012. This is an introduction to the special issue devoted to the seven papers published by the McKnight Cognitive Battery group.

Foster, T. and Notterpek L. (2012) *The business of the brain*. Village Journal at Haile Plantation Vol 8 (2) p 47-50.

Presentations at scientific meetings:

Chairman, and Session Organizer for a session on: *Glia and Neural Plasticity*. 25th Annual Winter Conference on Neural Plasticity.

Increasing hippocampal levels of estrogen receptor alpha via viral vector delivery enhances memory in aging rats in the absence of ovarian estrogen. (2012) Witty, C.F., Foster, T.C., Semple-Rowland, S.L., Daniel, J.M. Soc for Neurosci. 92.02/SS11.

Disruption of signaling from NMDARs to gene transcription contributes to age-related learning impairments (2012) Foster, T.C. Rani, A., Tchigrinova, O., Kumar, A. Soc for Neurosci. 104.09/CCC33.

Age related changes in central and circulating cytokines and their relationship to learning and memory. (2012) Speisman, R.B., Kumar, A., Rani, A., Asokan, A., Foster, T.C., Ormerod, B.K. Soc for Neurosci. 243.07/E52.

Ca^{2+} from VGCCs and NMDA receptors contributes to LTD induced by either synaptic activity or mGluR activation at CA3-CA1 hippocampal synapses during senescence. (2012) Kumar, A. and Foster, T.C. Soc for Neurosci. 336.09/E22.

Synaptic dysfunction and early cognitive impairment in a mouse model of AD. (2012) Montgomery, K.S., Kumar, A., Demars, K., Foster, T.C., and Bizon, J. Soc for Neurosci. 752.05/F19.

Epigenetic regulation of regional and age-related differences in estrogen receptor alpha expression in the hippocampus. (2012) Han, X. and Foster, T.C. Soc for Neurosci. 814.24/FFF64

Presentations at public (non-scientific) meetings or events:

Ask America's Ultimate Experts: Help me keep my brain young. Woman's World page 23, 9/3/2012.

Awards (other):

Award: UF Opportunity Grant Genomic Bases of Differential Aging in Hippocampal Circuits: Single Cell Approaches $100,000 2012-2013

Faculty. Please include abbreviated CV with publications for previous 12 months:

See page 85

Trainees:

Post doctoral:

Ashok Kumar (Research Associate)

Pre-doctoral:

Wei-Hua Lee, Ph.D. program
Mike Guidi, Ph.D. program
Linda Bean, Ph.D. program
Xiaoxia Han, transferred from Genetics to Statistics program

Other:

Asha Rani, Olga Tchigrinova

Clinical/Translational Programs:

New programs: NA

Update on existing clinical studies:

- In 2009 I took over as Chair for the committee overseeing the Age-Related Memory Loss (ARML) Program. Other members of the committee include Drs. Lucia Notterpek, Tetsua Ashizawa, and Christiaan Leeuwenburg.
- During the past year I have been a member of a search committee, Chaired by Dr Bizon and directed at hiring another faculty member for the ARML Program. Candidates have been interviewed and two candidates have returned for a second visit. Dr Eric Blalock will be back for a third visit at the end of February.
- We have two previous pilot projects (Bowers and Frazier) that have been refunded (100K each). The Frazier project has resulted in a grant submission. The project was scored but not funded. A second submission is planned for February.
- We have met with a new Psychology faculty member, Dr Natalie Ebner, whose research includes lifespan changes in cognitive processing of socially and age-related differences in perception of, attention to, and memory for human faces. After the meeting several recommendations were made including a mentoring system through the Pepper Center. She is planning a grant submission in early 2013 and the ARML committee will provide a letter of support including conditional financial support.
- Other proposals are currently being reviewed; however, I do not expect a decision until the middle of 2013.

- I am a member of the Institute on Aging and Pepper Center Executive Committee. I regularly attend meetings with this group where I promote research on age-related cognitive decline.

Technology transfer:

a. Patents applications : N/A
b. Revenue generated from technology : N/A

Budget update:

a. Status of matching funds, if applicable : N/A
b. Projected budget for coming year: Financial summaries start on page 63
c. Extramural funding: N/A

Educational Programs focusing on Age Related Memory Loss:

Scientific: N/A

Public:

Foster, T. and Notterpek L. (2012) The business of the brain. Village Journal at Haile Plantation Vol 8 (2) p 47-50.

Ask America's Ultimate Experts: Help me keep my brain young. Woman's World page 23, 9/3/2012.

Collaborative programs with other McKnight Institutes:

Acting in my capacity as an Associated Editor for the Journal *Frontiers in Aging Neuroscience* and as an author on several of the manuscripts, we have completed and published seven manuscripts for the Cognitive Battery McKnight Brain Research Foundation Working Group.

The MBRF Epigenomics Research Focus group meeting was held in Gainesville. The meeting resulted in a plan for an Inter-Institute program that is mission relevant, high impact, and practical. The plans call for shared resources for studies of the epigenetics of cognitive decline with aging. The goal is for discovery and therapeutics targeted to epigenomics. Another meeting will be scheduled for Dec or Jan.

Collaborative program with non-McKnight Institutes, institutions and research programs:

I have collaborated with Jill Daniel at Tulane University to examination over expression of estrogen receptor alpha as a mechanism to prevent age-related cognitive decline. This has resulted in one published manuscript (Wittya et al., 2013).

I have collaborated with Dr Kathy Magnusson at the University of Washington examination over expression of NMDAR subunit GluN2B as a mechanism to prevent age-related cognitive decline. This has resulted in one published manuscript Brim et al., 2012).

Briefly describe plans for future research and/or clinical initiatives:

- To publish work on 1) the effect of non-steroidal anti-inflammatory drugs on age-related cognitive decline and brain aging and 2) age-related memory impairment and the function of signaling cascades from NMDARs to histone acetylation.
- Collaborate on projects related to GABAB receptor function and aging of executive function (Bizon).
- Collaborate on projects related to DNA regulation and aging of single cells (Moroz).
- Collaborate on projects related to Ca^{2+} regulation and hippocampal aging (Frazier).
- Submission of a grant on mechanisms for age-related deficits in working memory versus reference memory.
- Set up meetings and organize for a possible program project grant for summer 2013.

If applicable, please provide endowment investment results for the report period:
See page 71

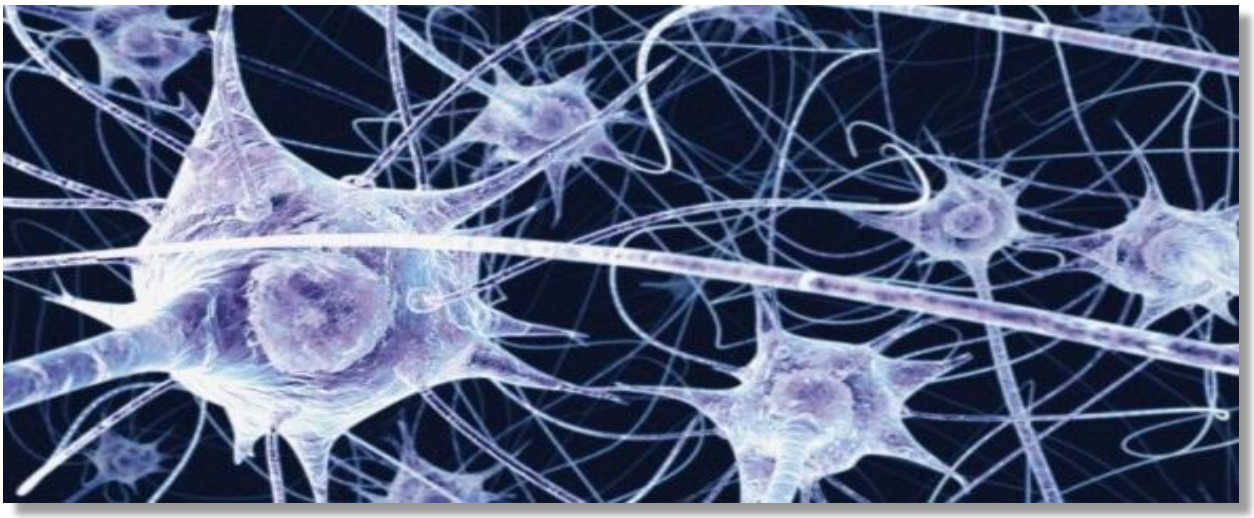
Were any funds used for a Prohibited Purpose during the report period?
No

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

Signature, date, and title of person submitting the report,

Thomas C. Foster, Ph.D.
Professor and Evelyn F. McKnight Chair for Research on Cognitive Aging and Memory



2012 Annual Report

Cognitive Aging and Memory Clinical Translational Research Program (CAM-CTRP)

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

UF | UNIVERSITY of FLORIDA
The Foundation for The Gator Nation

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January 9, 2013

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). Since the last report in December of 2012, recruitment of a Director to the Program was successfully completed. Dr. Ronald Cohen assumed the directorship on July 1, 2012. He was one selected from a large number of applicants for the position and from eleven finalist who came to the University of Florida to interview. The first half of 2012 was spent completing the hiring process, followed by Dr. Cohen's initial move and transition into Institute of Aging. During this period Dr. Cohen recruited his first graduate student, Talia Seider a graduate of UC-Berkley, and more recently a research assistant in the Memory Disorders Program at UCSF. He is co-mentoring another graduate student with Dr. Anton of the Institute, and also is serving as a dissertation committee member for three more advanced graduate students. A major emphasis has been placed on recruiting faculty to join the new CAM center. Thus far, five candidates have come for initial interviews, of whom three continue to be active as possible faculty recruits. The initial intent is to recruit two faculty members, ideally one at a junior faculty level, and the other more advanced in their career with evidence of a history of external funding from NIH in areas related to aging. We hope to make an offer in the coming month to one of the three candidates.

Several major initiatives have occurred in the first six months. These include: 1) R01 submission in October to NHLBI/NIDDK of a study to examine the effects of weight loss and improved metabolic function following bariatric surgery; 2) Successful submission of an ancillary LIFE study to collect multimodal neuroimaging data on the UF cohort to establish a normative neuroimaging database stratified by age and physical activity; 3) Submission with Dr. Pahor of two multicenter R01s in which age-associated memory and brain function will be examined in the context of the LIFE study (LIFE-Extension, LIFE-ARISE); 4) Collaboration between UF and Mt. Sinai in Miami on the development of ADCR; 5) Submission of a TBI-Aging project as part of a UF initiative related to the study of NFL athletes and also DOD populations; and 6) Initiation of a pilot project aimed at developing a R01 proposal to study brain function in the context of HIV and Aging. In addition, Dr. Cohen on behalf of the CAM-CTRP has begun collaborative efforts with the three other McKnight Institutes, including the working with Drs. Wright (Miami) and Alexander (Arizona) on a neuroimaging workgroup aimed at harmonizing MR imaging across these institutions, and integrating neuroimaging efforts with the cognitive and genomic initiatives currently underway.

Budgetary details are provided in the body of the progress report. We greatly appreciate the support and partnership of the MBRF and are very excited about the evolution of this new program over the coming years.

Sincerely,

Ronald A. Cohen, PhD, ABPP, ABCN
Professor, Aging, Neurology and Psychiatry
Director, CAM-CTRP

Marco Pahor, MD
Professor and Chair
Department of Aging and Geriatric Research
Director, Institute on Aging

CAM-CTRP - Annual Report

McKnight Brain Research Foundation
Sponsored Institutes and Research Programs
Report Period: 2012

Summary of scientific achievements since last report:

The mission of the CAM-CTRP is to provide a cutting-edge interdisciplinary clinical translational research program dedicated to advancing knowledge regarding human cognitive aging and memory, and translating findings into the development of clinical applications to slow, avert or restore the age-related cognitive decline and memory loss. The CAM-CTRP seeks to extend and translate basic science findings from the AMRL and other UF investigators, and from human neuroscience research conducted within the center. The CAM-CTRP is housed within the UF Institute on Aging and is closely affiliated with the McKnight Brain Institute, providing a nexus between these two institutes. Academically, it has primary affiliation with the Departments of Neurology, Neuroscience, Psychiatry and Aging and Geriatric Research and also with the Department of Clinical and Health Psychology within the School of Public Health. Accordingly, the CAM-CTRP is well positioned to interact with and collaborate with researchers from key academic departments important to its mission.

The development of the CAM-CTRP took a significant step forward with the arrival of Dr. Ronald Cohen to direct the program. He is an expert in age-associated cognitive and memory functioning, having served as co-director of the Alzheimer's and Related Disorders Clinic at Brown University for many years. He also has publishing many studies examining the neural bases of cognitive and memory loss in the elderly, including the contribution of specific risk and etiological factors, and the mechanisms mediating these effects.

In his current role as director of the CAM-CTRP, Dr. Cohen has taken initial steps to continue his ongoing lines of research and to extend these efforts by: 1) Initiating new lines of research that while linked to his earlier work focus explicitly on community dwelling older adults without preexisting neurological brain disease; 2) Extending ongoing lines of research at UF, including clinical trials (e.g., LIFE) that study on aging, but that have not explicitly focused on cognition or the brain; 3) Fostering collaborations with UF investigators previously supported by the MBRF (Bowers, Dotson, Marsiske, Manini), and new investigators (e.g., Natalie Ebner); 4) Recruit talented new faculty members with strong academic and research accomplishments related to cognitive aging and memory who will advance the mission of the program, providing synergy with existing faculty across UF; and 5) Facilitate efforts underway across the four McKnight Brain Institutes to achieve greater integration across institutes and to develop inter-Institute collaborative research that will make major contributions to clinical and neuroscience of the aging brain. These five initiatives are summarized below. Specific projects and scientific contributions are described in sections of the report that follow.

Extension of previous lines of research: Dr. Cohen has conducted a large number of past studies examining the influence of vascular and metabolic disturbances to the development of brain dysfunction and functional decline in the elderly. Much of this work has been conducted with specific clinical populations, such as cardiovascular disease, HIV, and Alzheimer's disease. Studies are needed that examine the mechanisms underlying age-related brain changes in healthy elderly adults without preexisting brain disease or severe medical illnesses. This effort has been initiated within the CAM-CTRP through a recently approved study which will collect neuroimaging, neurocognitive, and laboratory

biomarkers from 200 older adults (age > 70 years), of whom 60 will have extensive clinical, physical and behavioral data from the LIFE study. This represents a major effort that will result in a normative database and that will support future grant submissions.

Extending current research within the Institute on Aging and UF: The CAM-CTRP has taken a leadership role in three major research efforts over the past six months: 1) LIFE-Extension Study; 2) LIFE-ARISE; 3) UF-Mt. Sinai ADRC initiative; and 4) HIV-Alcohol and Aging Program. The LIFE-Extension Study is an important NIA-supported study of exercise effects on physical health in the elderly in which UF plays the lead role. Renewal of this project is now being undertaken. NIA has asked that the primary outcome be switched to cognitive function; specifically conversion to MCI. Dr. Cohen has played a key role in this regard, providing expertise on MCI and methodological support regarding approaches to assessing cognitive decline and also the implementation of neuroimaging measures. The LIFE-ARISE study is in response to a RFA from NIA to study novel approaches to preventing neurodegenerative disease in the elderly. Together with Dr. Pahor (PI) a proposal has been developed that would incorporate neuroimaging, including MRI and PET, laboratory biomarkers (e.g., CSF beta amyloid and tau) and other functional measures. LIFE-ARISE is a clinical trial in which the effects of exercise on neuroimaging and laboratory biomarkers will be examined relative to physical measures that have been assessed previously. Another effort that is currently underway has focused on developing an Alzheimer's Disease Research Center (ADRC) which would incorporate Mt. Sinai hospital in Miami as the clinical site, and UF as the primary academic site. Dr. Cohen has been developing this proposal with Drs. Golde, Nelson, Ashizawa, and Cottler from UF and Ronjon Duara from Mt. Sinai. This ADRC would be rather unique in that its focus would be pre-MCI and early MCI rather than AD per se. Finally, efforts to continue current research on HIV-aging effects on the brain have taken a step forward through collaboration that has been established with Dr. Robert Cook and successful submission of a proposal for pilot funding, which will enable a research cohort to be established. NIAAA has indicated their interest in funding a U-grant focused on HIV-alcohol and aging at UF.

Fostering CAM-CTRP collaborations: The CAM-CTRP has actively sought to fortify collaborations researchers who have been supported by MBRF (Bowers, Dotson, Marsiske, Manini) and to develop new collaborations. Ongoing studies include: 1) The effects of cognitive training and exercise in community dwelling older adults (Bowers, Marsiske, Cohen). Neuroimaging has been incorporated into the VITAL study to examine mechanisms of change; 2) Examination of cerebral and peripheral neuromuscular factors contributing to sarcopenia, metabolic disturbances, and fatigue (Manini, Cohen); 3) Examination of the neural bases of depression, apathy, and sedentary behavior in the elderly using neuroimaging methods (Dotson). Dr. Cohen is serving as a collaborator on this project, and is also serving on dissertation committees of two graduate students being mentored by Drs. Dotson and Manini. A new collaboration has been established with Dr. Natalie Ebner, whose research focuses on hormonal influences (oxytocin) on social behavior, risk taking and emotion in the elderly. She is now on the CAM-CTRP faculty, and will continue to engage in collaborative research integrating her cognitive neuroscience group and CAM-CTRP investigators.

Faculty Recruitment: Important to the success of the CAM-CTRP is establishing a core faculty dedicated to the mission of the program, interested in collaborating, and also promoting their own areas of research. Three directions have been taken in this regard. 1) A national search has been initiated for 2-3 faculty positions ranging in rank from Assistant to Associate Professor for individuals with strong background in neuroscience, aging and neuroimaging; 2) Faculty currently at UF with strong interests and background in areas of relevance to the CAM-CTRP have been approached and invited to submit letters of interest to join the faculty. There has been a great response to this effort, and 11 individuals have submitted vitas and asked to become members. This list is available in the appendix; 3) A major

effort is being directed at developing a post-doctoral research training program in the aging brain and neuroimaging that would provide a system for bringing in talented young faculty willing to write career development awards to kick start their academic career in this field.

McKnight Inter-Institutes Initiative: The CAM-CTRP is playing an active role in efforts to mobilize a large scale research project across the four McKnight Institutes, which will initially dependent on achieving standardization and harmonization across institutes. Dr. Cohen is overseeing UF efforts related to human neuroscience and translational research in this regard. This has involved working with Dr. Bowers who has agreed to lead the cognitive workgroup. Dr. Cohen is leading neuroimaging efforts for UF, along with investigators from Miami, Arizona, and UAB (Wright, Alexander and others). We are preparing two papers to be published in Journal of Neuroimaging that reviews structural and functional neuroimaging of age-related cognitive and memory decline. This will be followed by initial efforts to standardize MRI methods across sites and obtain preliminary data.

Publications in peer reviewed journals:

Phelan S, Hassenstab J, McCaffery JM, Sweet L, Raynor HA, Cohen RA, Wing RR. (2011). *Cognitive interference from food cues in weight loss maintainers, normal weight, and obese individuals*. Obesity Jan;*19*(1):69-73. *PMID:0539296.*

Jefferson AL, Holland CM, Tate DF, et al. (2011). *Atlas-derived perfusion correlates of white matter hyperintensities in patients with reduced cardiac output*. Neurobiol Aging. Jan;*32*(1):133-139. PMC 889176.

Harezlak J, Buchthal S, Taylor M, et al. (2011). *Persistence of HIV-associated cognitive impairment, inflammation, and neuronal injury in era of highly active antiretroviral treatment*. AIDS. Mar *13* ;25(5):625-633. *PMID: 21297425.*

Cohen RA, de la Monte S, Gongvatana A, et al. (2011). *Plasma cytokine concentrations associated with HIV/hepatitis C coinfection are related to attention, executive and psychomotor functioning*. J Neuroimmunol. Apr;*233*(1-2):204-210. *PMC 3074016.*

Tate DF, Conley J, Paul RH, et al. *Quantitative diffusion tensor imaging tractography metrics are associated with cognitive performance among HIV-infected patients*. Brain Imaging Behav. Mar 2010;4(1):68-79. *PMC 2909656.*

Stanek KM, Grieve SM, Brickman AM, Korgaonkar MS, Paul RH, Cohen RA, Gunstad JJ. (2011). *Obesity is associated with reduced white matter integrity in otherwise healthy adults*. Obesity Mar;*19*(3):500-4. *PMID:21183934.*

*La*ne EM, Paul RH, Moser DJ, Fletcher TD, Cohen RA. (2011). *Influence of Education on Subcortical Hyperintensities and Global Cognitive Status in Vascular Dementia.* J Int Neuropsychol *Soc*. Mar 9:1-6. *PMID:21385518.*

Bunea F, She Y, Ombao H, Gongvatana A, Devlin K, Cohen R.(2011). *Penalized least squares regression methods and applications to neuroimaging*. Neuroimage. Apr 15;55(4):1519-1527. *PMID:21167288.*

Gunstad J, Strain G, Devlin MJ, Cohen RA, et al. (2011). *Improved memory function 12 weeks after bariatric surgery*. Surg Obes Relat *Dis.* Jul-Aug *7*(4):465-472. *PMC 3117085.*

Liu J, Cohen RA, Gongvatana A, Sheinkopf SJ, Lester BM. (2011). *Impact of Prenatal Exposure to Cocaine and Tobacco on Diffusion Tensor Imaging and Sensation Seeking in Adolescents*. J Pediatr *159*(5):771-5. *PMID:21723565.*

Tate DF, Sampat M, Harezlak J, Cohen RA, Guttmann CR, Navia B. (2011). *Regional areas and widths of the midsagittal corpus callosum among HIV-infected patients on stable antiretroviral therapies for the HIV Neuroimaging Consortium.* J Neurovirol*. 17*(4):368-79. *PMID:21556960.*.

Gongvatana, A, Cohen RA, Correia S, Devlin KN, Miles, J, Kang H, Ombao H, Navia B, Laidlaw DH, Tashima K. (in press). *Clinical contributors to cerebral white matter integrity in HIV-infected individuals*. J Neurovirol *17*(5):477-86. *PMID:21965122.*

Okonkwo OC, Cohen RA, Gunstad J, Poppas A. (2011). *Cardiac Output, Blood Pressure Variability, and Cognitive Decline in Geriatric Cardiac Patients*. *J Cardiopulm Rehabil Prev.* Jun 23 2011. [31(5):290-7. *PMC 3171573.*

Garcia S, Spitznagel MB, Cohen R, Gunstad J et al. *Depression is associated with cognitive dysfunction in older adults with heart failure*. Cardiovasc Psychiatry Neurol.2011;2011: PMID: 22195274.

Spitznagel MB, Garcia S, Miller LA, Strain G, Devlin M, Wing R, Cohen R, Paul R, Crosby R, Mitchell JE, et al. *Cognitive function predicts weight loss after bariatric surgery*. Surg Obes Relat Dis. 2011 Oct 29. [Epub ahead of print] PMID: 22133580.

Miller LA, Gunstad J, Spitznagel MB, McCaffery J, McGeary J, Poppas A, Paul RH, Sweet LH, Cohen, RA. *CAMTA1 T polymorphism is associated with neuropsychological test performance in older adults with cardiovascular disease*. Psychogeriatrics. 2011;11(3):135-40. PMID: 21951953.

Devlin, K, Gogvatana A, Clark U, Chassman J, Navia B, Tashima K, Cohen, RA.(2012). *Neurocognitive effects of HIV, Hepatitis C, and substance use history*. J Int Neuropsychol *Soc.18*(1):68-78. *PMID:22132928.*

Alosco ML, Spitznagel MB, Cohen R, et al. (2012). *Cognitive Impairment Is Independently Associated With Reduced Instrumental Activities of Daily Living in Persons With Heart Failure*. J Cardiovasc Nurs*. May 9. 27*(1):44-50. *PMC 3175008*.

Alosco, M. L.Spitznagel, M. B.Raz, N.Cohen, R. Sweet, L. H.van Dulmen, M.Colbert, L. H.Josephson, R.Waechter, D.Hughes, J.Rosneck, J.Gunstad, J.(2012). *Cognitive reserve moderates the association between heart failure and cognitive impairment*. J Clin Exp Neuropsychol*, 34*(1):1-10.

Knecht KM, Alosco ML, Spitznagel MB, Cohen R, et al. Gunstad J et al. *Sleep Apnea and Cognitive Function in Heart Failure*. Cardiovasc Psychiatry Neurol. 2012;2012: PMID: 22745901.

Alosco ML, Spitznagel MB, van Dulmen M, Raz N, Cohen R, Gunstad J. *The additive effects of type-2 diabetes on cognitive function in older adults with heart failure*. Cardiol Res Pract. 2012; PMID: 22701196.

Keary TA, Gunstad J, Benitez A, Spitznagel MB, McCaffery J, McGeary JE, Poppas A, Paul RH, Sweet LH, Cohen RA. *TCF7L2 polymorphism and cognitive test performance in cardiovascular disease.* Psychogeriatrics. 2012 Jun;12(2):93-8.

Miller LA, Spitznagel MB, Alosco ML, Cohen RA, Sweet LH, Josephson R, Hughes J, Gunstad J. *Cognitive profiles in heart failure: a cluster analytic approach.* J Clin Exp Neuropsychol. 2012; 34(5):509-20. PMID: 22375800.

Galioto R, Spitznagel MB, Strain G, Devlin M, Cohen R, Paul R, Crosby RD, Mitchell JE, Gunstad J. *Cognitive function in morbidly obese individuals with and without binge eating disorder.* Compr Psychiatry. 2012 Jul;53(5):490-5. Epub 2011 Oct 28.

Hassenstab JJ, Sweet LH, Del Parigi A, McCaffery JM, Haley AP, Demos KE, Cohen RA, Wing RR. *Cortical thickness of the cognitive control network in obesity and successful weight loss maintenance: A preliminary MRI study.* Psychiatry Res. 2012 Apr 30;202(1):77-9. Epub 2012 May 16. PMID: 22595506.

Sweet LH, Hassenstab JJ, McCaffery JM, Raynor HA, Bond DS, Demos KE, Haley AP, Cohen RA, Del Parigi A, Wing RR. *Brain response to food stimulation in obese, normal weight, and successful weight loss maintainers.* Obesity (Silver Spring). 2012 May 9. Epub ahead of print] PMID: 22569002.

Clark US, Cohen RA, Sweet LH, Gongvatana A, Devlin KN, Hana GN, Westbrook ML, Mulligan RC, Jerskey BA, White TL, Navia B, Tashima KT. *Effects of HIV and Early Life Stress on Amygdala Morphometry and Neurocognitive Function.* J Int Neuropsychol Soc. 2012:1-12. PMID: 22621973.

Gunstad, J, Sweet, LH, Cohen, RA. *Cerebrovascular Perfusion among Older Adults is Moderated by Strength Training and Gender.* Journal of Cardioplumonary Rehabilitation and Prevention.

Zhu. T, Zhong J, Hu R, Tivarus H, Ekholm, S, Harezlak J, Ombao H, Navia, B, Cohen R, Schifitto G. *Patterns of white matter injury in HIV infection after partial immune reconstitution: a DTI tract-based spatial statistics study*. Journal Neurovirology (In press)

Five other papers are currently in press, 10 submitted for publication.

Books

1. Cohen, RA, Sweet, LH (Eds). Brain Imaging in Behavioral Medicine and Clinical Neuroscience. 2011: Springer: New York, NY.

2. Cohen, RA. Neuropsychology of Attention (2nd ed). (In press). Springer: New York, NY.

Presentations at scientific meetings:

University of Florida, Medicine Grand Rounds (Endocrinology): *Metabolic and vascular determinants of brain age-associated brain dysfunction*

University of Florida, Institute on Aging Symposium: *Metabolic and vascular determinants of brain age-associated brain dysfunction*.

Presentations at public (non-scientific) meetings or events:

Healthy cognitive aging: Alachua County Retired Teachers Association

NIH Review Work:

Permanent member BMIO review group for eight years: ended June 2011; NIA
NIA center grant reviewer. Summer 2011

Served on six NIH special emphasis panels in 2012

Chaired IRG on HIV-Aging

Awards (other):

N/A

Faculty. Please include abbreviated CV with publications for previous 12 months:

See page 85

Trainees:

Post-doctoral:

Brown University. Dr. Cohen's post-doctoral training has included six fellows at Brown University. He served as mentor for career development awards for three of these individuals and all were successful (Win Gongvatana: K99; Uraina Clark: K01; Jason Hastenstaub (K24), and Katherine Demos (K24). In addition, he is currently supervising Dr. Jessica Caldwell-Kirland on her current post-doctoral research at Brown.

University of Florida. Natalie Ebner, PhD. (Cohen, Mentor). Dr. Ebner, Asst. Professor, Department of Psychology, is conducting a research project on oxytocin effects on social bonding and emotional processing, and is extending this research to study aging. This work is very consistent with the mission of the CAM. Dr. Cohen is serving as a primary mentor and a member of her advisory committed. She has joined the faculty of the CAM.

Pre-doctoral:

Two graduate students in the Department of Clinical and Health Psychology are being mentored by Dr. Cohen (Talia Seider and Christy Karabetian). Three students from this department and also the school of public health have asked Dr. Cohen to serve on qualifying examination and dissertation committees.

Talia Seider: Neuropsychology track, DCHP. She is a graduate of UC Berkley and has worked in a Memory Clinic at UCSF prior to coming to UF. Her interests are cerebrovascular disease effects on cognition, neuroimaging and HIV. She is performing a WMH quantification project for her first year project.

Christy Karabeteian. Behavioral Medicine Track, DCHP. Her interest is in behavioral factors affecting obesity and weight loss. Her primary mentor has been Steve Anton, PhD. The CAM is proving partial support for her second year. She is conducting analyses of our bariatric surgery research data examining

effects on brain functioning and also HIV x aging effects of obesity and leptin on cognitive function and neuroimaging biomarkers.

Jacob Jones. Neuropsychology track, DCHP. He is being mentored by Dr. Dawn Bowers. Dr. Cohen will serve as a co-mentor and member of his dissertation committee.

Clinical/translational programs:

New programs:

Two new clinical/translational research programs have been planned with initial steps towards implementation over the next six months.

IOA Brain-Wellness Program: In an effort to catalyze clinical and translational research efforts directed at preserving cognitive and functional health in the elderly, the CAM has moved forward with the development of a Brain-Wellness Program. This clinical program will be part of the new clinical geriatric program that will be housed in the new IOA building. The development of the Brain-Wellness Program coincides with the arrival of Dr. Laurence Sodenberg from Vanderbilt, who will direct clinical geriatrics at UF. The Brain Wellness program will bring together clinicians and clinical researchers focused on age-associated memory and cognitive functioning across clinical disciplines, including Geriatrics, Neurology, Psychiatry, Neuropsychology, Behavioral Medicine, Speech and Language, and Social Work so as to provide an integrated multi-disciplinary program which will provide several clinical services: 1) Dementia screening for Psychiatry at four setting; 2) A multi-disciplinary outpatient clinic in the IOA clinic in which patients with subjective cognitive problems and cognitively healthy adults with concerns about preventing problems can be referred for assessment, management, and preventive interventions. The model for the clinic will be that each patient will have a primary clinician based on the origins of the referral, but will be evaluated by neurology, psychiatry, neuropsychology, and other clinical disciplines based on the nature on the case. A consensus conference will be help weekly with adjudication of diagnosis and planning of future assessments and interventions. This clinic will interface with the operations of the geriatric clinic that will share a common space. Patients diagnosed with dementia or other neurological conditions will be referred to the specialty clinics including the Movement Disorder Program and the Memory Disorders/AD program. Neurology, Psychiatry and Neuropsychology fellows with perform clinical assessments under the supervision of faculty from these departments. This clinic will serve as a rich source for future CAM based clinical research studies.

HIV-Aging:

Steps have been taken to build the infrastructure and academic support necessary for continuation of current lines of neuroHIV research conducted by Dr. Cohen at Brown University. Collaboration has been initiated with Dr. Robert Cook who is leading efforts at UF to develop a HIV consortium that may eventually lead to a CFAR. We have proposed a pilot project to facilitate this effort on which Dr. Cook and I will serve as co-PIs. A larger R01 grant is being planned to use functional imaging methods to study HIV effects on the aging brain with Drs. Cook, Ding (BRS) and several other UF faculty members.

Faculty Recruitment: The CAM is currently recruiting for two faculty members with expertise and a strong research record in neuroimaging, cognitive neuroscience and aging. Three candidates have come for first interviews. Dr. Kirk Erickson came for a second interview and an offer was made, though unfortunately he decided to stay at Univ. of Pitt. The other two candidates are still interested, but are not in a position to move forward at this point. Two other individuals are coming for first interviews; Drs. Richard Briggs and Adam Woods. Dr. Briggs was previously a professor at UF and has been at Univ.

Texas in Dallas for about 10 years. He is a well-established and highly recognized neuroimaging researcher and MR methods specialist with expertise in FMRI, ASL and MRS. Dr. Woods is completing a fellowship at Univ. Penn.

In addition, to external recruitment, a concerted effort has been directed at bringing together researchers from within the medical center and the university as a whole with strong interests and background in the aging brain. Several faculty already in the IOA have asked to be formally affiliated with CAM, including Drs. Steve Anton, Manini, and Leeuwenburgh. Several faculty members from the Department of Clinical and Health Psychology have become affiliate faculty, including: Drs. Dawn Bowers, Russell Bauer, Michael Marsiske, Cate Price, and Vonetta Dodson. Dr. Jamie Reilly from the Speech and Language Dept. is now an affiliate faculty member and collaborating with Dr. Cohen on semantic memory studies. Dr. Mingzao Ding from bioengineering asked to join CAM faculty, as did Dr. Song Lai, the AMRIS biophysicist in charge of the 3T scanner. Both are now affiliate faculty of the CAM. Most recently, Dr. Natalie Ebner, a cognitive neuroscientist has joined the CAM faculty and is being mentored by Dr. Cohen. The full list of current affiliate faculty is listed in Appendix 1.

Update on existing clinical studies:

HIV studies: All current clinical studies being conducted by Dr. Cohen as PI are being performed at the CFAR in Providence, RI. These studies are being directed by Dr. Cohen in the CAM, with some of the neuroimaging analyses performed in his laboratory here. This line of research will be continued with grants submitted through UF.

LIFE-ancillary study: The LIFE scientific advisory board approved the CAM proposal to conduct an ancillary study to collect multimodal neuroimaging data, neurocognitive measures (including NIH Toolbox), and select measures from the McKnight cognitive workgroup battery. We will also collect blood samples for biomarkers analysis. This study will provide a wealth of data from cognitively healthy older adults who are currently involved in the LIFE study, enabling physical activity and other clinical measures to be examined relative to neuroimaging to support subsequent grant submissions.

Technology transfer:

a. Patents applications : N/A
b. Revenue generated from technology : N/A

Budget update:

a. Status of matching funds, if applicable : N/A
b. Projected budget for coming year: Financial summaries start on page 63
c. Extramural funding: N/A

5 R34 DA031057-02 (Ronald Cohen, PI)
09/30/10-08/31/13
"Improving Adherence and Cognition in Substance-Using HIV Patients"

Substance abuse in the context of HIV is a major problem that affects clinical outcome and interferes with treatment adherence. This study examines the value of a computer-based cognitive training program (Vigorous Mind) to enhance attention and executive functioning as a means of improving organizational, planning ability, and treatment adherence. The study focuses on further development of this intervention.

Role: Principal Investigator

5 P01 AA019072-02 (Peter Monti, PI)
09/30/10-08/31/15
"Alcohol and HIV: Biobehavioral Interactions and Intervention"

This study focuses on the interactive effects of HIV and alcohol use on metabolic-vascular disturbances underlying brain dysfunction.
Role: Principal Investigator of Project 1

5 R01 MH074368-07 (Ronald Cohen, PI)
09/30/06–08/31/13 (no-cost extension)
"Age Effects on HIV-Associated Brain Dysfunction"

The goal of this project is 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.
Role: Principal Investigator

5 R01 HL089311-04 (John Gunstad, PI)
09/15/08-11/30/13
"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: Principal Investigator of Subcontract

5U01 CA1503878-03 (Rena Wing, PI)
09/28/09-08/31/14
"Increasing Sleep Duration: A Novel Appraoch to Weight Control"

The purpose of the project is to translate the basic science on sleep duration into a novel intervention to reduce obesity and obesity-related co-morbidities.
Role: Co-Investigator

Educational programs focusing on age related memory loss:

Scientific:

Submission of a T32 post-doctoral training grant is planned that will focus on neuroimaging in the study of age-associated memory and cognitive decline. This T32 training program would capitalize on existing expertise in neuroimaging, neuroscience, and aging within the MBI and IOA, as well as past success of the DCHP in obtaining T32 funding for pre-doctoral research training. This program would differ from the existing T32 program at UF in that it would focus exclusively on post-doctoral research training and preparing fellows for neuroimaging research careers focusing on the aging brain. The current T32 trains only pre-doctoral students and focuses on cognitive function only.

Public:
N/A

Collaborative programs with other McKnight Institutes, institutions programs:

A major effort emphasis has been directed at integration of the CAM with ongoing initiatives of the UF MBI and also the four McKnight Institutes. In particular, the CAM is coordinating the efforts of the Cognitive and Neuroimaging workgroups. Dr. Dawn Bowers is leading efforts on the Cognitive Workgroup, Dr. Dawn Bowers is leaning that workgroup and reports to Dr. Cohen. The Neuroimaging Imaging Workgroup met for the first time in New Orleans this summer. Dr. Cohen is the representative from UF on this workgroup. The plan is to generate a series of papers focusing on neuroimaging for the study of age-associated memory and cognitive function. Dr. Cohen is leading one of two papers on structural imaging methods.

Collaborative program with non-McKnight Institutes, institutions and research programs:

The CAM is collaborating with Dr. Ronjon Dura and his colleagues at Mt. Sinai in Miami and investigators from UF (Cohen, Golde, Nelson, Cottler, Ashezawa, Bauer) on the development of a UF-Mt. Sinai ADCC that would make use of large existing clinical populations of healthy normal and MCI patients, and UF providing scientific infrastructure, including resources of the CAM, IOA and MBI.

Briefly describe plans for future research and/or clinical initiatives:

Bariatric surgery grant. A R01 to study the effects of weight loss following bariatric surgery on brain structure and function among people with and without Type 2 diabetes was submitted and is under review by NIH. Dr. Cusi and several other faculty from UF are co-investigators on this project.

Neuroimaging the effects of increased cardiac output followingresynchronization on brain function. A R01 submission is planned for the spring that would involve faculty from cardiology, the CAM, and AMRIS.

TBI neuroimaging studies. On the request of Dr. Kevin Wang and other investigators at UF and the VA, a DOD proposal was submitted to study TBI effects sustained from blasts in combat versus professional football. A similar proposal was generated for a CTRI proposal to study ex- NFL players who had sustained multiple TBIs.

LIFE-Extension: The CAM has collaborated with Dr. Pahor on the LIFE-Extension proposal, specifically on the inclusion of cognitive measures not currently included in LIFE and also neuroimaging. Dr. Cohen is committing 10% effort to this study. The new endpoint for LIFE_ES is conversion to MCI.

LIFE-ARISE: The CAM is playing a primary role in a new proposal for a R01 study designed to examine the prevention of cognitive decline through exercise. The study will examine neuroimaging endpoints (hippocampal volume, resting-state BOLD), as well as memory and will attempt to delineate mechanisms and risk factors that predict who received neural benefits from physical activity.

Anti-inflammatory intervention study: A R01 project is being planned for a submission late next year to examine novel pharmacological approaches to reduce systemic inflammation that may reduce age-associated cognitive problems related to this factor.

P01 Project. A P01 project that would involve that CAM (Cohen), ARML (Foster), IOA (Pahor, Anton, Leeuwenburgh) is being planned for a submission next year. It will likely focus on vascular and metabolic influences on brain structure and function occurring in the context of aging. It will contain both human

and laboratory animal projects and cores to support the effort. We are contemplating an anti-inflammatory intervention to provide an experimental manipulation for this study.

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

Please describe any negative events (loss of personnel, space, budget, etc.) that occurred during the report period and the possible impact on carrying out the Gift Agreement:

We have experienced some difficulty in our initial recruitment efforts for external faculty to join the CAM. Dr. Kirk Erickson was very close to coming, but decided after two interviews and much deliberation to stay at Pitt. Dr. Simon Davis remains interested in the position, but is unable to move until his fiancé is closer to completing her dissertation at Duke. Dr. Greg Saminez-Larkin's wife just had a baby. He is taking time, until things settle on this front.

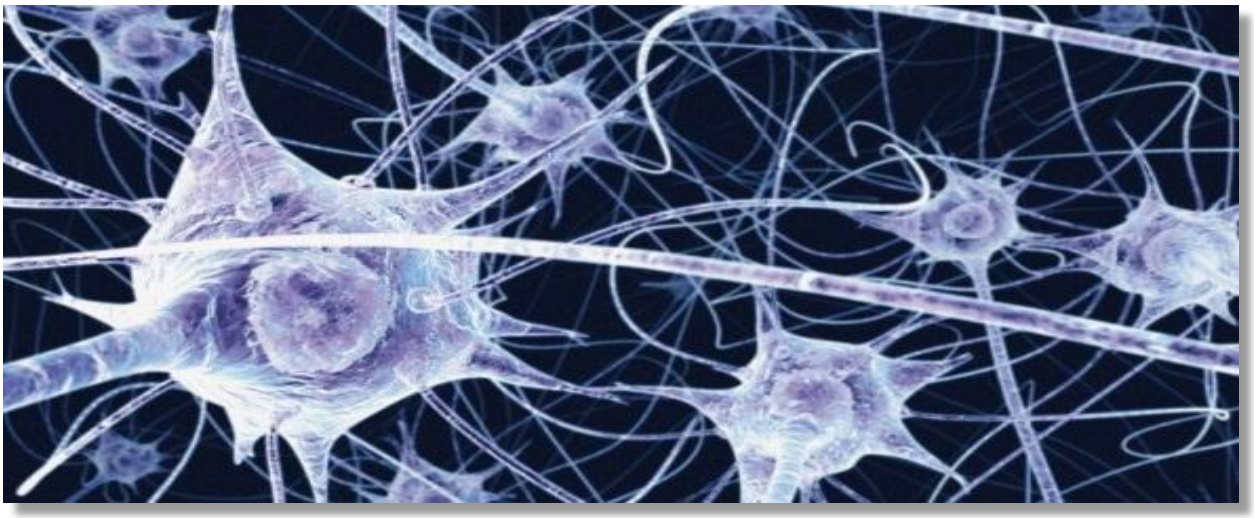
Please provide any general comments or thoughts not covered elsewhere – a response is not required. Please respond only if you would like to add something not otherwise covered elsewhere:

An issue that has impacted the decision making of at least one faculty candidate (Dr. Larkin) is the lack of PET imaging research facilities nearby. He has two R01s that involve PET. He cannot effectively pursue his line of work that involves dopamine imaging without resources in this area.

Signature, date, and title of person submitting the report,

January 9, 2013

Ronald A. Cohen, PhD, ABPP, ABCN
Director, Center for Cognitive Aging and Memory
Professor, Departments of Neurology, Psychiatry, and Aging and Geriatric Research
College of Medicine



2012 Annual Report

Program Financials

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

UF | UNIVERSITY of FLORIDA
The Foundation for The Gator Nation

UF UNIVERSITY of FLORIDA

Office of the Senior Vice President, Health Affairs
Finance and Planning

PO Box 100014
Gainesville, FL 32610-0014
352-273-5420 Phone
352-392-9395 Fax

January 9, 2012

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

Dear Trustees:

Enclosed are the following summary Income and Expenditure statements for the year ending December 31, 2012:

- McKnight Brain Research Grant (F008057/58)
- McKnight Chair (F007889/90)
- CAM-CTRP Budget (F016327)

These reports have been prepared by the respective units and reviewed by me. I believe they represent their financial activities for the 2012 calendar year.

Thank you for supporting the advancement of UF's Age-related Memory Loss and Cognitive Aging and Memory Programs. Let me know if you have any questions about this information.

Sincerely,

Russell E. Armistead, MBA, CPA
Associate Vice President for Finance & Planning
UF Health Science Center

McKnight Brain Research Grant

Age-related Memory Loss Program

Financial Summary

January 1, 2012 to December 31, 2012

Foundation Spendable Account		**Amount**
Endowment income transferred in:		
March 31, 2012	$	256,575
June 30,2012		256,575
Sept 30, 2012		256,575
Dec 31, 2012		256,575
Total endowment income transferred in		1,026,300
Transferred out:		
Dr. Bowers (VITAL Project), adjustment		465
Transferred to UF Peoplesoft spendable accounts		- [1]
Total transferred out		465
Net change in foundation spendable account		1,025,835
Beginning balance, January 1, 2012		399,942
Ending balance, December 31, 2012	**$**	**1,425,777**

Allocation of ending balance:

Amount due to the Institute on Aging / CAM-CTRP[2]	$	784,804	
Remaining funds for Age Related Memory Loss		640,973	**$ 1,425,777**

UF PeopleSoft Accounts		**Amount**
Received from foundation spendable account	$	- [1]
Transfers / awards to seed grants:		
Dr. Bowers (VITAL Project)		100,000
Dr. Frazier seed grant		100,000
Total transfers to seed grants		200,000
Expenditures:		
Dr. Ormerod seed grant, ended May 31, 2012		21,530
Recruitment expenses		10,016
Travel, Publications, and Other		18,591
Total expenditures		50,137
Total transfers out and expenditures		250,137
Net change in UF Peoplesoft accounts		(250,137)
Beginning balance, January 1, 2012		750,969
Ending balance, December 31, 2012	**$**	**500,832**

[1] No transfers to UF Peoplesoft accounts in 2012

[2] Accumulation of the Institute on Aging's portion of the endowment interest income (50%), per the 2009 Gift Agreement Amendment. Scheduled to be transferred in Jan 2013.

McKnight Brain Research Grant

Age-related Memory Loss Program

Seed Grant Balances

January 1, 2012 to December 31, 2012

Dr. Bowers - VITAL Project (UF PeopleSoft Accounts)		**Amount**
Received from MBRF grant spendable UF account	$	100,000
Expenditures:		
Salary expenses		115,515
Operating expenses (lab supplies, services, and other)		11,547
Total expenditures		127,062
Net change in UF Peoplesoft accounts		(27,062)
Beginning balance, January 1, 2012		61,605
Ending balance, December 31, 2012	**$**	**34,543**

Dr. Frazier - Seed Grant (UF PeopleSoft Accounts)		**Amount**
Received from MBRF grant spendable UF account	$	100,000
Expenditures:		
Salary expenses		53,626
Operating expenses (lab supplies, services, and other)		4,687
Total expenditures		58,313
Net change in UF Peoplesoft accounts		41,687
Beginning balance, January 1, 2012		67,815
Ending balance, December 31, 2012	**$**	**109,502**

Dr. Ormerod - Seed Grant (UF PeopleSoft Accounts)		**Amount**
Received from MBRF grant spendable UF account	$	-
Expenditures:		
Operating expenses (lab supplies, services, and other)		21,530
Total expenditures		21,530
Net change in UF Peoplesoft accounts		(21,530)
Beginning balance, January 1, 2012		21,530
Ending balance, December 31, 2012	**$**	**-**

McKnight Endowed Chair

Tom Foster, PhD

Financial Summary

January 1, 2012 to December 31, 2012

Foundation Spendable Account		**Amount**	
Endowment income transferred in:			
March 31, 2012	$	39,121	
June 30,2012		39,121	
Sept 30, 2012		39,121	
Dec 31, 2012		39,121	
Total endowment income transferred in		156,484	
Transferred to UF Peoplesoft spendable accounts[1]		200,000	[1]
Net change in foundation spendable account		(43,516)	
Beginning balance, January 1, 2012		134,363	
Ending balance, December 31, 2012	**$**	**90,847**	

UF PeopleSoft Accounts		**Amount**	
Received from foundation spendable account	$	200,000	[1]
Expenditures:			
Faculty and research staff salaries		160,768	
Research equipment, supplies, and services		65,353	
Travel and other		12,502	
Total expenditures		238,623	
Net change in UF Peoplesoft accounts		(38,623)	
Beginning balance, January 1, 2012		251,640	
Ending balance, Dec 31, 2012	**$**	**213,017**	

[1]Transfers to UF Peoplesoft accounts in 2012

Cognitive Aging & Memory

Clinical Translational Research Program

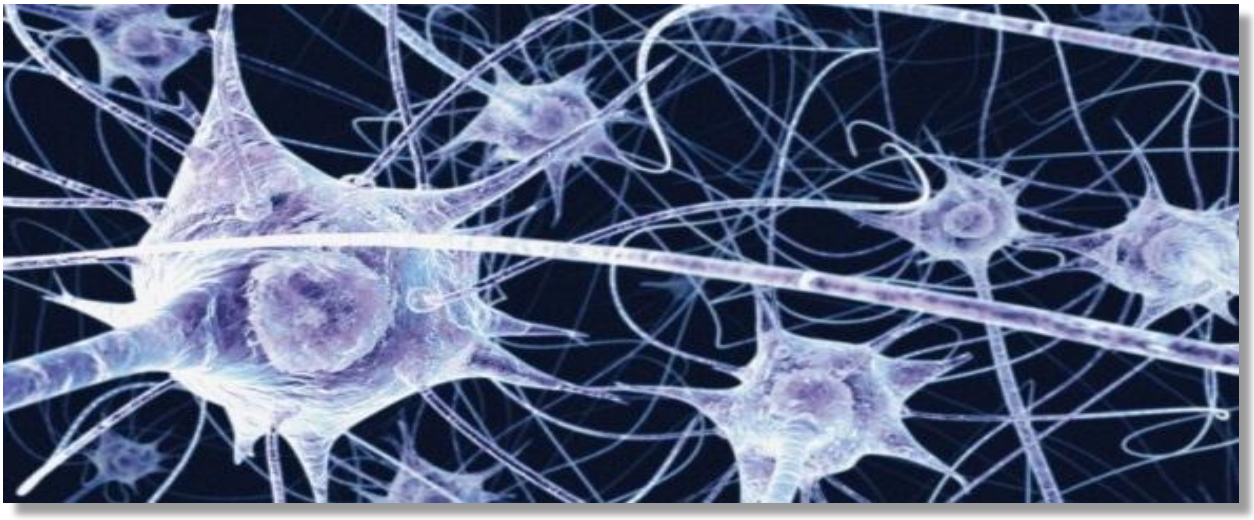
Financial Summary

January 1, 2012 to December 31, 2012

Foundation Spendable Account		**Amount**	
Transferred to UF Peoplesoft spendable accounts[1]	$	400,000	[1]
Net change in foundation spendable account		(400,000)	
Beginning balance, January 1, 2012		2,375,906	
Ending balance, December 31, 2012	**$**	**1,975,906**	
Due from McKnight Brain Research Grant:			
Endowment interest income to be transferred		784,804	
Total funds available to CAM-CTRP, December 31, 2012		**2,760,710**	

UF PeopleSoft Accounts		**Amount**	
Received from foundation spendable account	$	400,000	[1]
Expenditures:			
Faculty and research staff salaries		174,914	
Research equipment, supplies, and services		24,423	
Travel and other		20,183	
Total expenditures		219,520	
Net change in UF Peoplesoft accounts		180,480	
Beginning balance, January 1, 2012		99,973	
Ending balance, Dec 31, 2012	**$**	**280,453**	

[1]Transfers to UF Peoplesoft accounts in 2012



2012 Annual Report

UF Foundation Endowment Reports

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

UF | UNIVERSITY of FLORIDA
The Foundation for The Gator Nation

Evelyn F. McKnight Brain Research Grant

We would like to take this opportunity to express our gratitude for your generous support of UF through this endowment. University of Florida endowments are a vital asset to the ongoing success and sustainability of future research, teaching, and service programs. By establishing predictable, recurring revenue streams, endowments impact programs that cultivate growth in our students and faculty for generations to follow. We are pleased to provide you with the following financial report for Fiscal Year 2012/2013.

BOOK VALUE as of 09/30/12	**$25,967,781**
MARKET VALUE as of 09/30/12	**$29,730,252**
PROJECTED SPENDABLE INCOME for 2012/13	**$1,026,301**

ENDOWMENT MANAGEMENT

Endowment assets are invested through the University of Florida Investment Corporation (UFICO), created in 2004 to manage UF's investment portfolios. UFICO is headed by a Chief Investments Officer who reports to a volunteer Board of Directors and to the President of the University of Florida.

FOR MORE INFORMATION, CONTACT:

Thomas J. Mitchell
Vice President, Development and Alumni Affairs
(352) 392-5407 (tmitchell@uff.ufl.edu)

Cindy Belknap
Director of Stewardship and Donor Relations
(352) 846-3444 (cbelknap@uff.ufl.edu)

(Fund # 008057)

Evelyn F. McKnight Chair for Brain Research in Memory Loss

We would like to take this opportunity to express our gratitude for your generous support of UF through this endowment. University of Florida endowments are a vital asset to the ongoing success and sustainability of future research, teaching, and service programs. By establishing predictable, recurring revenue streams, endowments impact programs that cultivate growth in our students and faculty for generations to follow. We are pleased to provide you with the following financial report for Fiscal Year 2012/2013.

BOOK VALUE as of 09/30/12	**$3,995,677**
MARKET VALUE as of 09/30/12	**$4,533,120**
PROJECTED SPENDABLE INCOME for 2012/13	**$156,485**

ENDOWMENT MANAGEMENT

Endowment assets are invested through the University of Florida Investment Corporation (UFICO), created in 2004 to manage UF's investment portfolios. UFICO is headed by a Chief Investments Officer who reports to a volunteer Board of Directors and to the President of the University of Florida.

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(352) 846-3444 (cbelknap@uff.ufl.edu)

(Fund # 007889)

McKnight Brain Research Foundation

Evelyn F. McKnight Brain Research Grant (008057)

Spendable Fund Transfers since endowment inception

FY 2012/2013	**$256,575**	**(09/30/12 YTD)**
FY 2011/2012	**$1,026,301**	
FY 2010/2011	**$971,846**	
FY 2009/2010	**$941,689**	
FY 2008/2009	**$1,086,475**	
FY 2007/2008	**$1,172,824**	
FY 2006/2007	**$1,056,031**	
FY 2005/2006	**$881,347**	
FY 2004/2005	**$843,131**	
FY 2003/2004	**$729,335**	
FY 2002/2003	**$651,801**	
FY 2001/2002	**$657,852**	
FY 2000/2001	**$648,384**	
TOTAL	**$10,923,591**	

Evelyn F. McKnight Chair for Brain Research in Memory Loss (007889)

Spendable Fund Transfers since endowment inception

FY 2012/2013	**$39,121**	**(09/30/12 YTD)**
FY 2011/2012	**$156,485**	
FY 2010/2011	**$148,182**	
FY 2009/2010	**$143,584**	
FY 2008/2009	**$165,660**	
FY 2007/2008	**$178,827**	
FY 2006/2007	**$161,019**	
FY 2005/2006	**$134,384**	
FY 2004/2005	**$127,813**	
FY 2003/2004	**$124,127**	
FY 2002/2003	**$125,768**	
FY 2001/2002	**$100,869**	
FY 2000/2001	**$99,417**	
FY 1999/2000	**$3,438**	
TOTAL	**$1,708,694**	

FOR MORE INFORMATION, CONTACT:

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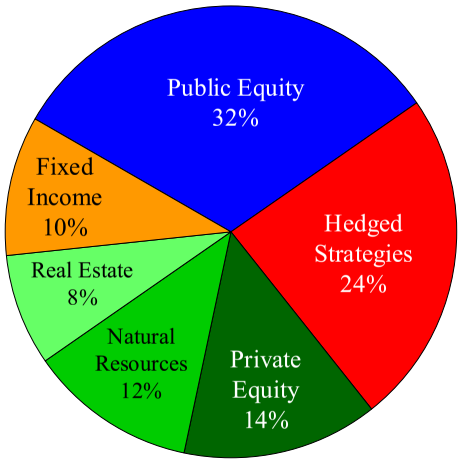
McKnight Brain Research Foundation

UF Investment Corporation Update
December 2012

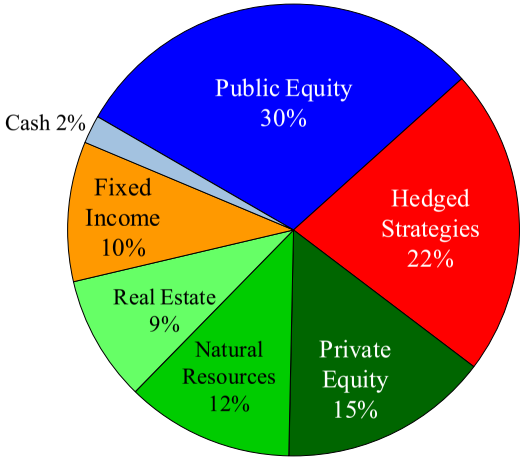
UF | UNIVERSITY of FLORIDA INVESTMENT CORPORATION

Endowment Asset Allocation Review

Strategic Target Allocation



Active Target Allocation



Actions in 3rd and 4th Quarters:

- Decreased allocation to US Equity
- Increased allocation to Emerging Markets
- Increased allocation to Fixed Income via futures position
- Increased Cash position

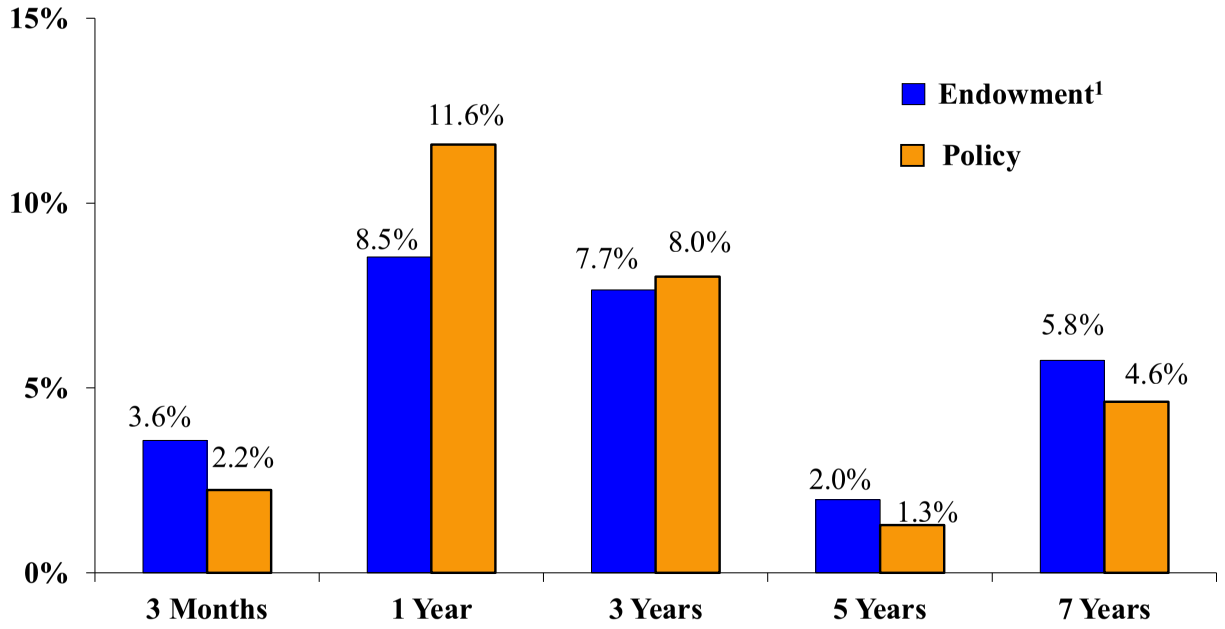
Asset Class	Active Target	Actual 6/30/2012	Actual 9/30/2012	Actual 11/30/2012
Public Equity	30.0%	29.3%	29.4%	30.4%
Hedged Strategies	22.0%	20.9%	20.5%	19.5%
Private Equity	15.0%	16.1%	15.4%	15.1%
Natural Resources	12.0%	11.5%	12.1%	12.3%
Real Estate	9.0%	10.6%	10.0%	9.8%
Fixed Income	10.0%	9.2%	9.6%	9.6%
Opportunistic	0.0%	0.0%	0.1%	0.8%
Cash	2.0%	2.4%	2.9%	2.5%
Total	100.0%	100.0%	100.0%	100.0%

Current Portfolio Positioning

- Public Equity
  - Underweight strategic target allocation
  - Underweight Europe, US
  - Overweight Canada, Emerging Markets
- Fixed Income
  - Slight underweight strategic target allocation
  - Overweight nominals / Underweight inflation-linked
  - Short duration
- Hedged Strategies
  - Underweight strategic target allocation
- Private Markets
  - Overweight strategic allocation to Real Estate
  - Overweight strategic allocation to Private Equity
- Cash
  - Overweight strategic target allocation

UFF Endowment Portfolio
Performance

Endowment Pool Returns
Trailing Period Returns as of November 30, 2012



[1] Actual returns are net of all investment management fees, but gross of UFF annual management fees.

UFF Endowment Portfolio
Performance

As of November 30, 2012

UFF Endowment	1-Quarter	1-Year	3-Years	5-Years	7-Years	ITD[2]
Public Equity	**3.77%**	**10.89%**	**8.11%**	**0.04%**	**4.81%**	**5.97%**
MSCI ACWI	*3.77%*	*13.33%*	*6.56%*	*-1.82%*	*3.55%*	*5.27%*
Hedged Strategies	**-0.67%**	**-0.18%**	**1.94%**	**-0.61%**	**3.43%**	**4.59%**
HFRI FoF Diversified	*0.92%*	*3.16%*	*1.55%*	*-1.67%*	*1.76%*	*2.72%*
Fixed Income	**1.70%**	**8.54%**	**7.80%**	**5.95%**	**6.44%**	**6.27%**
Benchmark [1]	*0.97%*	*5.52%*	*6.98%*	*6.69%*	*6.52%*	*6.20%*
Private Equity	**1.84%**	**8.95%**	**15.72%**	**7.86%**	**11.41%**	**11.93%**
S&P 500 + 3%	*2.02%*	*19.57%*	*14.56%*	*4.38%*	*7.10%*	*7.89%*
Natural Resources	**18.38%**	**25.06%**	**17.00%**	**12.03%**	**8.14%**	**9.69%**
S&P 500 + 3%	*2.02%*	*19.57%*	*14.56%*	*4.38%*	*7.10%*	*7.89%*
Real Estate	**1.57%**	**4.88%**	**3.89%**	**-2.46%**	**2.63%**	**3.62%**
NCREIF	*2.34%*	*11.00%*	*10.90%*	*2.26%*	*6.39%*	*7.94%*
Cash	**0.07%**	**0.38%**	**0.30%**	**1.00%**	**2.09%**	**2.19%**
Citi 90 Day T-bills	*0.02%*	*0.07%*	*0.09%*	*0.51%*	*1.70%*	*1.82%*
Total Endowment Fund	**3.58%**	**8.54%**	**7.65%**	**1.98%**	**5.75%**	**6.45%**
Policy Benchmark	*2.24%*	*11.58%*	*8.01%*	*1.29%*	*4.63%*	*5.55%*

Notes:

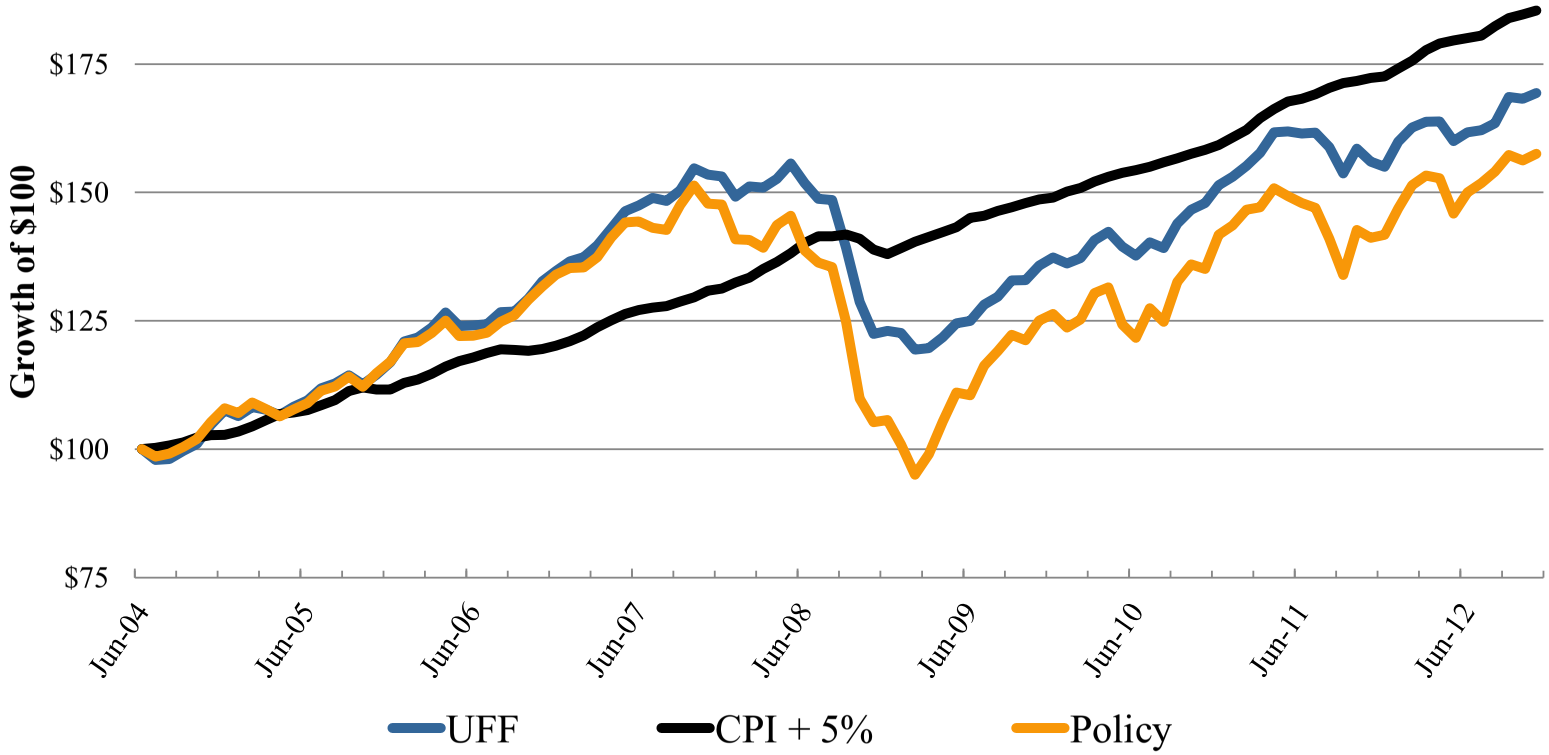
- Returns are annualized for periods over 1 year
- Shaded areas represent periods of outperformance

[1] Fixed Income Benchmark: 7/1/04-6/30/11 - 100% Barclays Universal; As of 7/1/11 - 50% Barclays Gov't Index / 50% Barclays US Inflation Protected

[2] UFICO's inception date of July 1, 2004

UFF Endowment Portfolio
Performance

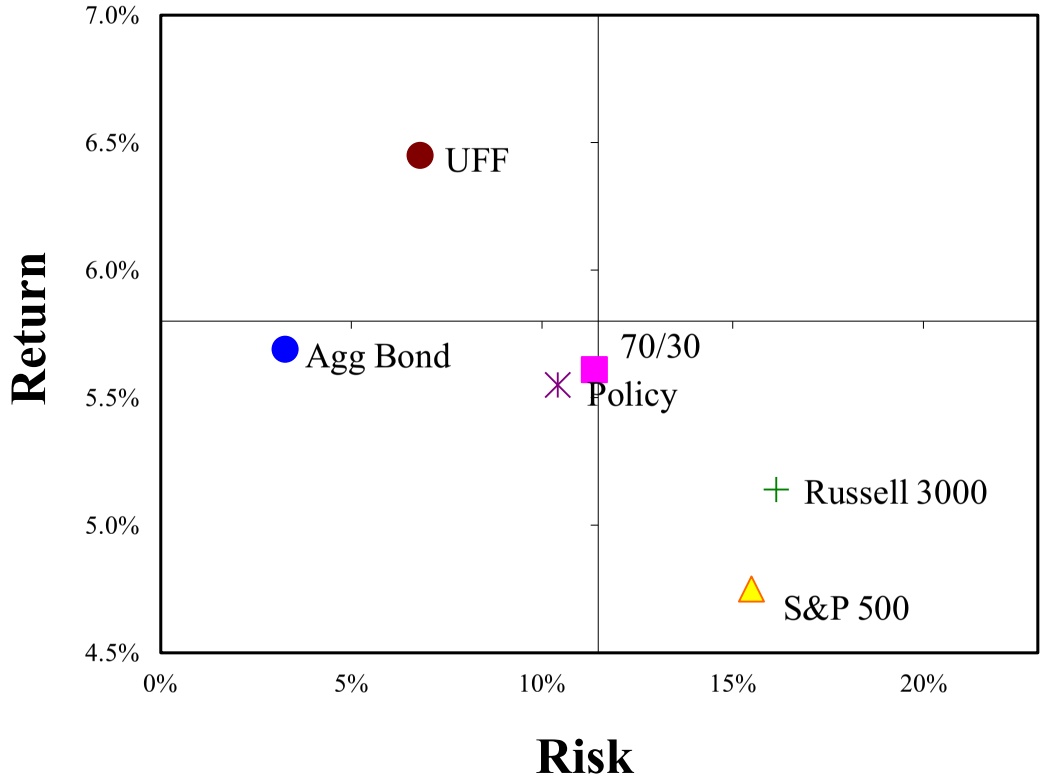
Purchasing Power
Inception[1] to November 30, 2012



[1] UFICO inception of June 2004.

UFF Endowment Portfolio
Risk

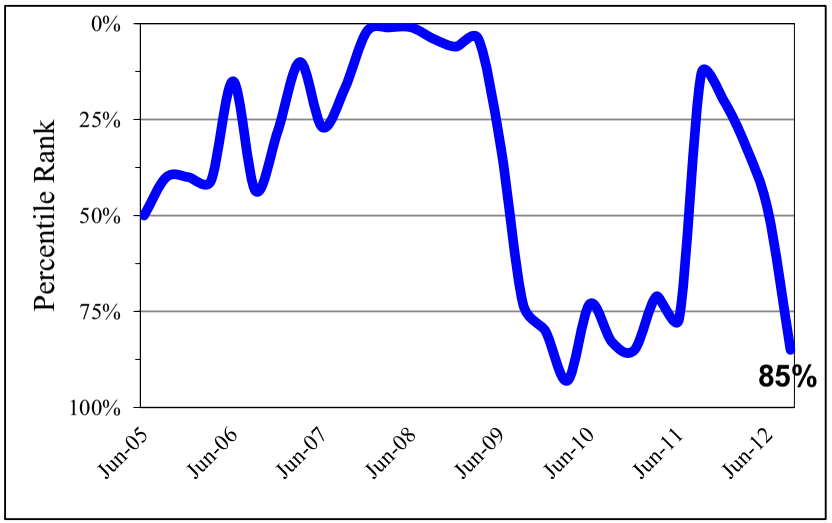
Risk / Return
UFICO Inception to 11/30/2012



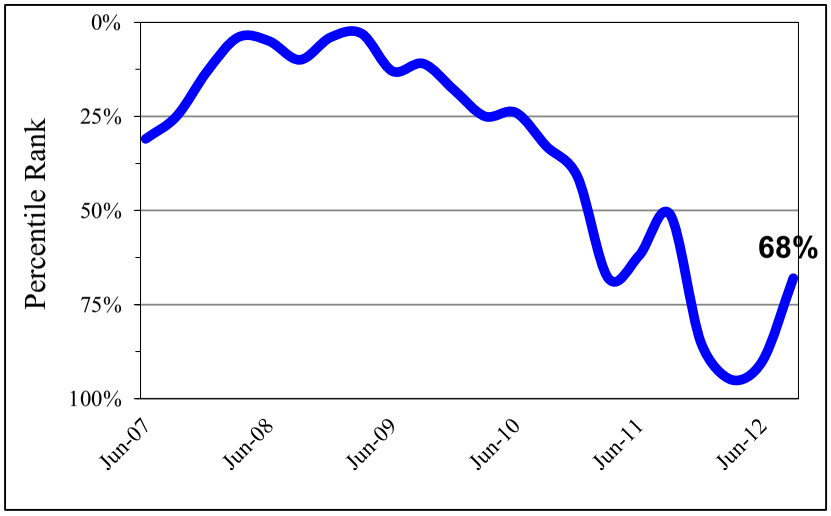
UFF Endowment Portfolio
Peer Comparison

Callan Associates
Mid-size Endowment & Foundation Universe
As of September 30, 2012

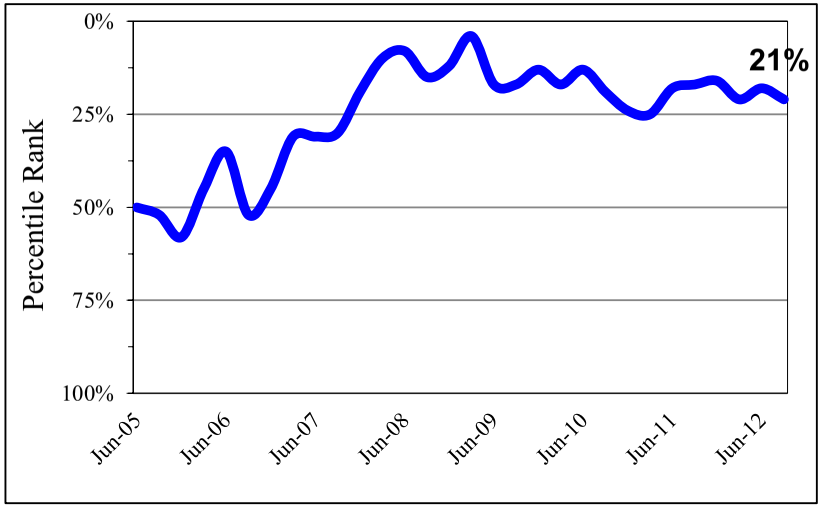
Rolling One-Year Percentile Rank

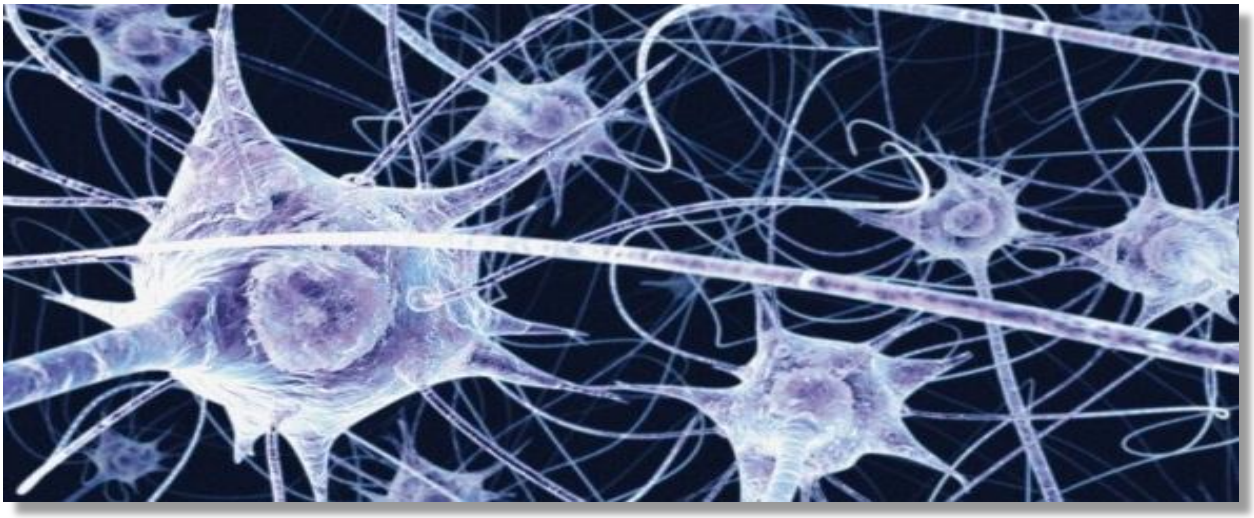


Rolling Three-Year Percentile Rank



UFICO Inception-to-Date





2012 Annual Report

Faculty Biographical Sketches

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

UF | UNIVERSITY of FLORIDA
The Foundation for The Gator Nation

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES**..

NAME	POSITION TITLE
Ashizawa, Tetsuo	Chairman and Professor, Neurology
eRA COMMONS USER NAME TEASHIZA	

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

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
Keio University, Kanagawa, Japan	Premed	1967-1969	Premed
Keio University School of Medicine, Tokyo, Japan	M.D.	1969-1973	Medicine
U.S. Naval Hospital, Yokosuka, Japan	Intern	1973-1974	Rotating Internship
Allegheny General Hospital, Pittsburgh, PA	Intern	1974-1975	Internal Medicine
Baylor College of Medicine, Houston, Texas	Resident	1975-1978	Neurology
Baylor College of Medicine, Houston, Texas	Fellow	1978-1979	Neuromuscular Disease
Baylor College of Medicine, Houston, Texas	Fellow	1979-1981	Neurochemistry

A. Personal Statement

The goal of the proposed research is to develop a high throughput screening assay for small molecules for treatment of spinocerebellar ataxia type 10 (SCA10) using a novel vector in which binding of hnRNP K to the 3'UTR of the reporter gene mRNA alters its translation. I have identified a large expansion of an ATTCT pentanucleotide repeat in intron 9 of the *ATXN10* gene on chromosome 22. I have developed cell culture and transgenic mouse models of SCA10 and obtained brain tissues from a rare SCA10 autopsy case, and investigated the pathogenic mechanism of SCA10. I found that the mutant intron containing the expanded AUUCU repeat is correctly spliced but not degraded, and accumulated AUUCU repeats become toxic to cells by sequestering hnRNP K, leading to cell death. Because the number of ATTCT's is polymorphic, the SCA10 pentanucleotide repeat is unlikely to be included in any open reading frame. I also found that the SCA10 mutation neither changes the splicing pattern of the *ATXN10* transcript nor alters expression levels of surrounding genes. We confirmed that the Ataxin 10 mRNA and protein are not altered in patients' cells. Thus, SCA10 can be considered as a prototype of RNA-gain-of-function disorders and the optimal target for treatments based on RNA inhibition. I have obtained preliminary data showing that overexpression of hnRNP K partially reverses the pathogenic process in cells expressing expanded AUUCU repeats. With this background, I believe blocking the interaction between hnRNP K and the mutant AUUCU repeat RNA will be a useful approach to alleviate the pathogenic mechanism of SCA10. Thus, I am in an excellent position to collaborate with Dr. Karen McFarland to develop the high throughput screening assay for treatment of SCA10.

B. Positions and Honors.

Positions and Employment

1981-2002	Assistant Professor (1981-1992), Associate Professor with tenure (1992-1997) and Professor with tenure, Department of Neurology Baylor College of Medicine, Houston, TX
1985-2002	Assistant Chief (Acting Chief,1988), Neurology Care Line, VA Medical Center, Houston, TX
2002-2009	Chair, John Sealy Professor, Dept. of Neurology, University of Texas Medical Branch (UTMB), School of Medicine, Galveston, TX
2002-present	Adjunct Professor, Department of Neurology, Baylor College of Medicine. Houston, TX
2009-present	Chair, Melvin Greer Professor, Dept of Neurology, University of Florida, Gainesville, FL
2010-present	Executive Director, McKnight Brain Institute, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

1998	Founder, International Myotonic Dystrophy Consortium (IDMC)
2001-present	Member, Scientific Advisory Committee, National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy, (NIAMS, NINDS, P.I.: Richard Moxley III)
2005, 2006	Chair, Scientific Review Group ZNS1 SRB-R 16 1/ZNS1 SRB-A (37), NIH Gene Discovery

2007-2011 Member, Scientific Advisory Board for A Genetic Linkage Study for GTS (TSAICG; 2U01NS040024-06A1; P.I. David Pauls)
2007-2011 Regular Member, NIH Cell Death in Neurodegeneration (CDIN) Study Section
2008-present Member, Scientific Advisory Committee, Myotonic Dystrophy Foundation
2009-2012 Chair, Steering Committee, the Clinical Research Consortium for Spinocerebellar Ataxias (NIH/NINDS, ORDR) and member, the Rare Disease Clinical Research Network Steering Committee (NIH/ORDR)
2009-present Member, Myotonic Dystrophy Therapeutic Development, Marigold Foundation
2010-present Lead Author, Panel for the American Academy of Neurology Evidence-based Guidelines for Myotonic Muscular Dystrophy
2010-present President, Strategic and Therapeutic Development Committee (COSET) for Myotonic Dystrophy Gene Therapy, Association Française contre les Myopathies (AFM); regular member, AFM COSET

Honors

2004 "Promoting Excellence In End-of-Life-Care": the Huntington's Disease Peer Workgroup, The Robert Wood Johnson Foundation
2005 "The Team Hope Award" for Medical Leadership: the Huntington's Disease Society of America
2006 "Medical Research Award": the Myotonic Dystrophy Assistance & Awareness Support Group
2009 "Steinert Medal": the IDMC7
2011 "Faculty member": Alpha Omega Alpha (AOA)
2011 "Best Doctors in America"

C. Selected Peer-reviewed publications (in chronological order). From 143 peer-reviewed papers.

Most relevant to the current application

1. Matsuura, T., Yamagata, T., Burgess, D.L., Rasmussen, A., Grewal, R.P., Watase, K., Khajavi, M., McCall, A., Caleb F. Davis, C.F., Zu, L., Achari, M., Pulst, S.M., Alonso, E., Noebels, J.L., Nelson, D.L., Zoghbi, H.Y., Ashizawa, T. (2000) Large Expansion of ATTCT Pentanucleotide Repeat in Spinocerebellar Ataxia Type 10. Nature Genetics, 26,191-194.
2. Matsuura T, Fang P, Lin X, Khajavi M, Tsuji K, Rasmussen A, Grewal RP, Achari M, Alonso ME, Pulst SM, Zoghbi HY, Nelson DL, Roa BB, Ashizawa T. (2004) Somatic and germline instability of the ATTCT repeat in spinocerebellar ataxia type 10. American Journal of Human Genetics, 74, 1216-1224.
3. Wakamiya M, Matsuura T, Liu Y, Schuster GC, Gao R, Xu W, Sarkar PS, Lin X, Ashizawa T. (2006) The role of ataxin 10 in the pathogenesis of spinocerebellar ataxia type 10**.** Neurology, 67, 607-613.
4. White MC, Gao R; Xu W, Mandal SM, Lim JG, Hazra TK, Wakamiya M, Edwards SF, Raskin S, Teive HAG, Zoghbi HY, Sarkar PS, Ashizawa T. (2010) Inactivation of hnRNP K by Expanded Intronic AUUCU Repeat Induces Apoptosis via Translocation of PKCδ to Mitochondria in Spinocerebellar Ataxia 10. PLoS Genetics, 6, e1000984.
5. White M, Xia G, Gao R, Wakamiya M, Sarkar PS, McFarland KN, Ashizawa T. (2012) Transgenic mice with SCA10 pentanucleotide repeats show motor phenotype and susceptibility to seizure. Journal of Neuroscience Research, 90, 706-714.

Additional publications of importance to the field (in chronological order)

1. Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY, Lee CC. (1997) Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α 1A-voltage-dependent calcium channel. Nature Genetics, 15, 62-69.
2. Monckton DG, Cayuela ML, Gould FK, Brock GJR, de Silva R, Ashizawa T. (1999) Massive (CAG)n DNA repeat expansions in the sperm of spinocerebellar ataxia type 7 males. Human Molecular Genetics, 8, 2473-2478.
3. Teive HAG, Roa BB, Raskin S, Fang P, Arruda WO, Neto YC, Gao, R., Werneck LW, Ashizawa T. (2004) Clinical Phenotype of Brazilian Families with Spinocerebellar Ataxia 10. Neurology, 63, 1509-1512.

4. Gatto EM, Gao R, White MC, Uribe Roca MC, Etcheverry JL, Persi G, Poderoso JJ, Ashizawa T. (2007) Ethnic origin and extrapyramidal signs in an Argentinean spinocerebellar ataxia type 10 family. Neurology, 69, 216-218.
5. Gao R, Matsuura T, Coolbaugh M, et al., Ashizawa T, Lin X. (2007) Instability of expanded CAG/CAA repeats in spinocerebellar ataxia type 17. European Journal of Human Genetics, 16, 215-222. [PMC Journal – in process]
6. Teive HA, Munhoz RP, Raskin S, Arruda WO, et al. and Ashizawa T. (2010) Spinocerebellar ataxia type 10: Frequency of epilepsy in a large sample of Brazilian patients. Movement Disorders, 25, 2875-2878.
7. Teive HA, Munhoz RP, Ashizawa T. (2011) New gene of spinocerebellar ataxia. Brain, 134, e179. [PMID:21357611]
8. Cherng N, Shishkin AA, Schlager LI, Tuck RH, Sloan L, Matera R, Sarkar PS, Ashizawa T, Freudenreich CH, Mirkin SM. (2011) Expansions, contractions, and fragility of the spinocerebellar ataxia type 10 pentanucleotide repeat in yeast. Proceedings of the National Academy of Science USA, 108, 2843-2848.
9. Teive HA, Arruda WO, Raskin S, Munhoz RP, Zavala JA, Werneck LC, Ashizawa T. (2011) Symptom onset of spinocerebellar ataxia type 10 in pregnancy and puerperium. Journal of Clinical Neuroscience, 18, 437-438.
10. Subramony SH, Ashizawa T, Langford L, McKenna R, Avvaru B, Siddique T, Vedanarayanan V. (2011) Confirmation of the severe phenotypic effect of serine at codon 41 of the superoxide dismutase 1 gene. Muscle Nerve, 44, 499-502.

D. Research Support

Ongoing Research Support

1RC1NS068897-01 Ashizawa (PI) 09/30/2009-09/29/2012 NCE
NIH
Clinical Research Consortium for Spinocerebellar Ataxias
The goal of this grant is to establish infrastructure and resources for clinical research on spinocerebellar ataxias. This application is to continue this project.
Role: PI

N/A S.H. Subramony (PI) 07/01/2009-06/30/2012
National Ataxia Foundation
New initiative for clinical research on ataxia
The goal of this grant is to establish and maintain the National Ataxia Registry, which supplements the DMCC's Contact Registry by verifying the diagnosis of the specific type of spinocerebellar ataxias by medical documents.
Role: Co-PI

Completed Research Support

2R01NS041547-06 Ashizawa (PI) 05/01/2006-03/30/2010
Pathogenic Mechanism of Spinocerebellar Ataxia Type 10
The goal of this grant was to investigate the molecular mechanism of spinocerebellar ataxia type 10 in patient-derived tissues/cells, transgenic mouse models and transfected cell culture models. The project is closed, and a new translational proposal is being prepared.
Role: PI

1U01NS050733-01A1 Newsom-Davis (PI) 09/24/2005-08/31/2010
Thymectomy in non-thymomatous MG patients on Prednisone.
The goal of this study was to determine the efficacy of thymectomy in reducing the dose of oral prednisone that is required to adequately treat patients with myasthenia gravis.
Role: site PI

5U01NS052592-05 Cudkowicz (PI) 09/30/2005-11/30/2011
Coenzyme Q10 in Huntington's Disease
The goal of this study was to determine the efficacy of coenzyme Q10 in Huntington's disease. When I left UTMB for UF on 03/31/2009, I transferred the site-PI from me to Dr. George Jackson at UTMB.
Role: site PI

N/A Ashizawa (PI) 09/01/2009-03/31/2010
Muscular Dystrophy Association
7th Internatinal Myotonic Dystrophy Consortium meeting (IDCM7)
The goal of this grant is to support the 7th International Myotonic Dystrophy Consortium meeting held in Wuertzberg, Germany in September 10-12, 2009.
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Jennifer L. Bizon	
eRA COMMONS USER NAME (credential, e.g., agency login) jbizon	Associate 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)*	MM/YY	FIELD OF STUDY
University of North Carolina at Chapel Hill	BS	1993	Psychology
University of California, Irvine	PhD	1998	Neurobiology and Behavior
Johns Hopkins University	Post-doc	1998-2002	Neuroscience

A. Personal Statement

My research program is broadly focused on understanding brain aging and its implications for cognitive functions, including learning, memory, and executive processes. Our central approach involves integrating neuroanatomical, biochemical, and/or pharmacological techniques with cognitive/behavioral variables to better understand how aging alters corticolimbic inhibitory and neuromodulatory circuits, and how such alterations contribute to decline of function across multiple cognitive domains. We are particularly interested in how changes in these systems contribute to age-related decline of executive functions, including working memory, cognitive flexibility, and decision making. A key element of our approach involves the consideration of individual differences in cognitive aging, which can be leveraged to identify and to better understand the relevant cognitive and neural mechanisms that underlie both impaired and successful cognitive outcomes. Our ultimate goal is to target effective compensatory strategies and to develop new approaches for promoting successful cognitive aging.

B. Positions and Honors

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-present Associate Professor of Neuroscience and Psychiatry, University of Florida College of Medicine
2011-present Co-director of Neuroscience Graduate Program, University of Florida College of Medicine

Honors and Professional Activities

Graduated with Highest Honors (Psychology) UNC-Chapel Hill (1993)
Individual NRSA, NIMH F31 pre-doctoral award (1995-1998)
Individual NRSA, NIA F32 post-doctoral award (2001-2003)
Leadership and Service Award, Faculty of Neuroscience, Texas A&M University (2008)
Montague Center for Teaching Excellence Award (2008), College of Liberal Arts, Texas A&M University
Editor, Animal Models of Human Cognitive Aging (2008), *Humana* (*Wiley) Press*

Editorial Board, *Neurobiology of Aging* (2008-present)
Advisory Board, Alzheimer's Drug Discovery Foundation (2010-present)
NIH Special Emphasis Review Panel (ZAG1 ZIJ-5), Bethesda, MD (2009)
NSF Review Panel (Modulatory Brain Systems), Rockville MD (2011)
NIH Review Panel (CNNT) Washington DC (2010, 2011)
NIH Review Panel (CDIN) Washington DC (2012)
Ad Hoc Reviewer for National Science Foundation (2009-present)
McKnight Cognitive Test Battery Working Group (2011-present)

C. Selected Peer-reviewed Publications:

1. McQuail, JA, Bañuelos, LaSarge, CL, Nicolle, MM, Bizon, J.L. $GABA_B$ receptor GTP-binding is decreased in the prefrontal cortex but not the hippocampus of aged rats. ***Neurobiology of Aging***. 33(6):1124.e1-12.
2. Bañuelos, C., Gilbert, R.J., Montgomery, K.S., Fincher, A.S., Wang, H. Frye, G.D., Setlow, B., Bizon. J.L. (2012) Spatial learning impairments in a human third trimester model of binge alcohol exposure in rat. ***Behavioral Pharmacology*** 2012 Feb;23(1):54-65.
3. Gilbert, RJ, Mitchell, M, Simon, NW, Bañuelos, C, Setlow, S, & Bizon, JL. Risk, reward, and decision-making in a rodent model of cognitive aging ***Frontiers in Neuroscience****. 5, 144 Epub. 2012 Jan 3.
4. Lim, C-S Kim, Y-J ,Hwang,Y-K, Bañuelos, C., Bizon, J.L. and Jung-Soo Han *(2012) Decreased interactions in* PKA-GR *signaling in the hippocampus after* selective removal of the basal forebrain cholinergic input. ***Hippocampus****.* Mar;22(3):455-65.
5. Huie, J.R., Garraway, S.M., Baumbauer, K.M., Hoy, K.C., Beas, B.S., Montgomery, K.S., Bizon, J.L., Grau, J.W. (2012) Brain-derived neurotrophic factor (BDNF) promotes adaptive plasticity within the spinal cord and mediates the beneficial effects of controllable stimulation. ***Neuroscience****.* 200: 74-90.
6. Bañuelos, C., LaSarge, C.L., McQuail, JA, Hartman, JA, Gilbert, RJ, Ormerod, BK, Bizon, J.L. Age-related changes in basal forebrain cholingergic and GABAergic neuron number: Relationship with spatial impairment. *In Press.* ***Neurobiology of Aging****.*
7. Mendez, I, Gilbert, RJ, Bizon, JL., Setlow, B. Effects of acute administration of nicotinic and muscarinic cholinergic agonists and antagonists on different forms of cost-benefit decision making. *In Press.* ***Psychopharmacology****.*
8. Alexander, G.E., Ryan, L., Bowers, D. Foster, TC, Bizon, J.L., Geldmacher, D.S., Glisky, E.L. Characterizing cognitive aging in humans with links to animal models. *In Press.* ***Frontiers in Aging Neuroscience****.* 2012;4:21. Epub 2012 Sep 12.
9. Bizon, J.L., Foster, TC, Alexander, GE, Glisky, EL, Characterizing Cognitive Aging of Working memory and executive function in animal models. 2012;4:19. ***Frontiers in Aging Neuroscience****.* 2012;4:12. Epub 2012 Sep 12
10. Foster, T.C., DeFazio, Bizon, J.L., Characterizing cognitive aging of spatial and contextual memory in animal models. ***Frontiers in Aging Neuroscience****.*
11. Roberson, ED, DeFazio, RA, Barnes, CA, Alexander GE, Bizon, JL, Bowers, D, Foster, TC, Glisky, EL, Levin, BE, Ryan, L, Wright, CB, Geldmacher, DS. Challenges and Opportunities for characterizing cognitive aging across species. ***Frontiers in Aging Neuroscience***. 2012;4:6. Epub 2012 Sep 12.
12. Mendez, I, Damborsky, JC, Winzer Serhan, UH, Bizon, JL & Setlow, B. α4β2* and α7 Nicotinic Acetylcholine Receptor Binding Predicts Choice Preference in Two Cost Benefit Decision Making Tasks. In *Press.* ***Neuroscience*****.**
13. Beas, B.S., Setlow, B., Bizon, J.L. Distinct manifestations of executive dysfunction in aged rats Submitted. ***Neurobiology of Aging***.

D. Research Support.

Ongoing:

R01 AG029421 (Jennifer L. Bizon, PI) 8/1/07-6/30/12 (no cost extension through 6/13) 35% effort
National Institute on Aging
"Basal Forebrain and Cognitive Aging: Novel Experimental and Therapeutic Avenues"
The goal of this project is to determine the contributions of GABAergic and cholinergic basal forebrain projection neurons and their cortical targets to age-related cognitive decline.

R01 DA024671 (B. Setlow PI, Bizon co-PI) 4/1/09-3/31/14 15% effort
National Institute on Drug Abuse
"Neural mechanisms of enduring cocaine effects on impulsive choice"
The goal of this project is to understand the long-term effects of cocaine use on decision making and to begin to elucidate the neurobiology associated with impulsivity resulting from psychostimulant drug use.

NSF 1224019 Pending
"Neurocognitive Mechanisms of Cost-Benefit Decision Making 4/1/14-4/1/17 17% effort
National Science Foundation
The goal of this project is to determine how age-related decline in affective/cognitive processes and dopamine signaling contribute to individual differences in delay- and risk-based decision making.

Completed:

F31-AG037286-01 (Karienn Montgomery student. Bizon sponsor) 8/1/10-8/1/12 n/a
National Institute of Aging
Transfer Learning in Mice: Implications for improved diagnosis and treatment of Alzheimer's Disease
The goal of this study is to design novel behavioral assessments that are very sensitive to age-related pathology and that are highly translational.

R01-NS041548 (Grau PI, Bizon, co-I) 4/07-3/11 15% effort
National Institute of Neurological Disorder and Diseases
"Learning within the spinal cord: clinical implications"
The goal of this grant is to study the role of BDNF in spinal cord learning. No overlap.

R01-DA13188 (PJ Wellman PI, Bizon co-I) 8/01/07-6/30/10 15% effort
National Institute of Drug Abuse
"Heavy Metal and Drug Self-Administration: Mechanisms"
The goal of this project is to determine how exposure to heavy metals (lead, cadmium) during development affects vulnerability to drug abuse during adulthood.

F31- NS059324 (Candi Lynn LaSarge student, Bizon sponsor) 6/1/08- 12/1/10 n/a
National Institute of Neurological Disorders and Stroke
"The Role of Basal Forebrain in Mild Cognitive Impairment"
The goal of the project under this training fellowship is to determine how changes in basal forebrain anatomy are related to cognitive dysfunction in aging.

R01-AA012386 (G Frye PI, Bizon co-I) 8/1/08-8/1/10 10% effort
National Institute on Alcohol Abuse and Alcoholism
"CNS Development GABAARs and Vulnerability to Ethanol"
The goal of this project is to determine how developmental exposure to alcohol affects basal forebrain neuronal function and cognition later in life.

F31- DA023331 (NW Simon student, Bizon co-sponsor) 2/1/08-2/1/10 n/a

National Institute on Drug Abuse
"Long-term cocaine effects on impulsive choice and orbitofrontal cortex activity"
The goal of the project under this training fellowship is to determine how chronic cocaine exposure affects impulsive decision-making and orbitofrontal cortex function.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Dawn Bowers, Ph.D.	Professor, Clinical & Health Psychology
eRA COMMONS USER NAME (credential, e.g., agency login) dbowers	

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)*	MM/YY	FIELD OF STUDY
Emory University, Atlanta GA		1968-1970	Psychology
University of Florida, Gainesville FL	B.A.	08/1972	Psychology
University of Florida, Gainesville FL	M.S.	12/1974	Clinical Psych/Neuropsy
University of Florida, Gainesville FL	Ph.D.	12/1978	Clinical Psych/Neuropsy
University of Florida, Gainesville FL	Post-doc	12/1979	Behavioral Neurology

A. Personal Statement

I am a university professor and a clinical neuropsychologist-cognitive neuroscientist. I have longstanding research and clinical expertise in cognitive and emotional changes that are associated with neurologic disease and aging, particularly apathy, depression, memory and executive function. Current research focuses on emotion regulation and executive function, psychophysiologic signatures of apathy and depression, and interactive effects of cognitive training and exercise in cohorts of elderly adults. I have been a funded researcher for many years, including two recently completed clinical trials one for treatment of apathy using rTMS and another for treatment of "'masked faces" in individuals with Parkinson disease. My Cognitive Neuroscience Laboratory at the McKnight Brain Institute includes five doctoral students, who are using various tools (startle, pupillometry, ERP, computational modeling, advanced statistical approaches) to better understand mechanisms that underlie emotional and cognitive changes in older adults including those with dopaminergic depletion disorders. As such, I have experimental tools /approaches and statistical expertise that can help facilitate hypothesis-driven research including such as the important innovative approach to execise/cognitive training being proposed in this project.

B. Positions and Honors

Positions & 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]
1998- Director, Cognitive Neuroscience Laboratory, UF McKnight Brain Institute
2006- Division Chief, Neuropsychology Area, Dept. Clinical & Health Psychology
2006-2009 UF Foundation Research Professor
2012 Fellow, American Psychological Association, Division 40 (Neuropsychology)
2012-15 Board of Governors, International Neuropsychological Society

Other Experience & Professional Memberships

2012- Merit Review Panel for Mental Health and Behavioral Sciences – B (MHBB)
Department of Veterans Affairs

2011-	Member, NIH SBBIR2013/01 ZRG1 ETTN-K (10) B – Small Business: Clinical Clinical Neurophysiology, Devices, Neuroprosthetics, & Biosensors
5/ 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)].
2009-10	Ad hoc Member, NIH Adult Psychopathology & Disorders of Aging Study Section
2006	Member, NIH Special Emphasis Panel (ZRR1 BT-801), Interdisciplinary Research Consortium
2005	Member, NIH Special Emphasis Panel (2006/01) Cognition and Perception Study Section
2004-05	Ad hoc Member, NIH Biobehavioral Mechanisms of Emotion, Stress, and Health Study Section
2000-	Editorial Boards, The Clinical Neuropsychologist, Journal of International Neuropsychological Society
1999-2003	Special Review Panel, Minority Research Infrastructure Support Program (MRISP), NIMH
1995-1998:	Member, Merit Review Committee, Mental Health &d Behavioral Science, Dept. Veterans Affairs
Membership	American Psychological Association (Divisions 12 and 40), International Neuropsychology Society, American Academy of Neurology, Society for Neuroscience, Cognitive Neuroscience Society
Journal Reviews:	*Neuropsychologia, Lancet, Neurology, New England Journal of Medicine, Cortex, Movement Disorders, Journal of International Neuropsychological Society, The Clinical Neuropsychologist, J. Neurology, Neuropsychiatry, & Neurosurgery, Neuropsychology, Neuropsychologia, J. Cognitive Neursocience, JCED, Parkinson Disease and Parkinsonism*

C. Selected Peer-reviewed Publications (2012)
(Students in italics)

Alexander, G., Ryan L., **Bowers, D,** Foster, T., Bizon, T., Geldmacher D.S., Glisky, E. Charactering cognitive aging in humans with links to animal models. (2012) *Frontiers in Aging Neuroscience.* 4, 21. doi: 10.3389/fnagi.2012.00021

Dietz, J., Jones, J., Bradley, M., Okun, M.S., Perlstein, W., **Bowers, D**. (2012, in press). The late positive potential, emotion, and apathy in Parkinson's disease. *Neuropsychologia.*

Dietz, J., Noecker, A., McIntyre, C.C., Mikos, A., **Bowers, D**., Foote, K., Okun, M.S. (2012, in press). Stimulation region within the GPI does not affect verbal fluency: Results from field modeling. *Brain Stimulation.* Jun 16. [Epub ahead of print]

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

Kay, D., Kirsch-Darrow, L., Zahodne, L. **Bowers, D**. (2012). Dimensions of apathy in Parkinson's disease: Factor analysis of the Apathy Scale. *Journal of Parkinson's Disease*. 2 (2), 161-166.

Kluger, B., Parra, V., Jacobson, CE, Garvan, C., Rodriguez, r., Fernandez, H., Fogel, A., Skoblar, B., **Bowers, D**., Okun, M.S. (in press, 2012). Prevalence of fatigue following deep brain stimulation in Parkinson's disease and association with quality of life*. Parkinson's Disease.* Epub 2012 May 13.PMID:22666631

Limotai, N., Oyama, G., Go, C., Bernal, C., Ong, T., Moum, S., Bhidayasir, R., Foote, F., **Bowers, D**., Ward, H., Okun, M.S. (2012). Addiction-like manifestations and Parkinson's disease: A large single Center Nine Year Experience*. International J. Neuroscience,* 122, 145-153. PMID 22023411t.

Naugle, K., Hass, C.J., , **Bowers, D.,** Janelle, C. (2012). Emotional state affects gait initiation in individuals with Parkinson disease. *Cognitive, Affective, and Behavioral Neuroscience.* 12, 207-219. PMID: 22194236

Okun, MS, Foote, K.D., Wu, S.S., Ward, H.E., **Bowers, D**., Rodriguez, R.L., Malaty, I., Goodman, W.K., Gilbert, D.M., Walker, H.C., Mink, J.W., Merritt, S., Morishita, T., Sanchez, J.C. (2012). A Trial of Scheduled Deep Brain Stimulation for Tourette Syndrome: Moving Away From Continuous DBS Paradigms. Archives of Neurology. Oct 8: 1-10

Oyama, G, Rodriguez, R., *Jones, J.,* Swartz, C., Merrit, S., Runger, R., Hubmann, M., Delgado, A., Simon, E., Doniger, G**., Bowers, D**., Foote, K., Okun, M.S. (2012). Selection of DBS candidates in private neurology practices: Referral may be simpler than a computerized triage system. *Neuromodulation,* 15(3):246-50; discussion 250. PMID: 22376158

Roberson, E.D., DeFazio, A., Barnes, C.A., Alexander, G.E., Bizon, J.L., **Bowers, D**., Foster, T.C., Glisky, E.L., Levin, B.e., Ryan, L., Wrights, C.B., Geldmacher, D.S. (2012). Challenges and opportunities for characterizing cognitive aging across species. *Frontiers in Aging Neuroscience.* **4**:6. doi: 10.3389/fnagi.2012.00006.

Zahodne, L., Bernal-Pacheco, O., **Bowers, D**., Ward, H., *Oyama, G., Limotai, N., Velez-Lago,* F, Rodriguez, R., Malaty, I, McFarland, N., Okun, M.S. (2012) Are selective serotonin re-uptake inhibitors associated with greater apathy in Parkinson's disease? *J. Neuropsychiatry and Clinical Neuroscience.* 24(3):326-30

Zahodne, L., **Marsiske, M**., Okun, M.S., and **Bowers, D.** (2012). Components of depression in Parkinson disease**.** J. Geriatric Psychiatry & Neurology. 25, 131-7 PMID:22859701

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. N*europsychology*, 26, 71-80. PMID: 22142359

Zahodne, L.B., **Marsiske, M.M., Bowers, D**. (2012, in press). A latent class analysis of psychological disturbance in Parkinson disease. *International Journal of Geriatric Psychiatry.*

Chapters

Bowers, D., *Jones, J., Dietz, J.* (2012, in press). Assessment of Emotion. In J. Synder, Nussbaum, and Parsons, M. (eds). *Pocket Handbook of Neuropsychological Assessment*.

Bauer, R.M., **Bowers, D**. (2012, in press). Intellectual Antecedents to the Boston Process Approach to Neuropsychological Assessment. In D. Libon (Ed.), *Boston Process Approach to Neuropsychological Assessment.* New York: Oxford University Press.

D. Research Support

Current

R1NS079767 PI: Bowers 6/1/2012-5/30/2014

Emotion Regulation, Executive Function, and Parkinson Disease.

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

Role: PI

McKnight Brain Research Foundation PI: Bowers 11/01/2010-5/30/2013
Multimodal Platform for the Enhancement of Cognition in Normal Elderly
This study examines whether exercise predosing improves effects of cognitive training in older adults, the trajectory of change over time, and the extent to which pure "aerobic" vs exergames are more beneficial.

K23- NS060660 PI: Price 08/31/2007-08/29/2013
White Matter and Cognition in Parkinson Disease
This project examines the role of white matter integrity, as indexed by diffusion tensor imaging, in relationship to cognitive decline in patients with Parkinson disease.
Role: Primary Mentor

1 F31 NS073331-01 PI: Dietz 5/01/2011-6/30/2013
Psychophysiology of Emotion in Parkinson disease
This predoctoral NRSA examines temporal trajectory of psychophysiological and ERP changes associated with approach and avoidance in Parkinson disease.
Role: Mentor

Recently Completed

National Parkinson Foundation PI: Classen 7/01/2010 – 06/30/2012
The Role of Visual Attention in Driving Safety in Parkinson disease
This study examines the relationship between driving and various visuopercpetual/spatial indices of cognition.
Role: Co-I

1R34MH080764, PI: Okun 08/31/2009 -09/01/2012
Scheduled and Responsive Brain Stimulation for the Treatment of Tourette Syndrome.
This clinical trial examines the effectivess of a scheduled DBS protocol for treatment of symptoms in patients with intractable Tourettes syndrome.
Role: Co-I

R21-AG033284 Multiple PI:: Altman & Hass 8/2/2010-8/1/2012
Language and Executive function in Parkinson's disease: Effects of dual task and exercise.
This project examines the influence of aerobic exercise and multi-tasking on language processing in patients with Parkinson disease.
Role: Co-I

R01-NS50633 PI: Bowers 12/01/04-11/30/09
Masked Facies in Parkinson Disease: Mechanism and Treatment
In this study, we evaluated a behavioral treatment approach for improving facial expressivity in patients with parkinson's disease using a double-blind sham-controlled randomized clinical.
Role: PI

Michael J. Fox Foundation PI: Fernandez 10/31/2006-9/30/2009
Repetitive Transcranial Magnetic Stimulation for the Treatment of Apathy in Parkinson's Disease
The purpose of this study was to learn whether rTMS improved symptoms of apathy and depression in nondemented patients with idiopathic Parkinson disease. To do so, we conducted a double-blind placebo controlled randomized parallel group study.
Role: Co-PI

R01 MH62539 PI: Bowers 06/01/02 – 05/31/06
Digitizing the Face: Priming, TMS, and Hemispheric Asymmetries

This project examined three hypotheses regarding the basis forr hemifacial movement asymmetries using cognitive priming, single pulse transcranial magnetic stimulation, and computer based systems for digitizing dynamic facial signals.
Role: PI

F31- NRSA NS059142 PI: Kirsch 7/1/2007-8/30/2009
Apathy, neurocognitive functioning, and Parkinson disease
This predoctoral fellowship examined dissociation and overlap between depression and apathy in a large sample of nondemented Parkinson patients (N=300) using confirmatory factor analysis. The effects of apathy and depression on neurocognitive measures were examined using multiple hierarchical regression techniques.
Role: Primary Mentor

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Cohen, Ronald A.	Professor
eRA COMMONS USER NAME (credential, e.g., agency login) rcohen1	

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)*	MM/YY	FIELD OF STUDY
Tulane University	BSc	06/76	Psychology
Louisiana State University	Ph.D.	08/82	Psychology
UCLA Medical Center: Neuropsychiatric Institute	Internship	07/82	Psychology
University of Florida	Fellowship	07/83	Neuropsychology

A. Personal Statement

The proposed research will examine the effects bariatric surgery on cerebral metabolite, vascular and cognitive functions among in people with chronic morbid obesity. The study will also examine the extent to which changes in glucose metabolism and diabetes status post-surgery mediates these neural and cognitive changes. The research approach employs innovative multimodal neuroimaging methods, in conjunction with behavioral, neurocognitive and laboratory measures of systemic inflammatory and metabolic disturbances. I am particularly well suited to serve as the principal investigator on this project given my background in neuropsychology, clinical and cognitive neuroscience, and behavioral medicine. My track record of research aimed at effects of vascular and metabolic factors on brain structure and function has direct relevance to the proposed project, as these factors are the mechanisms hypothesized to underlie obesity-associated brain disturbance. I have conducted research and published in each of the areas underling this project, including a large number of studies of vascular and metabolic function related to CVD, obesity and metabolic syndrome. I am also involved in studies of exercise, and other behavioral risk factors, such as smoking. I have published over 175 peer-reviewed articles, and numerous book chapters on topics related to this project. I co-edited several books on topics related to areas of clinical neuropsychological research, including “Cardiovascular Disease and Neuropsychology,” which was just published by Oxford Press, and a new book in press “Brain Imaging in Behavioral Medicine and Clinical Neuroscience,” which is the first to address the use of neuroimaging methods for studying various problems in behavioral medicine including obesity. I am also the author of “Neuropsychology of Attention” which was one of the first books on this topic in the field.

Over the past decade, I have published a number of studies on cognitive dysfunction and brain disturbances associated with obesity, as well as neural bases of food as a reward in the context of obesity. This has included studies on bariatric surgery with my former fellow, Dr. John Gunstad (consultant on this project). I was a co-investigator on the NIH-funded longitudinal study of bariatric surgery outcome (LABS), upon which the current project is based. I have decades of research support to study attention, executive function and the brain. I have served as the principal investigator on a number of NIH-sponsored R01 projects with the MR methods to be used in the current study. These studies have examined vascular and metabolic mechanisms underlying brain dysfunction in both systemic medical disorders, such as CVD, as well as behavioral risk factors. I was a founding member of the magnetic resonance foundation (MRF) at Brown University, which oversees MRI based neuroimaging research at the university, and was a Professor in the Department of Psychiatry and Human Behavior at Brown University. I recently joined the faculty of the McKnight Institute on Aging at the University of Florida as an endowed professor to direct the Center for Cognitive Aging and Memory. In this position, I am able to coordinate resources and faculty from across the university to address issues related to the aging brain, including the influence of risk factors, such as obesity and diabetes, which will insure the successful completion of this project.

B. Positions and Honors

1992-1993	Associate Professor of Neurology, Univ. Mass. Medical School
1993-1996	Assistant Professor, Psychiatry-Human Behavior, Brown University
1993-2008	Director, Neuropsychology: The Miriam Hospital, Providence, RI.
1996-2004	Associate Professor, Psychiatry-Human Behavior, Brown University
1998-Current	Editorial Boards: Journal of the International Neuropsychological Society; Brain Imaging and Behavior; The Clinical Neuropsychologist, Stroke, JCRP.
2002-2011	Permanent Member, NIH – BMIO Study Section
2002-Current	Member, Executive Committee, Brown University, Magnetic Resonance Foundation
2004-Current	Professor, Brain Sciences Program, Brown Univ.
2004-Current	Professor of Psychiatry and Human Behavior, Brown University
2008-2012	Director, Neuropsychological Research, The Miriam Hospital
2012-Current	Director, Center for Cognitive Aging and Memory, The University of Florida
2012-Current	Evelyn McKnight Endowed Professor, Departments of Neurology, Psychiatry and Aging, the University of Florida

C. Selected Peer-reviewed Publications (selected from 176 peer-reviewed publications)

Most relevant to the current application

1. Gunstad J, Paul RH, **Cohen RA**, Tate DF, Spitznagel MB, Gordon E. Elevated body mass index is associated with executive dysfunction in otherwise healthy adults. *Compr Psychiatry.* Jan-Feb 2007;48(1):57-61.
2. **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. (2009). Vascular and cognitive functions associated with cardiovascular disease in the elderly. *J Clin Exp Neuropsychol, 31*:96-110.*PMC 2739675*
3. Jefferson AL, Holland CM, Tate DF, Guttmann, **Cohen RA**. Atlas-derived perfusion correlates of white matter hyperintensities in patients with reduced cardiac output. *Neurobiol Aging.* Mar 6 2009.
4. McCaffery JM, Haley AP, Sweet LH, **Cohen, RA** et al. (2009). 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. 90*(4):928-934. *PMC 2744621.*
5. **Cohen RA,** Harezlak J, Gongvatana A, et al. Cerebral metabolite abnormalities in human immunodeficiency virus are associated with cortical and subcortical volumes. *J Neurovirol.* Nov 2010;16(6):435-444.
6. **Cohen RA,** de la Monte S, Gongvatana A, et al. (2011). Plasma cytokine concentrations associated with HIV/hepatitis C coinfection are related to attention, executive and psychomotor functioning. *J Neuroimmunol.* Apr;*233*(1-2):204-210. *PMC 3074016.*
7. Okonkwo OC, **Cohen RA** Gunstad J, Tremont G, Alosco ML, Poppas A. (2010). Longitudinal trajectories of cognitive decline among older adults with cardiovascular disease *Cerebrovascular Disease.*30(4):362-373.PMCID: PMC3014862.
8. Gunstad J, Strain G, Devlin MJ, et al. (2011). Improved memory function 12 weeks after bariatric surgery. *Surg Obes Relat Dis.* Jul-Aug *7*(4):465-472. *PMC 3117085.*
9. Ott BR, **Cohen RA**, Gongvatana A, et al. (2010). Brain ventricular volume and cerebrospinal fluid biomarkers of Alzheimer's disease. *J Alzheimers Dis.20*(2):647-657. PMCID: PMC3078034.
10. Spitznagel MB, Garcia S, Miller LA, et al. Cognitive function predicts weight loss after bariatric surgery. *Surg Obes Relat Dis.* Oct 29 2011.
11. Alosco ML, Spitznagel MB, van Dulmen M, Raz N, **Cohen R**, Gunstad J. The additive effects of type-2 diabetes on cognitive function in older adults with heart failure. Cardiol Res Pract. 2012; PMID: 22701196.
12. Galioto R, Spitznagel MB, Strain G, Devlin M, **Cohen** R, Paul R, Crosby RD, Mitchell JE, **Gunstad** J. Cognitive function in morbidly obese individuals with and without binge eating disorder. Compr Psychiatry. 2012 Jul;53(5):490-5.
13. Hassenstab JJ, Sweet LH, Del Parigi A, McCaffery JM, Haley AP, Demos KE, **Cohen** RA, Wing RR. Cortical thickness of the cognitive control network in obesity and successful weight loss maintenance: A preliminary MRI study.Psychiatry Res. 2012 Apr 30;202(1):77-9. Epub 2012 May 16. PMID: 22595506.

14. Sweet LH, Hassenstab JJ, McCaffery JM, Raynor HA, Bond DS, Demos KE, Haley AP, **Cohen** RA, Del Parigi A, Wing RR. Brain response to food stimulation in obese, normal weight, and successful weight loss maintainers. Obesity (Silver Spring). 2012 May 9. Epub ahead of print] PMID: 22569002.
15. Miller, L., Crosby, R., Galioto, R., Strain, G., Devlin, M., Wing, R., **Cohen, R**. Mitchell, J., & Gunstad, J. (in press). Bariatric surgery patients exhibit improved memory function 12 months post-operatively. *Obesity.*

D. Research Support

Ongoing Research Support

5 R01 MH074368-05 (Ronald Cohen, PI) 09/30/06–08/31/13
"Age Effects on HIV-Associated Brain Dysfunction" NCE
The goal of this project is 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.
Role: Principal Investigator

5 R01 HL089311-04 (John Gunstad, PI) 09/15/08-11/30/12
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: Principal Investigator of Subcontract

5U01 CA1503878-03 (Rena Wing, PI) 09/28/09-08/31/14
"Increasing Sleep Duration: A Novel Appraoch to Weight Control"
The purpose of the project is to translate the basic science on sleep duration into a novel intervention to reduce obesity and obesity-related co-morbidities.
Role: Co-Investigator

5 R34 DA031057-02 (Ron Cohen, PI) 09/30/10-08/31/13
"Improving Adherence and Cognition in Substance-Using HIV Patients"
Substance abuse in the context of HIV infecton is a major problem that affects clinical outcome and interferes with adherence to treatment regimens. This study examines the value of a computer-based cognitive training program (Vigorous Mind) to enhance attention and executive functioning as a means of improving organizatonal and planning ability and ultimately treatment adherence. The study focuses on further development of the program for use in this population and initial testing to determine its acceptability and whether a larger scale clinical trial is warranted.
Role: Principal Investigator

5 P01 AA019072-02 (Peter Monti, PI) 09/30/10-08/31/15
"Alcohol and HIV: Biobehavioral Interactions and Intervention"
This study focuses on the interactive effects of HIV and alcohol use on metabolic-vascular disturbances underlying brain dysfunction.
Role: Principal Investigator of Substudy

Completed Research Support

5 R01 NS036524-10 (Brad Navia, PI) 05/23/05-04/30/10
NINDS/Subcontract from Tufts University
"Proton MRS Studies of Cerebral Injury in HIV Infection"
The major goals of this project are to examine the MRS and MRI correlates of cognitive function in the context of antiretroviral therapy
Role: PI of Subcontract

3 R01 NS036524-07S1 (Brad Navia, PI) 07/01/06-04/30/10
NINDS/Subcontract from Tufts University
“Brain Morphometric Studies of Cerebral Injury in HIV Infection”
(Supplement to “Proton MRS Studies of Cerebral Injury in HIV Infection”)
Role: Principal Investigator of Subcontract

5 P50 CA084719-11 (Jeanne McCaffery, PI of sub) 09/30/99-07/31/10
NCI/Subcontract from Butler Hospital
“Nicotine Dependence: Phenotype, Endophenotype & Contexts”
The major goal is to characterize the relationship of cigarette smoking to fMRI responses on smoking related tasks. Dr. Cohen’s role is to provide expertise related to the functional imaging analyses and interpretation of data from these paradigms.
Role: Co-Investigator

5 R01 HL084178-03 (Lawrence Sweet, PI) 01/25/07-11/30/11
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: Principal Investigator of Subcontract

5 R01 DA020725-03 (Tara White, PI) 10/01/07-05/31/11
NIDA/Subcontract from Brown University
“Imaging Individual Differences in Amphetamine Effects”
This study examines the relationship between personality factors and response to risk and award following intake of amphetamines.
Role: Principal Investigator of Subcontract

2 R56 DK075119-05A1 (John Gunstad, PI) 09/01/06-08/31/12
NIDDK/Subcontract from Kent State
“Extended Effects of Bariatric Surgery on Cognitive Function”
This study examines the effects obesity and rapid loss of weight associated bariatric surgery.
Role: Principal Investigator of Subcontract

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	POSITION TITLE
Vonetta M. Dotson	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) dotsonv	

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)*	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 Assistant 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 is also a Claude C. Pepper scholar. 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-present	Assistant Professor, Department of Clinical and Health Psychology, University of Florida, Gainesville, FL
2011-present	Joint Assistant Professor, Department of Neuroscience, University of Florida, Gainesville, FL

Honors

2012	Claude D. Pepper Scholar
2010	Claude D. Pepper Affiliated Scholar
2007	Recipient of National Institute on Aging Summer Institute on Aging Research travel fellowship
2007	Accepted to attend the American Psychological Association's Advanced Training Institute on Structural Equation Modeling for Longitudinal Research
2006	Recipient of the Institute for Learning in Retirement Graduate Aging Research Award
2006	Accepted to attend the American Psychological Association's Advanced Training Institute on Functional Magnetic Resonance Imaging
2005	Accepted into the Society for Neuroscience's Neuroscience Scholars Program
2004	Recipient of National Institute on Aging Technical Assistance Workshop travel fellowship
2004-2005	National Institute on Aging funded Predoctoral Fellow

2003-2005 University of Florida Institute on Aging Trainee
2000-2004 University of Florida Graduate Minority Fellowship
1998-1999 The National Dean's List
1997-1999 Dean's List, St. Mary's University
1997 ACCD Foundation Scholars Award

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* [DOI 10.1007/s10862-008-9123-z].
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. NIHMS154777
12. Dotson, V.M., Baydoun, M.A., & Zonderman, A.B. (2010). Recurrent depressive symptoms and the incidence of dementia and MCI. *Neurology*.
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.
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). Late-life Depression and

Cerebrovascular Changes in Postmenopausal Women: The Women's Health Initiative MRI Study. Journal of Affective Disorders.

15. Dotson, V.M., Zonderman, A.B., Kraut, M.A., & Resnick, S.M. (2012). Temporal Relationships between Depressive Symptoms and White Matter Hyperintensities in Older Men and Women. *International Journal of Geriatric Psychiatry*. DOI: 10.1002/gps.3791.
16. Dotson, V.M., Sozda, C.N., Marsiske, M., & Perlstein, W.M. (in press) Within-session Practice Eliminates Age Differences in Cognitive Control. *Aging, Neuropsychology and Cognition*.

D. Research Support

Physical, Cognitive and Mental Health in Social Context
Principal Investigator: Michael Marsiske, Ph.D.
5T32AG020499-07
NIH/NIA
Role: Research Fellow/Trainee
2004 - 2005

Double Jeopardy: Cognitive Decline in Depression and Aging
Principle Investigator: Vonetta M. Dotson, Ph.D.
AG024539-01
NIH/NIA
Role: PI
9/04 - 6/06

Effect of Exercise on Memory in Geriatric Depression: An fMRI Pilot Study
Principal Investigator: Vonetta M. Dotson, Ph.D.
McKnight Brain Research Foundation
Role: PI
3/11-2/13

Diversity Supplement to the Lifestyle Interventions and Independence for Elders (LIFE) Study (PI)
Principle Investigator: Marco Pahor, M.D.
U01 AG022376
NIH/NIA
Role: PI for diversity supplement
2/12-2/14

BIOGRAPHICAL SKETCH

NAME Thomas Foster	POSITION TITLE Professor of Neuroscience
eRA COMMONS USER NAME (credential, e.g., agency login)	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	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 brain mechanisms of aging and their relationship with age-related cognitive decline. 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 103 publications on memory mechanisms and the aging brain. As part of this work we are funded to examine electrophysiological and transcriptional markers of aging in relation to hippocampal function. Our published research demonstrates that age-related memory impairments are linked to changes in cell excitability (i.e. afterhyperpolarization), synaptic plasticity, and altered gene expression. We have previously examined variability in cell excitability during aging in relation to cognitive decline. We have published a number of papers examining the regulation of gene expression in hippocampal tissue during aging, including descriptions of regional differences. Recent work points to altered redox signaling in mediating senescent physiology. Other major projects in the lab examine the role of estrogen receptors in protecting against age-related cognitive decline.

B. Position and Honors

1. 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

2. Academic Honors and Awards

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

C. Selected Peer-reviewed Publications

Publications in peer reviewed journals accepted or published in 2012.

Foster, T.C. (2012) Dissecting age-related cognitive decline in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca2+ channels in senescent synaptic plasticity. Progress in Neurobiology, 96:283-303, PMID: 22307057.

Han, X., Aenlle, Bean, L.A., Rani, A., Semple-Rowland, S.L., K., Kumar, A., and **Foster**, T.C. Role of estrogen receptor alpha and beta in preserving hippocampal function during aging. Journal of Neuroscience, in press.

Charizanis, K., Lee, K-Y., Batra, R., Goodwin, M., Zhang, C., Yuan, Y., Shiue, L., Cline, M., Scotti, M.M., Xia, G., Kumar, A., Ashizawa, T., Clark, H.B., Kimura, T., Takahashi, M.P., Fujimura, H., Jinnai, K., Yoshikawa, H., Gomes-Pereira, M., Gourdon, M. Sakai, N., Nishino, S., **Foster**, T.C., Ares Jr, M., Darnell, R.B., and Swanson, M. (2012) Muscleblind-Like 2 Mediated Alternative Splicing in the Developing Brain and Dysregulation in Myotonic Dystrophy, Neuron, 75:437-450, PMID: 22884328.

Speisman, R.B., Kumar, A., Rani, A., Pastoriza, J.M. Severance, J.E. **Foster**, T.C., and Ormerod, B.K. (2013) Environmental enrichment protects neurogenesis and performance in a short water maze task from the effects of age. Neurobiology of Aging, 34, 263-274.

Speisman, R.B., Kumar, A., Rani, **Foster**, T.C., and Ormerod, B.K. (2013) Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. Brain, Behavior and Immunity, in press, PMID: 23078985.

Brim, B., Haskell, R., Awedikian, R., Elinwood, N.M., Jin, L., Kumar, A., **Foster**, T.C., and Magnusson, K. (2013) Memory in aged mice is rescued by enhanced expression of the GluN2B subunit of the NMDA receptor. Behavioural Brain Research, in press, PMID: 23103326.

Wittya, C.F., **Foste**r, T.C., Semple-Rowland , S.L., and Daniel, J.M. (2012) Increasing hippocampal estrogen receptor alpha levels via viral vectors increases MAP kinase activation and enhances memory in aging rats in the absence of ovarian estrogens. PLOS ONE, in press.

Boye1, S.L., Peshenko, I.V., Huang, W.C., Min, S.H., McDoom, I., Liu, X., Dyka, F.M., **Foster**, T.C., Umino, Y., Karan, S., Jacobson ,S.G., Baehr, W., Dizhoor, A., Hauswirth, W.W., and Boye, S.E.. (2013) AAV-mediated gene therapy in the guanylate cyclase (RetGC1/RetGC2) double knockout mouse model of Leber congenital amaurosis. Human Gene Thrapy, in press.

Roberson, E.D, DeFazio, R.A., Geldmacher, D.S, Alexander, G.E., Barnes, C.A., **Foster**, T.C., Bizon, J.L., Glisky, E.L., Ryan, L., Levei, B.E., Wright, C.B., and Bowers, D. (2012) Challenges and Opportunities in Characterizing Cognitive Aging Across Species. Frontiers in Aging Neuroscience, 4:6 doi: 10.3389/fnagi.2012.0006, Epub 9/12/2012.

Foster, T.C., DeFazio, R.A., and Bizon, J.L. (2012) Characterizing cognitive aging of spatial and contextual memory in animal models. Frontiers in Aging Neuroscience, 4:12 doi: 10.3389/fnagi.2012.00012, Epub 9/12/2012, PMCID: PMC3439636.

Bizon, J.L., **Foster**, T.C., Glisky, E.L., Alexander, G.E., (2012) Characterizing Cognitive Aging of Working Memory and Executive Function in Animal Models. Frontiers in Aging Neuroscience, 4:19 doi: 10.3389/fnagi.2012.00019, Epub 9/12/2012.

Publications (chapters/reviews/commentaries, not peer reviewed)

Guidi, M. and **Foster**, T.C. (2012) Animal model of memory and cognitive disorders. In F.H. Kobeissy (Ed.) Psychiatric disorders: Methods and Protocols, Volume 829: 145-153. Methods in Molecular Biology Springer, USA (Humana Press, Inc), PMID: 22231811.

Craft, S., **Foste**r, T.C., Landfield, P.W., Maier, S.F., Resnick, S.M. and Yaffe, K. (2012) Session III: Mechanisms of Age-Related Cognitive Change and Targets for Intervention: Inflammatory, Oxidative, and Metabolic Processes. Journal of Gerontology: Biological Sciences, in press PMID: 22570133.

Foster, T.C. (2012) Challenges and opportunities in characterizing cognitive aging across species. Frontiers in Aging Neuroscience, doi: 10.3389/fnagi.2012.00033, Epub 11/07/2012. This is an introduction the to the special issue devoted to the seven papers published by the McKnight Cognitive Battery group.

Foster, T. and Notterpek L. (2012) The business of the brain. Village Journal at Haile Plantation Vol 8 (2) p 47-50.

D. Research Support

Ongoing Research Support

R01 AG037984 Foster (PI) 9/15/2010 to 7/31/2016
This project will examine the hypothesis that the ratio of ERα/ERβ interacts with the level of E2 during aging to regulate memory and transcription of genes for neuroprotection and synaptogenesis.
Role: PI

R37 AG036800 Foster (PI) 10/05/2010 to 08/31/2014
The major goals of this project are to examine the hypothesis that age-related changes in NMDA receptor function impair signaling cascades, which lead to impaired transcription and memory deficits.
Role: PI

UF Opportunity Grant Foster (co-PI) 2012 to 2013
Genomic Bases of Differential Aging in Hippocampal Circuits: Single Cell Approaches

Research Completed

R01 AG14979 Foster (PI) 6/01/07 to 5/31/12
The major goals of this project are to examine the hypothesis that age-related changes in oxidative stress mediate changes in Ca^{2+} homeostasis, neuroplasticity, and memory deficits.
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 FOUR PAGES.**

NAME	POSITION TITLE
Frazier, Charles Jason	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) CJFRAZIER	

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)*	MM/YY	FIELD OF STUDY
Oberlin College, Oberlin, OH	B.A.	1991	Neuroscience
University of Colorado HSC, Denver, CO	Ph.D.	1997	Neuroscience

Please refer to the application instructions in order to complete sections A, B, C, and D of the Biographical Sketch.

A. Personal Statement

The work proposed in this grant is based on an innovative combination of cellular neurophysiology, two-photon based laser scanning epifluorescence microscopy, and computational neuroscience. My lab specializes in cellular neurophysiology, and I have nearly 15 years of experience with the core techniques. Further, in 2008 my lab finished construction of a rig capable of performing experiments in cellular neurophysiology while also simultaneously collecting optical data using two-photon based laser scanning epifluorescence microscopy. To date we have published one manuscript that relies on a straightforward combination of these technologies (specifically, two-photon guided minimal stimulation). Further, in the current application, I have provided preliminary data in to indicate that we are also prepared and able to meet the technical challenges involved in imaging calcium in dendritic spines of backfilled neurons. Finally, I have recruited a collaborator, Dr. Sachin Talathi, who has strong expertise in the area of computational neuroscience. I expect he will rather easily be able to construct the dynamic models of SK channel currents required for this project. In fact, his expertise in computational neuroscience, combined with my expertise in cellular neurophysiology and optical imaging, should be more than sufficient to support all aspects of this project, including the integration of dynamic clamp.

In addition to emphasizing our technical expertise, I would also like to point out that an interest in the neurobiology of age related memory dysfunction is one of the things (dating all the way back to undergraduate school) that first attracted me to science. It is also the reason I began my doctoral work with an emphasis on studying cholinergic systems in the hippocampus. Although my lab has focused less directly on this topic in recent years, I am excited about the potential to have an opportunity to begin a project such as this one. In fact, to be blatantly honest, this project has a powerful dual appeal to me. One undeniably attractive aspect is that it will allow us to effectively leverage the full capabilities of our recently acquired technology. In so doing, we expect to execute an innovative series of experiments that will address an important problem in a way that currently exceeds the technical capabilities of many labs. I find that both attractive and motivating. At the same time, another powerful draw is that this project will also allow me to once again devote significant time and effort to a problem of clear relevance to our understanding of age related memory dysfunction.

B. Positions and Honors

Positions and Employment

1998 – 2000 **Postdoctoral Fellow**, Department of Physiology and Biophysics, Case-Western Reserve
2000 - 2003 **Postdoctoral Fellow**, Department of Pharmacology and Therapeutics, University of Florida
2003 - 2010 **Assistant Professor**, Department of Pharmacodynamics, University of Florida
2003 – 2010 **Adjunct Assistant Professor**, Department of Neuroscience, University of Florida
2010 - present **Associate Professor**, Department of Pharmacodynamics, University of Florida
2010 - present **Adjunct Associate Professor**, Department of Neuroscience, University of Florida

Other Experience and Professional Memberships

1993-present	Member, Society for Neuroscience
1998-2000	Member, Biophysical Society
2006-2009	Course Director, GMS6022, Cell signaling in the nervous system. Required course in the Neuroscience graduate program in UF College of Medicine.
2006-present	Member, UF HSC Student Conduct Committee
2008-present	Member, COP Academic and Professional Standards Committee

Honors

2006	Nominated, College of Pharmacy Teacher of the Year
2007	Nominated, College of Pharmacy Teacher of the Year
2009	Nominated, College of Pharmacy Teacher of the Year
2010	Jack Wessel Award for Excellence as an Assistant Professor

C. Selected Peer-reviewed Publications

1. Frazier CJ, Rollins YD, Breese CR, Leonard S, Freedman R, Dunwiddie TV (1998) Acetylcholine activates an α-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *Journal of Neuroscience* 18:1187-1195.
2. Frazier CJ, Buhler AV, Weiner JL, Dunwiddie TV (1998) Synaptic Potentials Mediated via alpha-Bungarotoxin-Sensitive Nicotinic Acetylcholine Receptors in Rat Hippocampal Interneurons. *Journal of Neuroscience* 18:8228-8235.
3. Frazier CJ, George EG, and Jones SW (2000) Apparent change in ion selectivity caused by changes in intracellular K(+) during whole-cell recording. *Biophysical Journal* 78 (4):1872-1880.
4. Frazier CJ, Serrano JR, George EG, Yu X, Viswanathan A, Perez-Reyes E, and Jones SW (2001) Gating kinetics of the α1I T-Type Calcium Channel. *Journal of General Physiology* 118(5):457-470.
5. Frazier CJ, Strowbridge BW, Papke RL (2003) Nicotinic receptors on local circuit neurons in dentate gyrus: a potential role in regulation of granule cell excitability. *Journal of Neurophysiology* 89(6):3018-28.
6. Hofmann ME, Nahir B, Frazier CJ (2006) Endocannabinoid mediated depolarization-induced suppression of inhibition in hilar mossy cells of the rat dentate gyrus. *J. Neurophysiol*ogy 96(5):2501-12.
7. Nahir B, Bhatia C, Frazier CJ (2007) Presynaptic inhibition of excitatory afferents to hilar mossy cells. *J. Neurophysiology* 97(6):4036-47.
8. Frazier CJ (2007) Endocannabinoids in the dentate gyrus (Review). In: "The Dentate Gyrus: A comprehensive guide to structure, Function and Clinical Implications", Scharfman HE, Editor, *Progress in Brain Research*, Volume 163, Chapter 19, 319-337.
9. Hofmann ME, Nahir B, Frazier CJ (2008) Excitatory afferents to CA3 pyramidal cells display differential sensitivity to CB1 dependent inhibition of synaptic transmission. *Neuropharmacology* 55(7):1140-1146.
10. Nahir B, Lindsly C, Frazier CJ (2010) mGlur-Mediated and endocannabinoid-dependent long-term depression in the hilar region of the rat dentate gyrus. *Neuropharmacology,* 58:712-721.
11. Hofmann ME and Frazier CJ (2010) Muscarinic receptor activation modulates the excitability of hilar mossy cells through the induction of an afterdepolarization. *Brain Research,* 1318:42-51.
12. Lindsly C and Frazier CJ (2010) Two distinct and activity dependent mechanisms contribute to autoreceptor mediated inhibition of GABAergic afferents to hilar mossy cells. *Journal of Physiology*, 588:2801-2822.

Submitted Manuscripts

1. Bhatia C, Hofmann ME, Frazier CJ Cannabinoid receptor agonists potentiate action potential independent release of GABA in the dentate gyrus through a CB1 receptor independent mechanism. Under revision after 'provisional acceptance' at the *Journal of Physiology*.

D. Research Support

Ongoing Research Support

NA

Pending Research Support

R21DA029828-01A1 Frazier (PI) Awaiting Feb. 2010 Council Meeting
CB1R independent effects of cannabinoids on synaptic physiology in the CNS.
This project focuses on an unusual form of CB1R independent effect of cannabinoids on action potential independent synaptic transmission that my lab has identified in the hilar region of the dentate gyrus. Priority score: 23 (percentile not calculated)
Role: Principal Investigator

Completed Research Support

R01 DA019576 Frazier (PI) 7/01/2005-06/30/2010
Endocannabinoids and tonic GABA in the dentate gyrus.
Half of this project focuses on CB1 receptor dependent forms of short term synaptic plasticity, while the other half focuses on regulation of ambient GABA in the CSF and subsequent activation of high affinity GABA receptors. A hypothetical link between these mechanisms is proposed, and experiments are designed to identify a role for these systems in drug abuse. The A1 of the our competing renewal for this project will be reviewed in Feb. 2010.
Role: Principal Investigator

Frazier (PI) 2007 Opportunity Incentive Seed Funds 5/1/2007-04/30/2008
University of Florida Division of Sponsored Research
Construction of the first 2-photon based laser scanning epifluorescence microscope at the University of Florida.
Role: Principal Investigator

Frazier (PI) 2005-2006
Epilepsy Foundation of America
Short-term plasticity of synaptic inputs to hilar mossy cells.
This project supported the study of CB1 activation subsequent to excitation of hilar mossy cells, and contributed to preliminary data section of our currently funded R01.
Role: Principal Investigator

Frazier (PI) 2003 - 2005
Evelyn F. McKnight Brain Research Grant Program (Intramural), University of Florida
Muscarinic modulation of intrinsic membrane properties and synaptic plasticity in the dentate gyrus.
This project involved characterization of a muscarinic ADP in the dentate gyrus, and addressed several other questions involving cholinergic effects on intrinsic membrane properties of specific neurons, and on synaptic transmission at well identified synapses.
Role: Principal Investigator

Frazier (Co-Investigator) 2001 - 2003
Agency: Evelyn F. McKnight Brain Research Grant Program (Intramural), University of Florida
The role of the septo-hippocampal cholinergic system in age-related memory dysfunction.
This project examined the effects of nicotinic receptor activation in several specific neurons both before and after septal deafferentation.
Role: Co-Investigator (R.L. Papke, PI)

NS10828 Frazier (PI) (NRSA) 1999 - 2002
NIH/NINDS
Inactivation of delayed rectifier potassium channels.
This study examined ion selectivity during inactivation of Kv2.1.
Role: Principal Investigator (S.W. Jones, Mentor)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Leonid L. Moroz	**Professor of Neuroscience, Genomics, Chemistry & Biology**
eRA COMMONS USER NAME (credential, e.g., agency login) **MorozL**	

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)*	MM/YY	FIELD OF STUDY
Institute of Developmental Biology, Moscow, USSR	Ph.D.	1989	Physiology, Develop. Biol.
University of Leeds, Leeds, UK	Postdoc.	1994	Physiology
Dept. Molecular Physiol., Univ. of Illinois, Urbana,IL	Researcher	1997	Neuroscience
Beckman Institute, Univ. of Illinois, Urbana, IL	Researcher	1998	Bioanalytical Chemistry
Dept. Neuroscience & Brain Institute, Univ. of Florida, Gainesville, FL and Whitney Laboratory for Marine Bioscience, Univ. of Florida, St. Augustine, FL	Professor	1998-present	Neuroscience, Zoology, Chemistry, Genomics & Nanotechnology

A. Personal Statement

This proposal focuses on systematic determination of properties of the transcriptomes, genomes and epigenomes of single cells, characterized neural populations including well-defined memory forming circuits in molluscs. To do so, we will develop and implement the innovative single-cell genomic approaches and physiological measurements using tools of nanotechnology and singe molecule measurements. Specifically, we plan to integrate transcriptomic and DNA methylation profiling to underestand the logic of gene regulation as neurons learn and remember, regenerate and age. This data will be used to identify genes involved in memory mechanisms and reconstructing signal transduction pathways to generate a nearly complete computational portrait of a neuron. I have a broad background in comparative, integrative, cellular and molecular physiology and neuroscience, having spent more than 20 years working with various groups of experimental models in biology and medicine with specific training and expertise in key research areas for this application. As PI or co-Investigator on several previous University- and NIH funded grants, I laid the groundwork for this proposed research by developing single cell cDNA libraries for genomic, methylome and transcriptome profiling in *Aplysia* relevant to studies of the genomic bases of neuronal identity, plasticity, development and toxicology. In addition, I successfully administered NIH and NSF projects including, together with Drs. Ju and Kandel, a large multi-institutional collaborative project, The NIH Center of Excellence in Genomic Sciences. I also collaborated with researchers at Columbia University, UCLA, UIUC, European Centers, and other Institutions, and produced several peer-reviewed publications from each project. As a result of these previous experiences, I am aware of the importance of frequent communication among project members and of constructing a realistic research plan, timeline, and budget. I have a demonstrated record of successful and productive research projects in an area of high relevance for this application, and my experiences have prepared me to be a PI & lead the project.

B. Positions and Honors

Positions and Employment

1993-1994 Postdoctoral Researcher, Department of Physiology, University of Leeds, Leeds, UK.
1994-1997 Postdoctoral Researcher, Dept. Molecular and Integrative Physiology, University of Illinois, IL.
1997-1998 Research Specialist in Life Sciences, Beckman Institute, Dept. Chemistry, Urbana, IL.
1998 -2003 Assistant Professor of Neuroscience, Dept. Neuroscience University of Florida, Gainesville, FL.
2003 -2006 Associate Professor of Neuroscience, Dept. Neuroscience University of Florida, Gainesville, FL.
2006 - Professor of Neuroscience, Chemistry & Biology; Depts. Neuroscience, Chemistry, Biology; Brain Institute, Genetic Institute & The Whitney Laboratory, University of Florida, FL, USA
2011 - Professor of Genomics, Genetic and Genomic Institute, University of Florida, Gainesville, FL

Other Experience and Professional Memberships

1993- present Member, Society for Neuroscience
1998- present Member, Society for Integrative and Comparative Biology
2000- present NSF, NIH, ad hoc reviewer
2011 – Editorial Board, *J. Neurogenetics*

Honors

1989	European Training Program in Brain and Behavioral Research (ETP) Award
1992	European Training Program in Brain and Behavioral Research (ETP) Research Award
1993-1994	Royal Society Postdoctoral Fellow Award, UK
1995-2000	Howard Hughes Medical Institute: International Scholar
2000	NSF medal for research in Antarctica
2002	Packard Interdisciplinary Science Award
2005	NIH Science Award (Nitrite Research)
2005	McKnight Brain Research Foundation Award
2007	Faculty Achievement Recognition Honoree & Award
2012	McKnight Brain Research Foundation Award

C. Selected Peer-Reviewed Publications (from 123 peer reviewed publications)

Moroz L.L., Kohn A.B. (2013). Singe-Neuron Transcriptome and Methylome Sequencing for Epigenomic Analysis of Aging. ***Methods in Molecular Biology***, In Press

Puthanveettil SV et al., (2012). The Synaptic Transcriptome of *Aplysia*: Isolation and characterization of RNAs actively transported by kinesin complex from the cell body to synapses. ***Proc.Natl.Acad. Sci. USA*** In Press.

Kohn A.B., Moroz, T.P., Barnes, J.P., Netherton, M., Moroz, L.L. (2013) Single-Cell Semiconductor Sequencing. ***Methods in Molecular Biology*** In Press

Ptitsyn A and Moroz L.L. (2012). Algorithm for gain and loss of gene analysis in distantly related genomes. ***BMC Bioinformatics***. V. 15:S5. doi: 10.1186/1471-2105-13-S15-S5.

Yalan Z., et al. (2012). Regulation of neuronal excitability by interaction of Fragile X Mental Retardation Protein with Slack potassium channels. ***J. Neuroscience*** 32(44):15318 –15327

Moroz LL. *Aplysia*: A quick quide. (2011). ***Current Biology***, 21(2):R60-61.

Philippe H, et al. (2011). Acoelomorph flatworms are deuterostomes related to *Xenoturbella*. ***Nature*** 470:255-258

Koket et al.(2011). Phylogenomics reveals deep molluscan relationships. ***Nature***, 477(7365):452-456

Moroz, L.L. et al… Kandel ER. (2006) Neuronal transcriptome of *Aplysia*: Neuronal compartments and circuitry. ***Cell***;127:1453-1467.

Moroz LL, Kohn AB (2011). The diversity of enzymatic and non-enzymatic pathways of nitric oxide synthesis and signaling: from evolutionary biology to memory mechanisms. ***Frontiers in Biosciences***: 17:2008-51.

Heyland A, Vue Z, Voolstra CR, Medina M, and Moroz LL. (2011) Developmental transcriptome of *Aplysia californica*'. ***J Exp Zool*** *B* ***Mol Dev Evol*** 316B(2):113-134.

Moroz, L.L. Kohn, A. (2010). Do different neurons age differently? Direct genome-wide analysis of aging in identified cholinergic neurons. ***Frontiers in Neuroscience***, *6(2), 1-18.*

Walters, E.T., Moroz, L.L. (2009). Moluskan memory of injury: Evolutionary insights into chronic pain and neurological disorders. ***Brain, Behavior and Evolution***, v.74 (3): 206-218.

Moroz, L.L. (2009). On the independent origin of complex brains and neurons. ***Brain, Behavior and Evolution***, v.74(3): 177-190.

Lee YS, et al. (2008). Transcriptome analysis and identification of regulators for long-term plasticity in *Aplysia kurodai*. ***Proc Natl Acad Sci*** *USA*;105(47):18602-18607.

Antonov, I., Ha, T., Antonova, I., Moroz, L.L., and Hawkins, R.D. (2007) Role of nitric oxide in classical conditioning of siphon withdrawal in *Aplysia****. J. Neurosci****.* 27, 10993-11002.

Moroz, L.L., et al. (2005) Direct single cell determination of nitric oxide synthase related metabolites in identified nitrergic neurons. ***J. Inorganic Biochemistry***, 99(4): 929-939.

Gillette, R., Huang, R., Hatcher, N., Moroz, L.L. (2000). Cost-benefit analysis potential in feeding behavior of a predatory snail by integration of hunger, taste and pain. ***Proc.Natl Acad Sci. USA***: 97, 3585-3590.

Bourlat, S.J., et al. (2006). Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. ***Nature***, 444, 85-88.

D. Research Support/ Ongoing Research Support

NIH/NIGM Genomic Bases of Behavioral Learning
1R01GM097502-02 (Moroz) 07/01/2011-05/31/2015

NIH/NIDA (Moroz) Spatial Organization of the Genome in Identified Neurons of Memory Circuits
R21DA030118 06/01/2010 – 02/28/2013

NIH/NINDS (Moroz) "Genomic Approaches to Deciphering Memory Circuits"
1R01MH097062-01A1 09/01/2012 - 08/31/2017

NSF (Moroz) Quest for the Earliest Transmitters: Signal Molecules in Ctenophores 03/01/2012 - 28/02/2016

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Brandi K. Ormerod	Assistant Professor, Biomedical Engineering 1600 Center Drive, Biomedical Sciences Bldg University of Florida, Gainesville, FL, 32611
eRA COMMONS USER NAME BORMEROD	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Queen's University, Kingston, ON, Canada	B.Sc.	1998	Biopsychology
University of British Columbia, BC, Canada	Ph.D.	2003	Neuroscience
Stanford University, Stanford, CA, USA	Postdoctoral	2006	Stem Cell Sciences

Positions and Employment

1996-1998 Undergraduate Research Assistant in Dr. Beninger's Behavioral Pharm Laboratory (Queen's)
1997-1998 Undergraduate Research Assistant in Dr. Weisman's Avian Bioaccoustics Laboratory (Queen's)
1997-1998 Undergraduate Teaching Assistant for 4th yr Behavioral Pharmacology (Queen's University)
1998-1999 Teaching asst for 2nd yr Psych of Gender/Laboratory Instructor for 3rd yr Biopsych (UBC)
1999-2000 Teaching asst for 2nd yr Biopsych and Gender Psych and 4th yr Neuroplasticity (UBC)
2000-2001 Teaching asst for 2nd yr Biopsych and Gender Psych, 3rd yr Stats, 4th yr Neuroplasticity (UBC)
2001-2002 Laboratory Instructor for 2nd yr Biopsych/Teaching asst for 3rd yr Biopsych/4th yr Neuroplasticity
2003-2006 Postdoctoral Stem Cell Researcher, Palmer Lab, Neurosurgery Dept, Stanford University
Since November 2006 - Assistant Professor, Biomedical Engineering Dept, University of Florida

- 2007 Fall – Developed and Taught EML4930 - Introduction to BME
- 2008-2010 Spring Terms – Developed and Taught BME 6938 - Foundations of Neural Engineering
- 2008 Fall – Taught BME 8654 – Problem-based Learning
- 2009-2011 Fall Terms – Developed and Taught BME 6938 – Stem Cell Engineering
- Since 2011 Spring Terms – Developed and Taught BME 3323L – Cell Engineering Laboratory
- Since 2011 Fall Terms – Stem Cell Engineering and Neural Foundations Taught in Alternating years.

Since November 2011 – Joint Faculty, Neuroscience Dept, University of Florida

Awards/Honors

1998 Faculty of Graduate Studies Travel Award (UBC; CDN$400)
1999 Natural Sciences and Engineering Research Council of Canada Scholarship A (UBC; $34,600)
2001 Natural Sciences and Engineering Research Council of Canada Scholarship B (UBC; $38,400)
2001 Killam Predoctoral Fellowship (UBC; CDN$44,000 - $24,000 top-up accepted)
2001 Killam Predoctoral Fellowship Travel Award (UBC; CDN$1,500)
2001 Alzheimers Society of Canada Predoctoral Fellowship (UBC; CDN$44,000 – declined)
2002 Invited to introduce UBC president Dr. Martha Piper at the first Nobel Awardee Michael Smith Honorary "Women at the Frontiers of EXXcellence" Conference opening ceremony
2003 UBC Brain Research Centre "3D Microscopy of Living Cells Course" scholarship (US$2,250)
2003 Society for Neuroscience Chapters Travel Award (US$735)
2003 Michael J. Fox Foundation Postdoctoral Fellowship (US$78,000)
2003 Natural Sciences and Engineering Research Council of Canada Fellowship (Stanford; $40,000)
2003 NIH CHOC Human Embryonic Stem Cell Course (Burnham Institute; Scholarship Bursary; $1,500)
2004-2006 Chair of the Stanford Brain Food Bimonthly Seminar Series
Since 2007 UF College of Engineering Faculty Track Graduate Student Mentor

Service

2007 Scientific Consultant on Aging and Cloning to the American Federation for Aging Research
Since 2007 Maryland Funded TEDCO Stem Cell Competition Peer Review Committee
Since 2007 Ad Hoc Reviewer for the University of Washington Alzheimer's Disease Research Center Competition
2008/9 UF Biomedical Engineering Faculty Search Committee
2008/9 UF Neuroscience Faculty Search Committee
Since 2009 Ad Hoc Reviewer for the National Science Foundation - Integrative Organismal Systems / BIO
Since 2010 Ad Hoc Reviewer for the US-Israel Binational Science Foundation
Since 2010 Ad Hoc Reviewer Agency for Science, Technology and Research (Korea)

Publications

Peer-reviewed Publications in 2012 (of 28)

1. Speisman, RB, Kumar A, Rani A, Pastoriza JM, Severance JE, Foster TC and Ormerod BK (2013). Environmental enrichment restores neurogenesis and rapid acquisition in aged rats. *Neurobiology of Aging*. 34:263-274.
2. Speisman RB, Kumar A, Rani A, Foster TC and Ormerod BK (2012). Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. *Brain, Behavior and Immunity*. Epub ahead of print.
3. Ormerod BK, Hanft SJ, Asokan A, Haditsch U, Lee SW and Palmer TD (2012). PPARγ activation prevents impairments in spatial memory and neurogenesis following transient illness. *Brain, Behavior and Immunity*. Epub ahead of print.
4. Lee SW, Haditsch U, Monje ML, Cord BJ, Guzman R, Kim S, Boettcher C, Priller J, Ormerod BK and Palmer TD (2012). Absence of CCL2 is sufficient to restore hippocampal neurogenesis following cranial irradiation. *Brain, Behavior and Immunity*. Epub ahead of print.
5. Ogle,WO, Speisman, RB, and Ormerod BK (2012). Potential of treating age-related depression and cognitive decline with nutraceutical approaches: A mini-review. *Gerontology*. Epub ahead of print.
6. Bañuelos, C, LaSarge, CL, McQuail J, Hartman JJ, Gilbert RJ, Ormerod BK, Bizon JL (2012). Age-related changes in rostral basal forebrain cholinergic and GABAergic projection neurons: relationship with spatial impairment. *Neurobiology of Aging*. Epub ahead of print.
7. Munikoti VV, Hoang-Minh, LB and Ormerod BK (2012). Enzymatic digestion improves the purity of harvested cerebral microvessels.. *Journal of Neuroscience Methods* 207:80-85.
8. Stephens CL, Toda H., Palmer TD, DeMarse TB and Ormerod BK (2012). Adult neural progenitor cells reactivate superbursting in mature neural networks. *Experimental Neurology,* 234:20-30.

Relevent Published Abstracts (of more than 42)

1. Price R.O., Ormerod B.K., Palmer T.D. (2004). Maternal inflammation during fetal brain development disrupts cortical neurogenesis. Soc. Neurosci. Abstr Vol. 30., 831.90.

2. Ormerod B.K., Hanft S., Palmer T.D. (2005). NSAID treatment protects adult hippocampal neurogenesis and hippocampus-dependent behavior from the deleterious effects of neuroinflammation. Soc Neurosci Abstr Vol 31., 141.70.

3. Meltzer L., Ormerod B., Palmer T., Deisseroth K. (2005). Driving activity-dependent neurogenesis via L-Type Calcium Channels: Circuit-level and behavioral consequences. Soc Neurosci Abstr Vol 31. 141.69.

4. Ormerod BK., Lee SW., Cord BJ., Palmer TD (2006). Absence of monocyte chemoattractant protein-1 protects neurogenesis in response to cranial irradiation. Soc Neurosci Abstr Vol 31. 385.16.

5. Stephens CL., DeMarse TB, Ormerod BK (2007). Fetal and adult neural progenitor cells develop network-wide activity: a 60 channel microelectrode array analysis. Soc Neurosci Abstr Vol 32. 344.80.

6. Ferguson MC, Rani A, Kumar A, Foster TC, Ormerod BK (2007). Neurogenesis is enhanced in the hippocampus of young and aged rats exposed to an enriched versus impoverished environment. Soc Neurosci Abstr Vol 32. 566.70.

7. Zachrisson O, Andersson A, Isacson R, Jeldes S, Mercer A, Nielsen E, Patrone C, Ronnholm H, Wikstrom LB, Di Monte DA, McCormack AL, Ormerod BK, Palmer TD, Zhao M, Delfani KK, Janson Lang AM and Haegerstrand A. (2007). Neurorestorative effects of PDGF-BB in rodent models of Parkinson s disease through stimulation of cell proliferation. Soc Neurosci Abstr Vol 32. 797.1.

8. Stephens CL, DeMarse TB, Ormerod BK (2008). Neural progenitor cells influence and are influenced by the activity of primary neurons cultured on microelectrode arrays. Soc Neurosci Abstr Vol 33.

9. Asokan A, Ormerod BK (2008) A bioplex strategy for identifying candidate molecules that affect adult hippocampal neurogenesis. Soc Neurosci Abstr Vol 33.

10. Ormerod BK, Hoang-Minh L, Kelly P, Jones BE, Palmer TD (2008). Is there a circadian effect on cell production in extra-hippocampal adult brain regions? Soc Neurosci Abstr Vol 33.

11. Stephens CL, DeMarse TB, Ormerod (2009). Spontaneous activity patterns of fetal and adult neural networks are altered by the addition of adult neural progenitor cells. Soc Neurosci Abstr Vol 34, 324.13.

12. Asokan A and Ormerod BK. (2009). Lipopolysaccharide induced activation of circulating inflammatory molecules exert a negative influence on hippocampal progenitor cell differentiation in the adult brain. Soc for Neurosci Abstr Vol 34.

13. Ormerod BK, Speisman RB, Kumar A, and Foster TC. (2009) Biomarkers predict successful versus unsuccessful aging in rats. Society for Neuroscience Abstr Vol 34.

14. Sandhu M.S., Ross, H.H., B. J. Dougherty B.J., Laywell E.D., Ormerod B.K. Reier, P.J., Fuller D.D. (2010). Respiratory changes following transplantation of post-natal neural precursors into high cervical hemilesions of the adult rat. Society for Neuroscience Abstr Vol 35. 469.8.

15. Asokan, A., Chandron, M, Khargonekar, P.P. and Ormerod, BK. (2010). A combined multiplex experimental and modeling approach reveals candidate neuroinflammatory cytokines that affect hippocampal progenitor cell behavior in adult mice. Society for Neuroscience Abstr Vol 35. 638.9.

16. Hoang-Minh, L.B. and Ormerod B.K. (2010). Day/night variations in neurogenesis may be due to changes in cell cycle kinetics in the dentate gyrus of adult. Society for Neuroscience Abstr Vol 35. 638.19.

17. Speisman R.B., Kumar A., Rani A., Severance J.E., Foster T.C., Ormerod B.K. (2010). Enrichment concomitantly improves hippocampal neurogenesis and spatial memory in aged rats. Society for Neurosci Abstr Vol 35. 710.10.

18. Stephens, C.L., DeMarse, T.B., Ormerod, B.K. (2010). Adult neural progenitor cell addition after oxygen-glucose deprivation on microelectrode arrays influences recovery of network activity. Society for Neurosci Abstr Vol 35; 760.8.

19. Munikoti, V. Asokan, A., Ormerod, B.K. (2010). Hippocampal neurogenesis and laminin expression are inversely regulated by lipopolysaccharide-induced neuroinflammation in adult female mice. Soc for Neurosci Abstr Vol 35; 839.15.

20. Asokan, A., Ball, A., Laird, C., Hermer, L., Ormerod B.K. (2011). The fast acting anti-depressant desvenlafaxine promotes new granule neuron maturation in the dentate gyrus of adult rats. Society for Neurosci Abstr Vol 36. 331.19.

21. Speisman, R.B., Kumar, A., Rani, A., Foster, T.C., Ormerod, B.K. (2011). Circulating and central inflammatory cytokines linked to hippocampal neurogenesis; both modified by running in aged rats. Soc Neurosci Abstr Vol 36. 331.20.

22. Speisman, R.B, Kumar, A., Rani, A., Asokan, A., Foster T.C., Ormerod. B.K. (2012). Multiplex Approach for Designing a Biomarker Assay for Age-related Cognitive Decline. Annual Meeting of the Biomedical Engineering Society, Atlanta, Georgia.

23. Speisman, R.B, Kumar, A., Rani, A., Asokan, A., Foster T.C., Ormerod. B.K. (2012). Age related changes in central and circulating cytokines and their relationship to learning and memory. Soc Neurosci Abstr Vol 37. 243.07.

C. Other contributions

Additional presentations and participation

Invited Talks - 17
Conference Presentations (not including published abstracts) – 28
Memberships in Professional Societies – 8
Ad Hoc Reviewer – 19 journals
Book Chapters - 2

D. Past Support

2007 ***- UF Seed Fund*** (Frazier, Ditto, Carney, Roper, ***Ormerod – CoPI***) - $100,000

Construction of the first two-photon based laser scanning epifluorescence microscope at the University of Florida: A cross-college and multidisciplinary effort.

The major goal of this award is complete construction of a system that will stimulate cross disciplinary collaboration using techniques for imaging live cells otherwise unavailable to UF researchers.

2007-2009 – ***McKnight Brain Institute - UF Award #FF20***
(Ormerod – PI)- $50,000

The effects of chromosomal sex and developmental age on cultured and transplanted neural progenitor cells

The major goal of this award is to determine parameters that promote optimal engraftment of stem cells into the diseased or damaged brain with emphasis on minimizing inflammation.

2008–2010 ***– Ruth K. Broad Biomedical Research Foundation Extramural Award***
(Ormerod - PI) - $180,000

Neural stem cells and inflammation: Implications for Alzheimers disease

The major goal of this award is to understand how inflammation is transduced into neuroinflammation and to identify candidate inflammatory molecules that impact neural progenitor/stem cell behavior.

2010-2011 – ***McKnight Brain Research Foundation – Age-related memory loss panel***
(Ormerod – PI) - $50,000

Biomarkers of age-related cognitive decline

The major goal of this award is to identify immunomodulatory/neuroimmunomodulatory markers of age-related cognitive decline in rats.

Current Support

2010 – 2014 ***NIH – NIA*** **1R37 AG036800-01** (Dr. Tom Foster – PI; Ormerod – 30% Co-PI) - $1,140,000

Signaling cascades and memory deficits during aging

The major goal of this MERIT award is to identify inflammatory and neuroinflammatory markers that predict age-related changes in NMDAr signaling and hippocampus-dependent behavior.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Matthew R. Sarkisian, Ph.D.	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) SARKISIAN01	

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)*	MM/YY	FIELD OF STUDY
Clemson University, Clemson, SC	B.S.	05/95	Biological Sciences
University of Connecticut, Storrs, CT	Ph.D.	12/01	Physiology and Neurobiology
Yale University, New Haven, CT	Postdoc	02/02-10/08	Neurobiology

Please refer to the application instructions in order to complete sections A, B, C, and D of the Biographical Sketch.

A. Personal Statement

The goal of my research program is to understand how mutations in genes expressed in the fetal brain lead to abnormal development and dysfunction of the cerebral cortex. Development of the cortex is a highly orchestrated process, the disruption of which sets the stage for numerous neurological disorders as well as many different types of abnormalities in learning and memory. Detailed analysis of the developing brains of animals carrying these mutations will allow us to identify the specific developmental events affected by these mutations, information that could lead to development of therapeutic strategies for these devastating neural diseases. Furthermore, our understanding of molecular mechanisms critical for establishing the plasticity of the immature brain may be useful towards protective strategies from effects of age-related memory loss. My pre- and postdoctoral training were with highly established, pioneering investigators in developmental neurobiology. This training helped me to bring new technologies and expertise to the University of Florida Neuroscience program to explore basic mechanisms of neurodevelopment and pathogenesis of the cerebral cortex.

B. Positions and Honors

Positions and Employment

05/95-08/97 Research Technician, Children's Hospital, Harvard Medical School, Boston, MA
08/97-12/01 Graduate Student, Dept. of Physiology & Neurobiology, Univ. of Connecticut, Storrs, CT
Fall 1999 Teaching Assistant, Dept. of Physiology & Neurobiology, Univ. of Connecticut, Storrs, CT
02/02-01/08 Postdoctoral Associate/Fellow, Dept of Neurobiology, Yale Univ. School of Medicine, New Haven, CT
02/08-10/08 Associate Research Scientist, Dept of Neurobiology, Yale Univ. School of Medicine, New Haven, CT
10/2008- Assistant Professor, Dept of Neuroscience, Univ. of Florida, Gainesville, FL

Honors and Research Awards

1995 George M. Savoy Junior Fellowship Award (Savoy Foundation for Epilepsy Research)
1996,2000 Armenian Students' Assoc. of America (ASA), Inc. Academic Scholarship
1999, 2000 University Predoctoral Fellowship, awarded by the UConn Neurosciences Steering Committee
2001 ASA Scholarship
2004-2005 James Hudson Brown-Alexander Brown Coxe Postdoctoral Fellowship (Yale University)
2006-2007 Eric W. Lothman Training Fellowship (Epilepsy Foundation of America Distinguished Postdoctoral Award)
2008 Awarded Graduate Faculty Status by the University of Florida

Professional Memberships

1998- Member of the Society for Neuroscience
2000-02,06 Member of the American Epilepsy Society
2007- Member of the New York Academy of Sciences

C. Selected Peer-reviewed Publications (past 12 months)

1. Cannon A, Yang B, Knight J, Farnham IM, Zhang Y, Wuertzer C., D'Alton S., Lin W., Castanedes-Casey M., Rousseau L, Scott B, Jurasic M, Yu Z, Bailey R, **Sarkisian MR**, Dickson DW, Petrucelli L, Lewis J. (2012) Neuronal sensitivity to TDP-43 is dependent on timing of overexpression. *Acta Neuropathologica* 123: 807-23.
2. Arellano JI, Guadiana SM, Breunig JJ, Rakic P, **Sarkisian MR**[§]. (2012) Development and distribution of neuronal cilia in mouse neocortex. *J Comp Neurol* 520: 848-873.
3. **Sarkisian MR**[§]**,** Siebzehnrubl D (2012) Abnormal levels of Gadd45alpha in developing neocortex impair neurite outgrowth. *PLoS ONE* 7(9): e44207.

- *co-first author publication, [§]corresponding author

D. Research Support

Current Research Support

1) Source: McKnight Brain Research Foundation at University of Florida
Type: Start-up funds
Funding period: 10/03/08-10/02/13
Role: PI

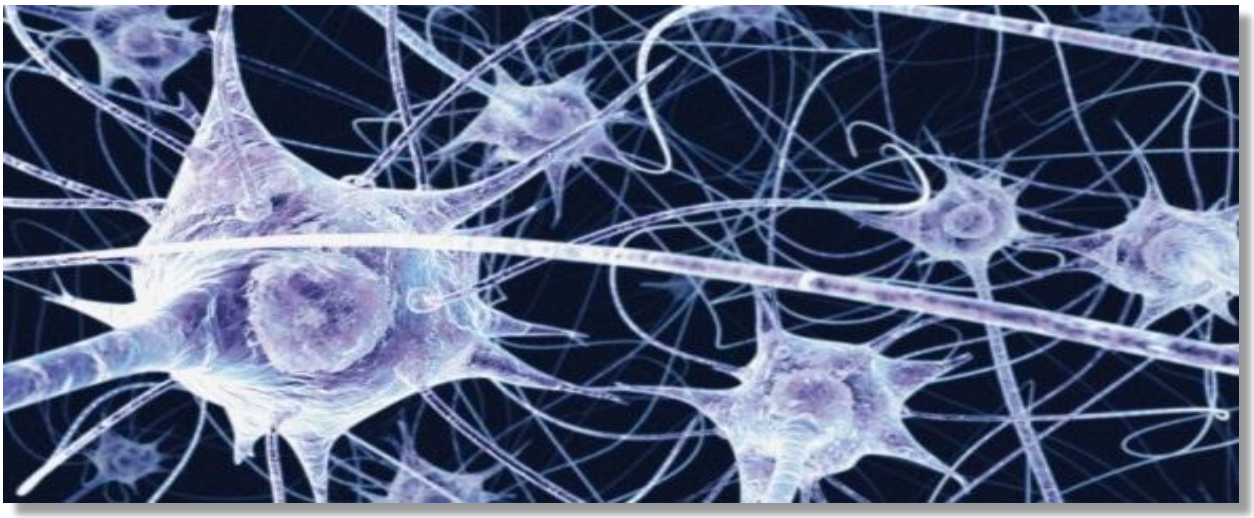
2) Source: University of Florida 2011 Research Opportunity Seed Fund Award from
Title: "Mechanisms of Abnormal Brain Development in the VPA Model of Autism"
Funding Period: 05/01/11-04/30/13
Role: Co-PI (with Dr. Mark Lewis, Psychiatry)

3) Source: American Cancer Society
Title: Identifying and Targeting Therapy Resistant Cells in Glioblastoma
Role: PI
Funding Period: 01/01/13-12/31/16

Completed Research Support

1) Source: American Cancer Society Chris DiMarco Institutional Research Grant Junior Investigator Award
Title: "Towards Inhibiting Ciliogenesis to Prevent Glioblastoma"
Funding Period: 12/01/10-11/30/11
Role: PI

2) Source: University of Florida McKnight Brain Institute Agency: Brain & Spinal Cord Injury Research Trust Fund (BSCIRTF)
Title: "A Comparison of Pathogenic Processes in Acute Spinal Cord Injury and ALS"
Funding Period: 07/01/11-06/30/12
Role: Co-PI (with Dr. David Borchelt, Neuroscience)



2012 Annual Report

Publications

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

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The Foundation for The Gator Nation

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Neurobiology of Aging 34 (2013) 845–862

NEUROBIOLOGY OF AGING

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Age-related changes in rostral basal forebrain cholinergic and GABAergic projection neurons: relationship with spatial impairment

Cristina Bañuelos[a,b], Candi L. LaSarge[c], Joseph A. McQuail[d], John J. Hartman[e], Ryan J. Gilbert[a,b], Brandi K. Ormerod[a,b,f], Jennifer L. Bizon[a,b,*]

[a] Department of Neuroscience, University of Florida College of Medicine, Gainesville, FL, USA
[b] McKnight Brain Institute, University of Florida, Gainesville, FL, USA
[c] Department of Anesthesia, Cincinnati Children's Hospital, Cincinnati, OH, USA
[d] Program in Neuroscience, Graduate School of Arts and Sciences, Wake Forest University, Winston-Salem, NC, USA
[e] Department of Psychology, Texas A&M University, College Station, TX, USA
[f] Department of Biomedical Engineering, University of Florida, Gainesville, FL, USA

Received 8 March 2012; received in revised form 15 June 2012; accepted 21 June 2012

Abstract

Both cholinergic and GABAergic projections from the rostral basal forebrain contribute to hippocampal function and mnemonic abilities. While dysfunction of cholinergic neurons has been heavily implicated in age-related memory decline, significantly less is known regarding how age-related changes in codistributed GABAergic projection neurons contribute to a decline in hippocampal-dependent spatial learning. In the current study, confocal stereology was used to quantify cholinergic (choline acetyltransferase [ChAT] immunopositive) neurons, GABAergic projection (glutamic decarboxylase 67 [GAD67] immunopositive) neurons, and total (neuronal nuclei [NeuN] immunopositive) neurons in the rostral basal forebrain of young and aged rats that were first characterized on a spatial learning task. ChAT immunopositive neurons were significantly but modestly reduced in aged rats. Although ChAT immunopositive neuron number was strongly correlated with spatial learning abilities among young rats, the reduction of ChAT immunopositive neurons was not associated with impaired spatial learning in aged rats. In contrast, the number of GAD67 immunopositive neurons was robustly and selectively elevated in aged rats that exhibited impaired spatial learning. Interestingly, the total number of rostral basal forebrain neurons was comparable in young and aged rats, regardless of their cognitive status. These data demonstrate differential effects of age on phenotypically distinct rostral basal forebrain projection neurons, and implicate dysregulated cholinergic and GABAergic septohippocampal circuitry in age-related mnemonic decline.

Keywords: Water maze; Stereology; Acetylcholine; GABA; Inhibitory; Spatial learning; Hippocampus; NeuN; Neuron number; Memory; Aging

1. Introduction

Explicit and spatial memory in humans is dependent upon the hippocampus and medial temporal lobe system, the function of which can decline precipitously with advanced age (Burke and Barnes, 2006; Della-Maggiore et al., 2002; Squire, 2004; Wilson et al., 2004). While aged individuals can exhibit learning and memory dysfunction similar to individuals with direct hippocampal damage (Gallagher and Rapp, 1997), neuron number in medial temporal lobe structures is stable across species during normal aging, even in subjects exhibiting profound mnemonic impairments (Calhoun et al., 1998; Rapp and Gallagher, 1996; Rapp et al., 2002; Rasmussen et al., 1996; Shamy et al., 2006; West et al., 2004). Despite the absence of frank hippocampal neuron loss, there is clear evidence that aging can negatively impact the processing of hippocampal-dependent spatial information (e.g., Barnes et al., 1997; Shen et al., 1997; Tanila et al., 1997) and that altered integrity of

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http://dx.doi.org/10.1016/j.neurobiolaging.2012.06.013

both cholinergic and GABAergic basal forebrain afferents to hippocampus may contribute to this functional decline (Gage et al., 1984; Gallagher and Nicolle, 1993; Smith and Pang, 2005; Ypsilanti et al., 2008).

Given their vulnerability to degeneration in Alzheimer's disease, basal forebrain cholinergic neurons have been studied extensively within the context of aging. Some studies have reported that cholinergic basal forebrain neurons decline with age, supporting the notion that their degeneration mediates age-related spatial learning impairments (Altavista et al., 1990; Armstrong et al., 1993; Bartus et al., 1982; De Lacalle et al., 1996; Durkin, 1992; Fadda et al., 2000; Fischer et al., 1989, 1992; Fragkouli et al., 2005; Gustilo et al., 1999). Notably, however, others have found no relationship between decline in cholinergic cell number and loss of cognitive abilities, and several recent studies have reported that cholinergic neuron number remains relatively stable at advanced ages (Lee et al., 1994; McQuail et al., 2011; Ypsilanti et al., 2008). The latter findings are consistent with those from studies showing that hippocampal-dependent spatial memory is largely spared following selective neurotoxic ablation of cholinergic neurons in rodents (Baxter et al., 1995).

It is becoming increasingly clear that corticopetal basal forebrain GABAergic neurons influence hippocampal physiology and hippocampal-supported cognition (Freund and Antal, 1988; Kiss et al., 1990a; Pang et al., 2001) and that the combined influence of cholinergic and GABAergic afferents is important for optimal hippocampal function. For example, septohippocampal connectivity is critical for the generation of hippocampal theta rhythms, 3–12-Hz oscillations which are strongly implicated in successful spatial cognition, memory processes, and sensorimotor integration (Bland and Colom, 1993; Bland and Oddie, 2001; Buzsáki, 2002; Colom, 2006; Rawlins et al., 1979; Winson, 1978). Lesion studies have shown that both cholinergic and GABAergic afferents from rostral basal forebrain neurons are critically important for generating these oscillations (Yoder and Pang, 2005). In addition, pronounced spatial learning impairments are produced by disruption of both cholinergic and GABAergic input to the hippocampus but not by disruption of either projection system in isolation (Baxter et al., 1995; Becker et al., 1980; Everitt and Robbins, 1997; McDonald and White, 1994; Pang et al., 2001; Parent and Baxter, 2004).

Alterations in inhibitory circuitry occur in a variety of neurodegenerative and neuropsychiatric diseases such as epilepsy, depression, schizophrenia, and autism, many of which are associated with abnormal cognitive function (Briggs and Galanopoulou, 2011; Gonzalez-Burgos et al., 2011; Lewis et al., 2005; Pizzarelli and Cherubini, 2011). Indeed, gamma aminobutyric acid (GABA)-mediated transmission appears crucial for processing information both within and between brain regions essential for mediating a variety of neurocognitive processes (Bartos et al., 2007; Volk and Lewis, 2002). While inhibitory circuitry is increasingly the focus of mechanistic studies associated with neurodegenerative diseases, considerably less attention has been paid to the anatomical integrity of GABAergic inhibitory circuits in normal aging (McKinney, 2005).

Nevertheless, there is emerging evidence that GABAergic indices change in normal aging. For example, interneurons in both prefrontal cortex and hippocampus of aged rats degenerate or cease to express the GABA-synthesizing enzyme, glutamic decarboxylase (GAD)67 (Shetty and Turner, 1998; Stanley and Shetty, 2004; Stranahan et al., 2012). Moreover, normal aging produces regionally specific changes in $GABA_B$ receptor expression and function (McQuail et al., 2012) and attenuated $GABA_A$ receptor activity and expression (Yu et al., 2006). Evoked GABA release is also reportedly decreased in the CA1 subregion of the aged rat hippocampus (Stanley et al., 2012). Finally, drugs targeting GABAergic signaling can improve cognitive functioning in both young and aged rats (Getova and Bowery, 1998, 2001; Helm et al., 2005; Lasarge et al., 2009; Mondadori et al., 1996a, 1996b).

This study was designed to determine if changes in the integrity of hippocampal-targeting basal forebrain cholinergic and GABAergic neurons are associated with loss of spatial learning abilities in aging. Confocal and stereological methods were employed to determine cholinergic (choline acetyltransferase [ChAT] immunopositive), GABAergic projection (GAD67 immunopositive) and total (NeuN immunopositive) neuron numbers in rostral basal forebrain of young and aged rats which were first characterized on a spatial learning task. The findings indicate that normal biological aging differentially impacts cholinergic and GABAergic projection neurons in rostral basal forebrain and suggest that alterations in inhibitory networks may be important contributors to age-related mnemonic dysfunction.

2. Methods

2.1. Subjects

Young adult (6 months; $n = 8$) and aged (24 months; $n = 16$) male F344 rats were obtained from the National Institute on Aging colony and housed in the vivarium in the Psychology Building at Texas A&M University for 2 weeks prior to the start of behavioral testing. This Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited vivarium was maintained at a consistent 25 °C with a 12:12 hour light/dark cycle (lights on at 8:00 AM). Rats had free access to food and water at all times. All rats in the study were screened for health problems including, but not limited to, cataracts, jaundice, food and water intake, and tumors. Sentinel rats, housed alongside the rats in this study, routinely tested negative for a range of pathogens. All animal procedures were conducted in accordance with approved institutional animal care procedures and National Institutes of Health guidelines.

2.2. Spatial learning assessment

2.2.1. Apparatus

Spatial learning abilities were assessed using the Morris water maze task as described previously (Bizon et al., 2009; LaSarge et al., 2007). The water maze apparatus consisted of a white circular tank (183 cm in diameter with a wall height of 58 cm) filled with water (27 °C) made opaque with the addition of nontoxic white tempera paint. A retractable white escape platform (12 cm diameter, HVS Image, Buckingham, UK) was submerged 2 cm below the water's surface near the center of the southwest quadrant of the maze. Black curtains, to which large white geometric shapes (extramaze cues) were affixed, surrounded the maze. Data were acquired via a video camera mounted above the maze which was connected to a DVD recorder and computer with a video tracking system and Water 2020 software (HVS Image).

2.2.2. Spatial reference memory (hidden platform) task

Rats' spatial learning abilities were tested according to methods developed by Gallagher and colleagues (Gallagher et al., 1993), with specific modifications for training F344 rats (Bizon et al., 2009; LaSarge et al., 2007). Briefly, rats received three training trials per day with a 30-second intertrial interval, over eight consecutive days. On each trial, rats were placed into the water facing the wall of the maze at one of four equally spaced start positions (north, south, east, or west). The start positions were varied in a pseudorandom fashion, such that all rats started from each of the locations approximately the same number of times. Rats were allowed to search until they found the hidden platform or until 90 seconds elapsed, at which time rats were guided to the escape platform by the experimenter. Rats remained on the platform for 30 seconds and then were placed in a holding chamber for a 30-second intertrial interval. Every sixth trial was a probe trial in which the platform was lowered to the bottom of the maze for the first 30 seconds of the trial, after which it was raised to allow the rats to escape.

2.2.3. Cued (visible platform) task

Following spatial reference memory training, rats were given a single session with six trials of cue training to assess sensorimotor abilities and motivation to escape. For cue training, rats were trained to escape to a visible platform (painted black and protruding 2 cm above the water's surface). Both the start position and platform location were varied on each trial, making the extramaze cues explicitly irrelevant to the platform location. On each trial, rats were allowed to search for the platform for 90 seconds and then were allowed to remain there for 30 seconds before a 30-second intertrial interval.

2.2.4. Behavioral and statistical analyses

Data files were created by the Water 2020 software (HVS Image) and exported to SPSS (version 16.0; SPSS, Inc., Cary, NC, USA) for analysis. Accuracy of performance on training and probe trials was assessed using a search error measure originally described by Gallagher et al. (1993). To calculate search error, the rat's distance from the platform location was sampled 10 times per second and these distances were averaged into 1-second bins. For training trials, cumulative search error was derived by summing these 1-second averages and then subtracting the optimal path between the start location and the platform location. For probe trials, a mean search error measure was derived by dividing cumulative search error by the 30-second duration of the probe trials. Training trial data were averaged into 4 blocks consisting of the 5 trials preceding each probe trial. Comparisons between groups on training trials were conducted using two-factor repeated measures analysis of variance (ANOVA) (age $\times$ training block) with Tukey's post hoc tests performed when warranted. In all statistical comparisons, p values ≤ 0.05 were considered significant.

To provide an overall measure of spatial learning ability for each rat, a "spatial learning index" (SLI) was calculated using mean search error from the interpolated probe trials as described previously (Bizon et al., 2009; Gallagher et al., 1993). Mean search error on probe trials was weighted and summed to provide the spatial learning index (Bizon et al., 2009). For some comparisons of cell number, aged rats were subgrouped on the basis of their spatial learning index. This classification approach has been successfully used in prior studies to identify and investigate structural and signaling alterations in the hippocampus and related circuitry that are relevant to decline of spatial learning abilities in aged rats (Bizon et al., 2001, 2004; Colombo et al., 1997; Foster and Kumar, 2007; Nicolle et al., 1999; Rapp and Gallagher, 1996). Aged rats that fell more than two times the standard deviation outside of the mean spatial learning index calculated for young adult rats were classified as "aged spatially impaired" (SLI $>$ 275) and all other aged rats (which fell within the range of the young adult cohort) were classified as "aged spatially-unimpaired" (SLI $\leq$ 275).

2.3. Immunofluorescent labeling of cholinergic, GABAergic projection, and total rostral basal forebrain neurons

One week after completion of behavioral testing, rats were rapidly euthanized with an overdose of pentobarbital and perfused transcardially with ice cold 0.9% saline followed by 4% paraformaldehyde. Brains were removed from the skull, postfixed for 24 hours in perfusate, and then cryoprotected in 20% sucrose in 0.1 M phosphate buffer. Systematic uniform random sampling was achieved by exhaustively sectioning brains coronally on a calibrated freezing stage microtome (35 μm) through the full rostrocaudal extent of the medial septum and vertical limb of the diagonal band of Broca (beginning just caudal to the olfactory bulbs and ending caudal to the crossing of the anterior commissure). A registered 1-in-4 series of sections was obtained for each animal and separate series (spaced at 140 μm intervals) were randomly assigned for processing to detect

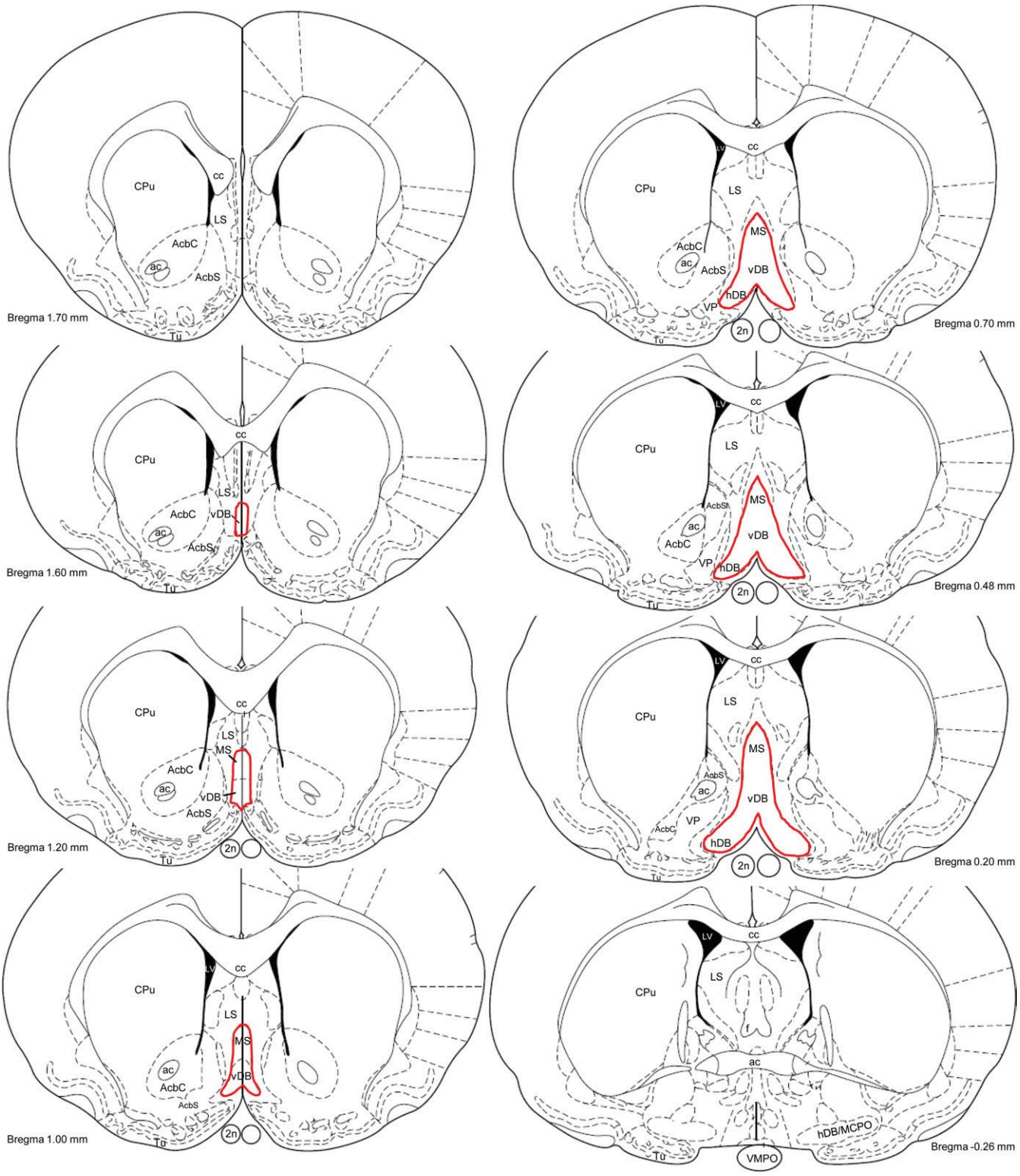

Fig. 1. Rostral basal forebrain boundaries used for cell count estimations. Schematic illustrations modified from Paxinos and Watson (2007) showing the rostrocaudal boundaries (extending from 1.7 mm anterior to −0.26 mm posterior of Bregma) and the delineation of rostral basal forebrain nuclei (red outline) used in the current study. The basal forebrain neurons that innervate the hippocampal formation are primarily localized within the medial septum (MS) and vertical limb of the diagonal band of Broca (vDB). Just caudal to the joining of the corpus callosum, these contiguous nuclei emerge along the midline with the vDB situated ventral to the MS. These nuclei are bordered laterally by the lateral septum and in more caudal sections by the medial edge of the nucleus accumbens shell (AcbS). The vDB extends ventrally to the medial intersection of the 2 hemispheres on the ventral edge of the tissue. In more caudal planes, the rostral-most portion of the horizontal limb of the diagonal band of Broca (hDB) emerges. Contiguous with the vDB, the hDB is bordered laterally by

rostral basal forebrain cholinergic (ChAT), GABAergic projection (GAD67) or total (NeuN) immunopositive neurons (Gundersen and Jensen, 1987). Sections were collected into cold 0.1 M phosphate-buffered saline and stored at 4 °C until stained immunohistochemically.

For immunohistochemistry, free-floating sections were washed several times in 0.1 M Tris-buffered saline (TBS; 100 mM Tris-HCl, 150 mM NaCl, pH 7.5), preincubated in a blocking solution containing 3% normal donkey serum and 0.3% Triton X-100 in 0.1 M TBS for 1 hour at room temperature and then incubated in blocking solution that contained rabbit anti-GAD67 (Bioworld Technology, Louis Park, MN, USA; 1:500); goat anti-ChAT (Millipore, Temecula, CA, USA; AB144P, 1:1000) or mouse anti-NeuN (Millipore; MAB377, 1:500) for 72 hours at 4 °C. After primary incubation, sections were washed in 0.1 M TBS, and incubated in 0.1 M TBS containing 2% normal donkey serum and the appropriate Alexa 488-conjugated secondary antibodies (Invitrogen, Carlsbad, CA, USA; 1:300) for 2 hours at room temperature in the dark. The sections were washed in 0.1 M TBS, and mounted onto Superfrost++ slides (Fisher Scientific, Pittsburgh, PA, USA). The sections were then coverslipped under ProLong Gold (Invitrogen), sealed with clear fingernail polish, and stored at 4 °C until analysis.

2.4. Stereological counts of ChAT, GAD67, and NeuN immunopositive neurons

2.4.1. Delineation of the rostral basal forebrain

The basal forebrain neurons that innervate the hippocampal formation are primarily localized within the medial septum (MS) and the vertical limb of the diagonal band of Broca (vDB) (Dutar et al., 1995; Lewis and Shute, 1967; McKinney et al., 1983; Meibach and Siegel, 1977; Segal and Landis, 1974; Swanson and Cowan, 1979). As shown in Fig. 1, these are contiguous nuclei that emerge along the midline just caudal to the joining of the corpus callosum. The vDB is positioned ventral to the MS and these nuclei are bordered laterally by the lateral septum and the medial edge of the nucleus accumbens shell. The vDB extends ventrally to the medial intersection of the two hemispheres on the ventral edge of the tissue section. In more caudal planes, the rostral-most portion of the horizontal limb of the diagonal band of Broca (hDB) emerges. Contiguous with the vDB, the hDB extends laterally along the medial edge of the nucleus accumbens shell and is bordered ventrally by the ventral pallidum and olfactory tubercle. The caudal-most edge of the vDB is rostral to the joining of the anterior commissure. After the crossing of the anterior commissure, the hDB (sometimes also referred to as the magnocellular preoptic area in this plane) is a clearly defined nucleus located in the ventral and lateral basal forebrain. As such, the crossing of the anterior commissure is often used as a boundary between rostral basal forebrain and more caudal neocortical-innervating basal forebrain nuclei (Colom et al., 2005; McQuail et al., 2011; Peterson et al., 1999; Ypsilanti et al., 2008). In the current study, counts were obtained from equally spaced (140 μm apart) sections throughout the entire rostrocaudal extent of the medial septum and vDB. Because there are not clear boundaries that allow the hDB to be reliably distinguished in the rostral basal forebrain, neurons within the hDB rostral to the crossing of the anterior commissure were also included in the population estimates. The rostral basal forebrain nuclei as a whole can be readily distinguished from surrounding structures (described above) within ChAT, GAD67, and NeuN immunolabeled material (Figs. 2–4).

2.4.2. Estimation of neuron number using the optical fractionator

Starting at a randomly selected level within the first sampling interval, the optical fractionator method (Gundersen, 1986; Peterson, 1999; West et al., 1991) was implemented using an Olympus Fluoview 300 confocal microscope, equipped with the appropriate filter sets, a CCD camera, and a computer-driven x, y, and z Ludl-motorized stage controlled with StereoInvestigator software (version 10; MBF BioScience, Williston, VT, USA). Regional boundaries for the rostral basal forebrain nuclei (shown in Fig. 1) were delineated at low power magnification (4×) in an evenly spaced series of immunolabeled sections. The series spanned the rostrocaudal extent of the MS and vDB, which are the basal forebrain nuclei that innervate the hippocampal formation (Dutar et al., 1995; Lewis and Shute, 1967; McKinney et al., 1983; Meibach and Siegel, 1977; Segal and Landis, 1974; Swanson and Cowan, 1979). This design yielded 7–8 sections for quantification (per 1-in-4 series) from each brain. The motorized stage of the microscope was moved in evenly spaced x-y intervals under the computer control, surveying the regions of interest in each section according to a systematic random sampling scheme (Table 1 for sampling details). Using a 60× oil-immersion objective (with 1.4 numerical aperture), section thickness was measured at each sampling site and z-stacks (comprised of 1 μm z-

the medial wall of the nucleus accumbens shell, and in more caudal sections, by the ventral pallidum and olfactory tubercle. The caudal-most edge of the vDB is just rostral to the joining of the anterior commissure (just prior to the Bregma −0.26 mm). At the crossing of the anterior commissure, the hDB (often referred to as magnocellular preoptic area in this plane of section) is located toward the ventral edge of the tissue and no basal forebrain nuclei remain along the midline. Because there are not clear boundaries reliably distinguishing the hDB from vDB in the rostral basal forebrain, immunopositive neurons localized within the hDB situated rostral to the crossing of the anterior commissure were included in the population estimates. Abbreviations: 2n, optic nerve; ac, anterior commissure; AcbC, nucleus accumbens core; cc, corpus callosum; CPu, caudate putamen; LS, lateral septum; LV, lateral ventricle; MCPO, magnocellular preoptic area; Tu, olfactory tubercle.

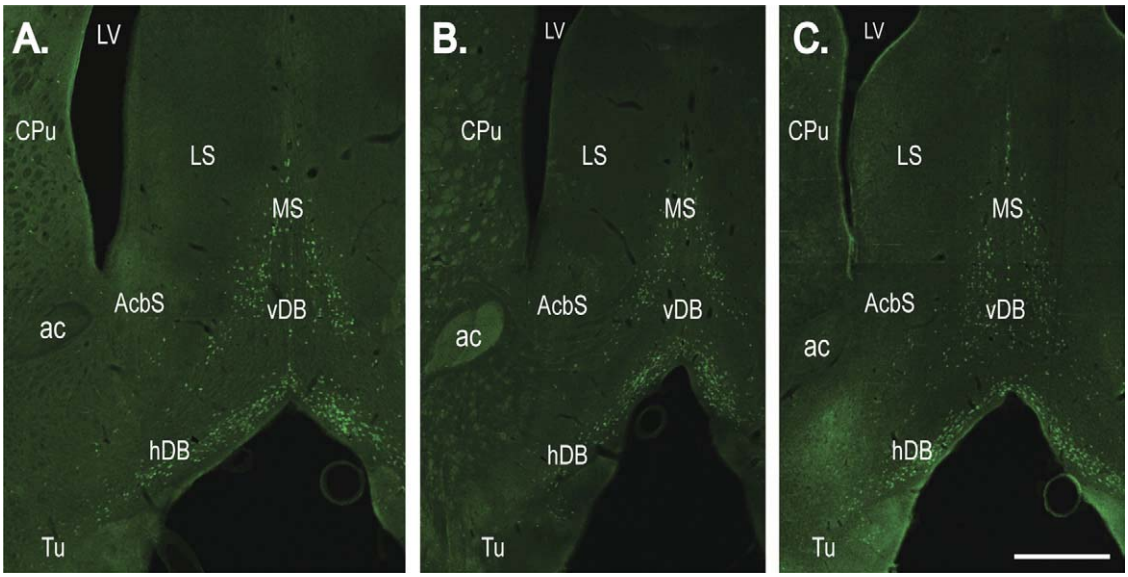

Fig. 2. Distribution of choline acetyltransferase (ChAT) immunopositive cells in rostral basal forebrain. Low magnification photomicrographs of ChAT immunolabeling in coronal sections taken through the rostral basal forebrain (approximately 0.5 mm anterior to bregma) of representative young (A), aged spatially unimpaired (B) and aged spatially impaired (C) rats. Staining was robust in individual neurons localized to rostral basal forebrain nuclei including the medial septum (MS), vertical limb of the diagonal band of Broca (vDB) and horizontal limb of the diagonal band of Broca (hDB), and was also evident in scattered interneurons distributed throughout the caudate putamen (CPu), nucleus accumbens (Acb) and olfactory tubercle (Tu). The dense immunolabeling of the ChAT immunopositive neurons within the MS, vDB, and hDB made these nuclei readily distinguishable from neighboring structures (including lateral septum, the medial wall of the nucleus accumbens shell [AcbS] and the olfactory tubercle) across age and cognitive groups. For orientation, white matter regions (i.e., corpus callosum and anterior commissure) and the lateral ventricle (LV) are labeled. Scale bar = 200 μm. Abbreviations: ac, anterior commissure; cc, corpus callosum; LS, lateral septum.

slices) were acquired through the full section thickness at the appropriate emission wavelengths. The mean section thickness was measured at 30.62 μm (coefficient of variation = 0.13), indicating an approximate 12.5% tissue shrinkage in the z-plane. This degree of shrinkage is significantly less than that observed from immunohistological procedures that require dehydration and is consistent with other reports of immunofluorescent tissue processing (Hart and Terenghi, 2004; Prasad and Richfield, 2010). Quantification was performed offline on the acquired z-stacks and was confined to an optical disector 25 μm in height which was positioned 3 μm below the surface of the tissue. The top-most nucleus associated with an immunopositive neuron was counted only when it first came into focus within the optical disector, provided it did not encroach on the exclusion lines of the counting frame (Gundersen, 1986; Sterio, 1984). The sufficiency of the guard zone was confirmed by plotting the distribution of the cells counted for each marker in the z-axis (Andersen and Gundersen, 1999; Dorph-Petersen et al., 2001, 2009). The number of cells counted was highly consistent across the disector height, indicating that the guard zone of 3 μm was sufficient to minimize the effects of superficial damage associated with tissue sectioning (i.e., to avoid lost caps [Gundersen, 1986], Supplementary Fig. 1). Moreover, the distribution of cells along the z-axis did not differ between age or cognitive groups (Gardella et al., 2003; Supplementary Fig. 1), confirming good antibody penetration throughout the full section thickness (see representative examples of cell positions within the disector in the orthogonal windows shown in Supplementary Fig. 1). The total number of ChAT, GAD67, and NeuN immunopositive cells in rostral basal forebrain was estimated using the optical fractionator method (West et al., 1991) in which the product of the cells counted in a known, uniformly random sample of the region of interest is multiplied by the reciprocal of the sampling fraction. The shrinkage robust version of the optical fractionator based upon the number weighted mean section thickness was used (Dorph-Petersen et al., 2001). Additional details, including stereological sampling parameters are provided in Table 1.

The precision of the stereological estimates was determined by estimating the coefficients of error (CE) using methods described by Gundersen et al. (1999). Equations used to generate the CEs are based on Gundersen's smoothness classification $m = 1$, as the areas defined for the cell counts changed smoothly from the rostral entrance of the MS to the caudal conclusion of the vDB. These CEs (ranging from 0.04 to 0.06) were less than half of the observed variation across subjects (coefficients of variation ranging from 0.16 to 0.2; Table 1), indicating that the sampling and counting parameters were sufficiently precise to detect frank biologically driven differences in neuronal population estimates among experimental groups (Boyce et al., 2010;

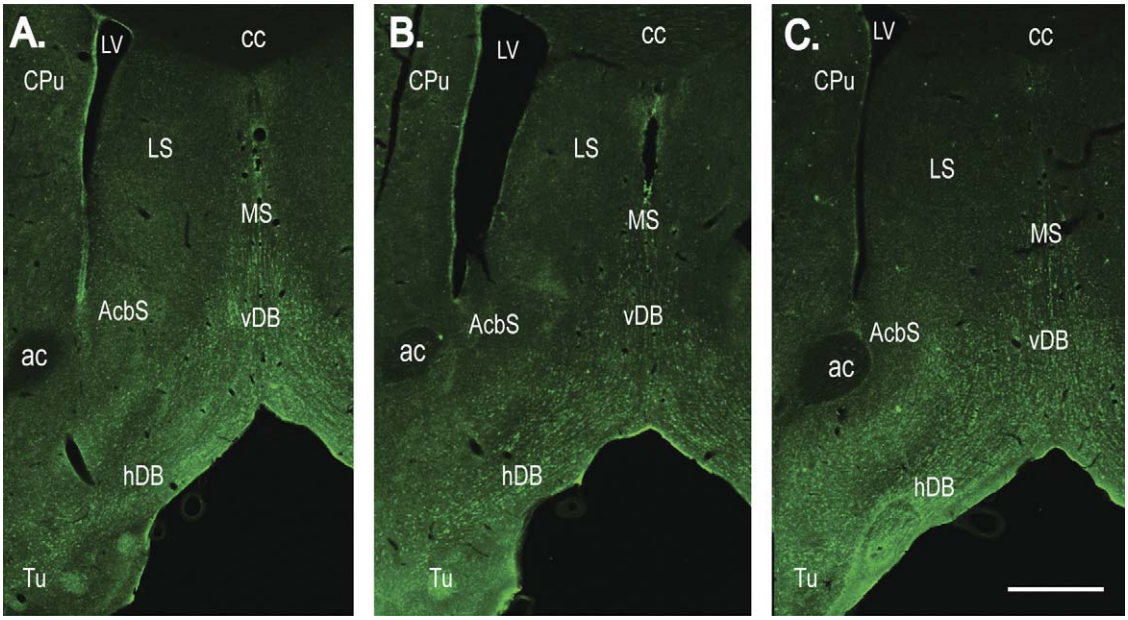

Fig. 3. Distribution of glutamic decarboxylase (GAD)67 immunopositive cells in rostral basal forebrain. Low magnification photomicrographs of GAD67 immunolabeling in coronal sections taken through the rostral basal forebrain (approximately 0.5 mm anterior to bregma) of representative young (A), aged spatially unimpaired (B) and aged spatially impaired (C) rats. GAD67 labeling was robust throughout rostral basal forebrain nuclei, including medial septum and the vertical and horizontal limbs of diagonal band of Broca (vDB and hDB, respectively). Somewhat diffuse GAD67 immunolabeling was also observed in lateral septum (LS) and in nucleus accumbens (Acb), whereas scattered individual GAD67 immunopositive cells were observed in the caudate putamen (CPu) and olfactory tubercle (Tu). Across age and cognitive groups, dense labeling within basal forebrain nuclei allowed these regions to be readily delineated from surrounding nuclei. For orientation, white matter regions (i.e., corpus callosum and anterior commissure) and the lateral ventricle (LV) are labeled. Scale bar = 200 μm. Abbreviations: ac, anterior commissure; AcbS, nucleus accumbens shell; cc, corpus callosum; MS, medial septum.

Dorph-Petersen et al., 2001; Gundersen and Jensen, 1987; Gundersen and Osterby, 1981; West, 1999).

3. Results

3.1. Cognitive performance in young and aged rats

In the spatial water maze task, a comparison of performance on training trials using the cumulative search error measure (two-factor ANOVA; age × trial block) indicated that both young and aged rats improved performance over the course of training trials (main effect of training trial block, $F(3,66) = 35.8$; $p < 0.0001$) but that aged rats were significantly impaired in finding the platform in comparison with young (main effect of age, $F(1,22) = 9.8$; $p < 0.005$, Fig. 5A). Notably, these differences were not present on the very first training trial ($F(1,22) = 0.77$; not significant [n.s.]), indicating that aged rats' initial search strategies were comparable with those of young rats. Performance on probe trials as assessed with the mean search error measure (two-factor ANOVA; age × probe trial) revealed results similar to those on training trials, in that search for the platform became more accurate as training progressed (main effect of probe trial, $F(3,66) = 13.8$; $p < 0.0001$) but aged rats were overall less proficient in their search than young rats (main effect of age, $F(1,22) = 7.34$; $p < 0.05$). In contrast to the spatial task, there was no impairment in the ability of aged rats to locate the visible escape platform during cue (visible platform) training (one-factor ANOVA, $F(1,22) = 1.4$; n.s.), indicating intact sensorimotor and motivational processes.

In order to relate population estimates to cognitive abilities, mean search error during probe trials was used to calculate a SLI for each rat as described above (Bizon et al., 2009; Gallagher et al., 1993). This measure, specifically designed to maximize individual differences in water maze performance within the context of aging, has been shown to correlate with age-related changes in numerous neurobiological substrates of spatial memory (Bizon et al., 2001, 2004; Colombo et al., 1997; Nicolle et al., 1999; Smith et al., 2000). Fig. 5B shows individual spatial learning indexes of young and aged rats. For some analyses, aged rats were subgrouped based on their SLI such that aged rats performing more than two times the standard deviation of the mean SLI of the young group (SLI > 275) were classified as "aged spatially impaired" ($n = 8$) and all other aged rats (SLI ≤ 275) were classified as "aged spatially unimpaired" ($n = 8$). A repeated measures ANOVA (training trial block × cognitive group) confirmed that cumulative search error across training blocks differed between these subgroups ($F(2,21) = 9.91$; $p < 0.01$) and Tukey's post hoc analyses indicated that aged spatially impaired rats performed significantly worse than both young and aged spatially unimpaired

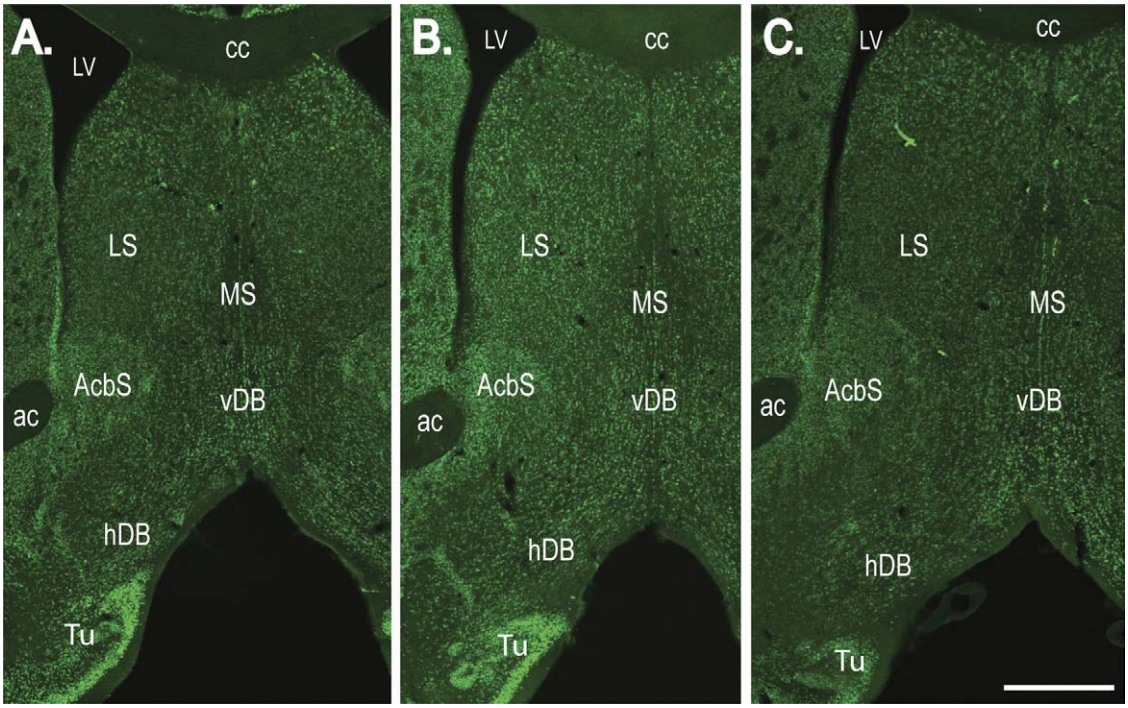

Fig. 4. Distribution of NeuN immunopositive cells in rostral basal forebrain. Low magnification photomicrographs of NeuN immunolabeling in coronal sections taken through the rostral basal forebrain (approximately 0.5 mm anterior to bregma) of representative young (A), aged spatially unimpaired (B), and aged spatially impaired (C) rats. NeuN expression is prominent in most neurons through rat forebrain, although differences in density and intensity of expression allow discrete nuclei to be clearly distinguished. NeuN immunolabeling is robust throughout the medial septum (MS) and the vertical and horizontal limbs of the diagonal band of Broca (vDB and hDB, respectively). It is also particularly prominent in lateral septum (LS) and the nucleus accumbens shell (AcbS), as well as in olfactory tubercle (Tu), allowing the boundaries of basal forebrain nuclei to be clearly delineated across age and cognitive groups. For orientation, white matter regions (i.e., corpus callosum [cc] and anterior commissure [ac]) and the lateral ventricle (LV) are labeled. Scale bar = 200 μm. Abbreviations: 2n, optic nerve; AcbC, nucleus accumbens core; CPu, caudate putamen; MCPO, magnocellular preoptic area.

rats ($ps < 0.05$); however, performance of young and aged spatially unimpaired rats did not differ (n.s.).

3.2. Distribution of ChAT, GAD67, and NeuN immunopositive neurons

Figs. 2–4 show low power photomicrographs of representative immunolabeling for ChAT (Fig. 2), GAD67 (Fig. 3), and NeuN (Fig. 4) in independent series of sections through the rostral basal forebrain of representative young, aged spatially unimpaired, and aged spatially impaired rats. Both cholinergic (ChAT immunopositive; Fig. 2) and GABAergic projection (GAD67 immunopositive, Fig. 3) neurons were distributed heterogeneously throughout the rostral basal forebrain nuclei and represented clear subpopulations of those neurons immunolabeled for NeuN (Fig. 4). For each label and across age and cognitive

Table 1
Sampling parameters used for estimating total number of rostral basal forebrain neurons

Object	Sampling grid (μm × μm)	Counting frame (μm × μm)	Disector height (μm)	Average object counted ± SD	Average CE[a]	CV
ChAT+ cells	336 × 448	140 × 140	25	292 ± 42.89	0.05	0.16
GAD67+ cells	448 × 448	110 × 100	25	679 ± 101.14	0.06	0.20
NeuN+ cells	400 × 400	89 × 89	25	781 ± 175.33	0.04	0.16

Total cell numbers were estimated using the formula: N (total number) = 1/section sampling fraction (ssf) × 1/area sampling fraction (asf) × 1/height sampling fraction (hsf) × number of immunopositive cells counted. The total number of cells counted for each subject and the sampling grid and counting frame used to generate the asf for each label are provided above. The ssf in the current study equaled ¼ in every case. The hsf was calculated using the mean weighted thickness (with section thickness measured at each sampling site).

Key: +, positive; SD, standard deviation; CE, coefficient of error; ChAT, choline acetyltransferase; CV, coefficient of variation; GAD, glutamic decarboxylase.

N = 24 total rats for ChAT and N = 23 for GAD67 and NeuN.

[a] Gundersen's CE, $m = 1$.

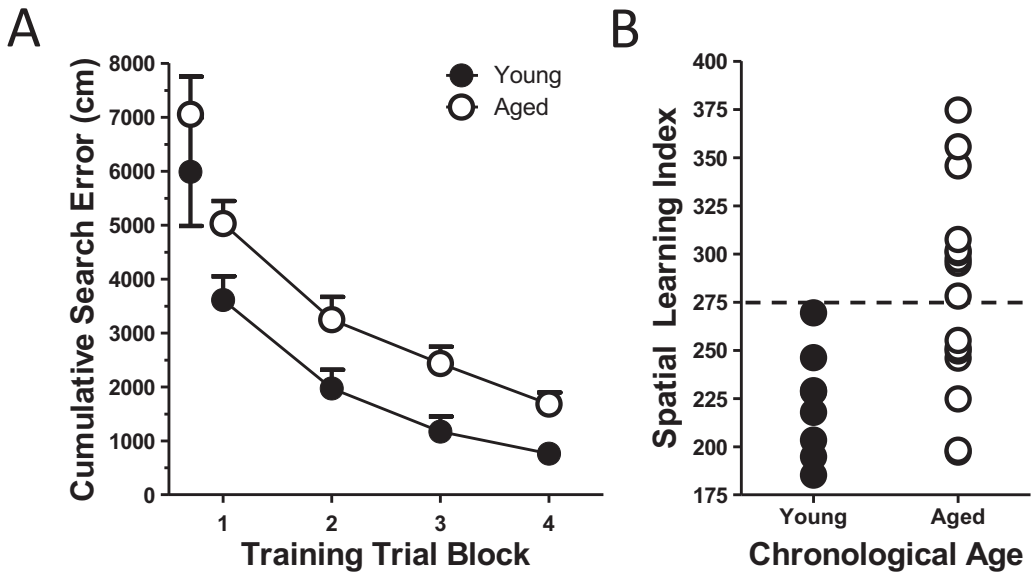

Fig. 5. Spatial learning in young and aged rats. (A) Young and aged rats did not differ on the first training trial, and both groups improved over the course of training. As a group, aged rats were significantly impaired relative to young in learning to swim to a hidden (submerged) platform within the water maze. (B) Spatial learning index (SLI) scores were calculated from probe trial performance to provide an overall index of spatial learning ability for each subject. Note that there was considerable variability among aged rats, with many performing within the range of young (aged spatially unimpaired rats; SLI $\leq$ 275) and others performing more than two times the standard deviation of mean young rat performance, demonstrating impairment (aged spatially impaired rats, SLI $>$ 275). See text for statistical analysis.

groups, the rostral basal forebrain nuclei could be readily distinguished from bordering structures (including the lateral septum, nucleus accumbens, and olfactory tubercle).

High magnification immunofluorescent labeling of ChAT, GAD67, and NeuN immunopositive neurons is shown in Fig. 6. ChAT immunopositive cells (Fig. 6A–C) were robustly labeled with well-defined nuclei and tended to be polygonal and fusiform in shape. Overall, ChAT immunopositive neurons appeared larger but less densely distributed than GAD67 immunopositive cells (Fig. 6D–F) in the same region. In agreement with previous reports (Brashear et al., 1986; Colom, 2006; Gritti et al., 2003, 2006), the GAD67 immunopositive cells exhibited diverse morphologies (oval, fusiform, or polygonal cell bodies were observed) and sizes (ranging from small oval cells to large multipolar cells). Labeling of ChAT and GAD67 was consistent with numerous neuroanatomical studies that have demonstrated that ChAT and GAD immunopositive neurons in the rostral basal forebrain are distinct nonoverlapping neuronal populations (Brashear et al., 1986; Formaggio et al., 2011; Gritti et al., 1993; Köhler et al., 1984; Semba, 2000). While the ChAT immunopositive cells were primarily clustered within the medial septum and along the ventral edge of the hDB, the GAD67 immunopositive cells were distributed more homogenously throughout the rostral basal forebrain subfields. As expected, NeuN immunopositive cells (which include both ChAT and GAD67 immunopositive neurons as well as a variety of other projection and interneurons) had discernible nuclei, were located throughout the rostral basal forebrain, and exhibited diverse sizes and morphologies; Fig. 6G–I). No obvious morphological differences in ChAT, GAD67, or NeuN immunopositive cells were detected at either low or high magnification in sections obtained from rats that differed in age or cognitive status.

3.3. Age and cognitive comparisons of ChAT, GAD67, and NeuN immunopositive cell numbers in the rostral basal forebrain

3.3.1. ChAT immunopositive neurons

A comparison between young and aged rats yielded a significant but modest decline in the number of ChAT immunopositive cells in aged rats relative to young (-12.5%; $t(22) = 2.00$; $p < 0.05$; Fig. 7A; Table 2). A comparison of ChAT immunopositive cells among young and aged rats subgrouped based upon spatial learning ability indicated a similar trend toward decreased ChAT immunopositive cell number with age ($F(2,21) = 2.23$; $p = 0.13$) but the magnitude of reduction in aged rats was comparable across aged spatially unimpaired (-14%) and aged spatially impaired (-12%) subgroups (Fig. 7B). In agreement with the observation that ChAT immunopositive cell number was reduced by a similar magnitude in both aged spatially unimpaired and aged spatially impaired rats, there was no reliable relationship between ChAT immunopositive cell number and spatial learning index among aged rats ($r = -0.10$; n.s.; Fig. 7D). Notably, however, a significant correlation between ChAT immunopositive cell number and spatial learning index

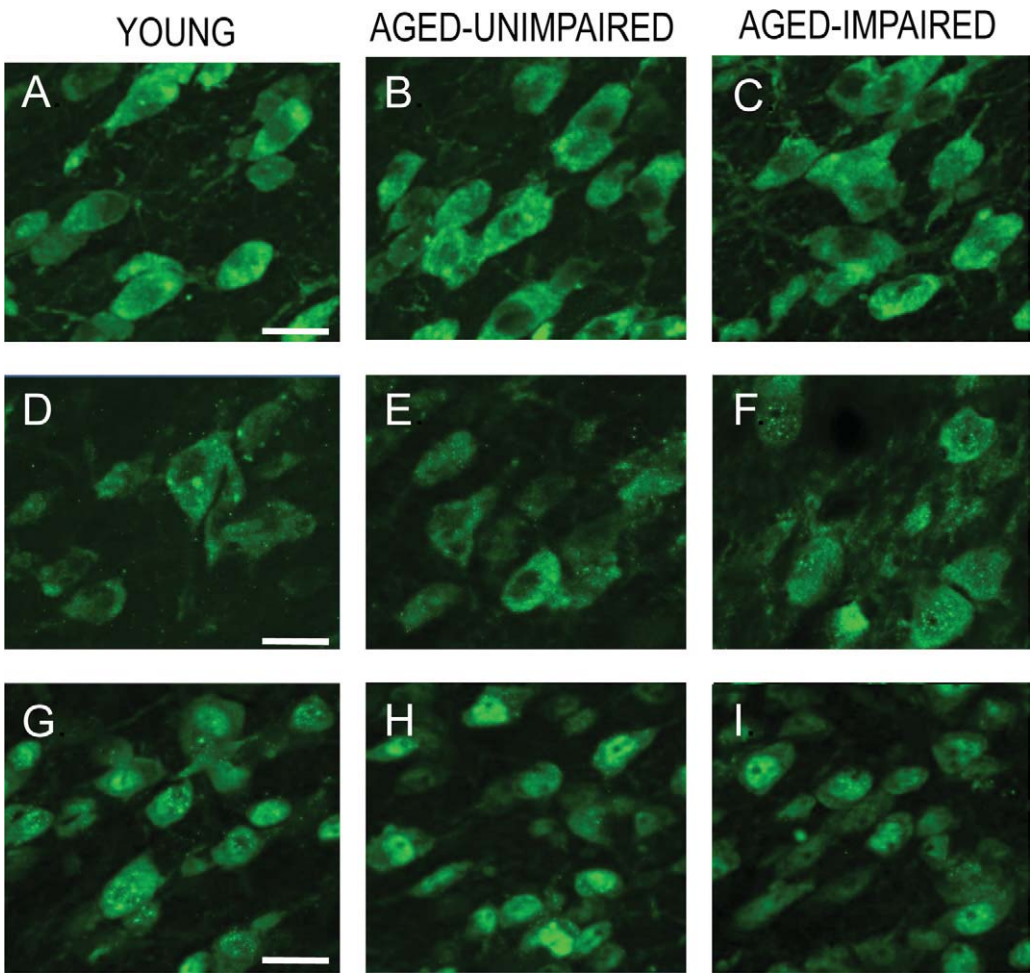

Fig. 6. High magnification immunofluorescent labeling of choline acetyltransferase (ChAT), glutamic decarboxylase (GAD)67, and NeuN immunopositive neurons. Representative ChAT (A–C), GAD67 (D–F), and NeuN (G–I) immunopositive cells in young (left), aged spatially unimpaired (middle), and aged spatially impaired rats (right). Across ChAT, GAD67, and NeuN material, immunopositive cells were robustly labeled and contained a well-defined nucleus. Overall, ChAT immunopositive cells tended to be polygonal and fusiform shaped whereas GAD67 immunopositive cells exhibited diverse morphologies and sizes (ranging from small oval cells to large multipolar cells). The NeuN immunopositive cells (which would include both ChAT and GAD67 immunopositive neurons as well as a variety of other projection and interneurons) had discernable nuclei and exhibited diverse sizes and morphologies. No obvious morphological differences between ChAT, GAD67, or NeuN immunopositive cells were detected from rats that differed in age or cognitive status. Scale bar = 20 μm.

was evident in young rats, such that higher ChAT immunopositive cell numbers were associated with better spatial learning performance ($r = -0.76$; $p < 0.05$; Fig. 7C).

3.3.2. GAD67 immunopositive neurons

A very different pattern of results was obtained from estimates of total GABAergic projection (GAD67 immunopositive) neuron numbers in the same subfields. As shown in Fig. 8A, GAD67 immunopositive cell number was greater in aged relative to young rats, although this difference was not statistically reliable ($t(21) = 0.74$; n.s.). However, a one-factor ANOVA comparing GAD67 immunopositive cell number in young and aged rats subgrouped on the basis of spatial learning ability revealed a highly significant difference among cognitive groups ($F(2,20) = 16.2$; $p < .0001$; Fig. 8B; Table 2). Post hoc comparisons confirmed a robust statistically significant increase in the number of GAD67 immunopositive cells specifically in aged spatially impaired rats relative to both young (+ 18%; $p < 0.005$) and aged spatially unimpaired (+ 25%; $p < 0.0001$) rats. The number of GAD67 immunopositive cells did not differ significantly between young and aged spatially unimpaired rats (n.s.). The strong relationship between elevated GAD67 immunopositive cell number and cognitive impairment was supported by a significant correlation among aged rats ($r = 0.60$; $p < 0.05$), such that higher numbers of GAD67 immunopositive cells were associated with higher spatial learning indexes (i.e., worse learning; Fig. 8D). Among young rats the relationship between spatial learning ability and GAD67 immunopositive cell number was in the same direction but did not reach statistical significance ($r = 0.49$; $p = 0.22$; Fig. 8C).

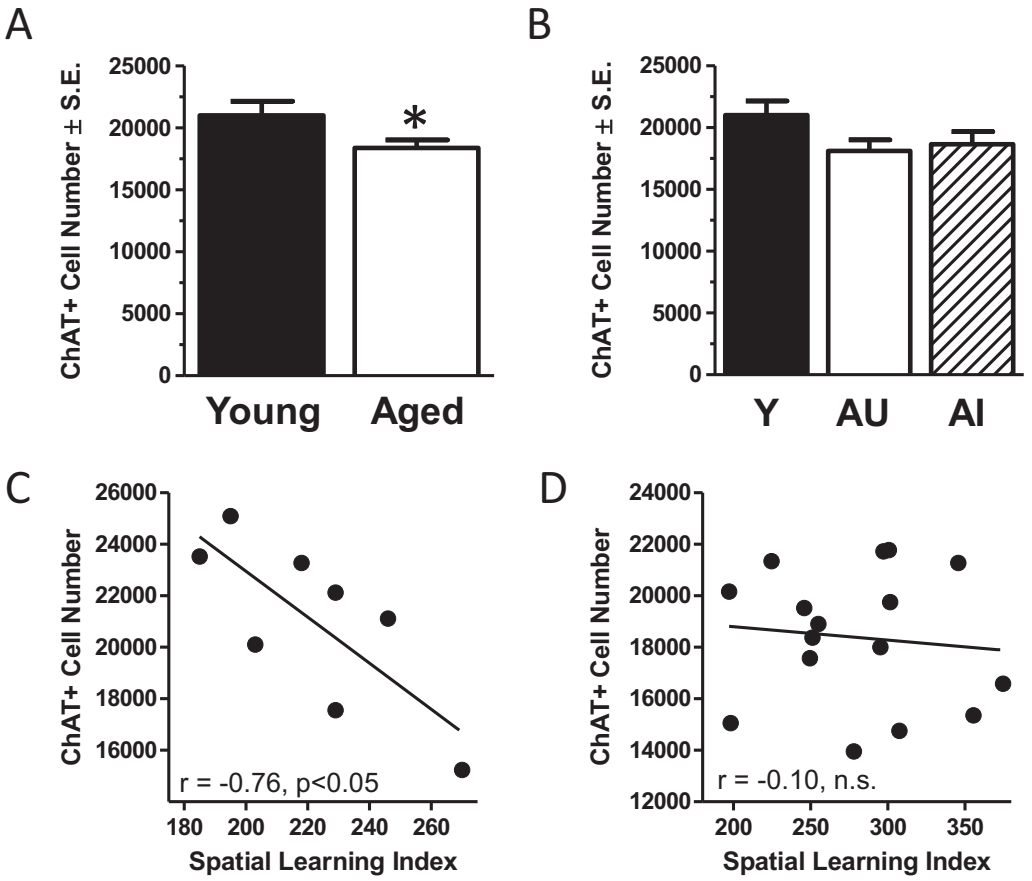

Fig. 7. Cholinergic (choline acetyltransferase [ChAT] immunopositive) cell number in the rostral basal forebrain of young (Y) and aged rats. (A) The number of ChAT immunopositive cells was modestly but significantly decreased in aged relative to young rats. (B) The reduction in ChAT immunopositive cells was of a similar magnitude (approximately −12.5%) in both aged spatially unimpaired (AU) and aged spatially impaired (AI) rats. (C) Scatter plot illustrating a strong relationship between ChAT immunopositive cell number and spatial learning index among young rats. (D) This relationship was not present among aged rats. See text for statistical analyses. * $p < 0.05$.

3.3.3. *NeuN immunopositive neurons*

To investigate whether age- and cognition-related alterations in ChAT immunopositive and GAD67 immunopositive cell estimates contributed to an overall difference in neuron number in rostral basal forebrain, NeuN immunopositive cells were also quantified. As shown in Fig. 9A, the mean estimated number of NeuN immunopositive cells was numerically lower in aged rats relative to young but this difference was not reliable ($t(1,21) = 1.2$; n.s.; Table 2). Likewise, no significant differences were evident using a one-factor ANOVA performed on young and aged rats subgrouped by cognitive ability ($F(2,20) = 0.78$; n.s.; Fig. 9B). As expected based on these analyses, no reliable correlations were observed between NeuN cell number and spatial learning indexes in young ($r = -0.13$; n.s.; Fig. 9C) or aged rats ($r = 0.26$; n.s.; Fig. 9D).

4. Discussion

This study was designed to test the hypothesis that coordinated age-related alterations in rostral basal forebrain cholin-

Table 2
Estimates of ChAT, GAD67, and NeuN immunopositive cells in rostral basal forebrain of young and aged behaviorally characterized rats

	Young	Aged, Total	Aged-SU	Aged-SI
ChAT+ cells (SD, *n*)	21003.47 (3284.54, 8)	**18383.71*** **(2626.83, 16)**	18113.89 (2515.56, 8)	18653.53 (2879.54, 8)
GAD+ cells (SD, *n*)	48586.17 (5564.82, 8)	51118.00 (8721.43, 15)	44459.45 (4408.62, 8)	**58727.78**** **(5317.29, 7)**
NeuN+ cells (SD, *n*)	140007.13 (19561.76, 8)	128798.47 (21035.71, 15)	124938.86 (22388.96, 7)	132175.63 (20674.01, 8)

Key: +, positive; ChAT, choline acetyltransferase; GAD, glutamic decarboxylase; SI, spatially impaired; SU, spatially unimpaired.
* $p < 0.05$ relative to young.
** $p < 0.001$ relative to both young and aged unimpaired.

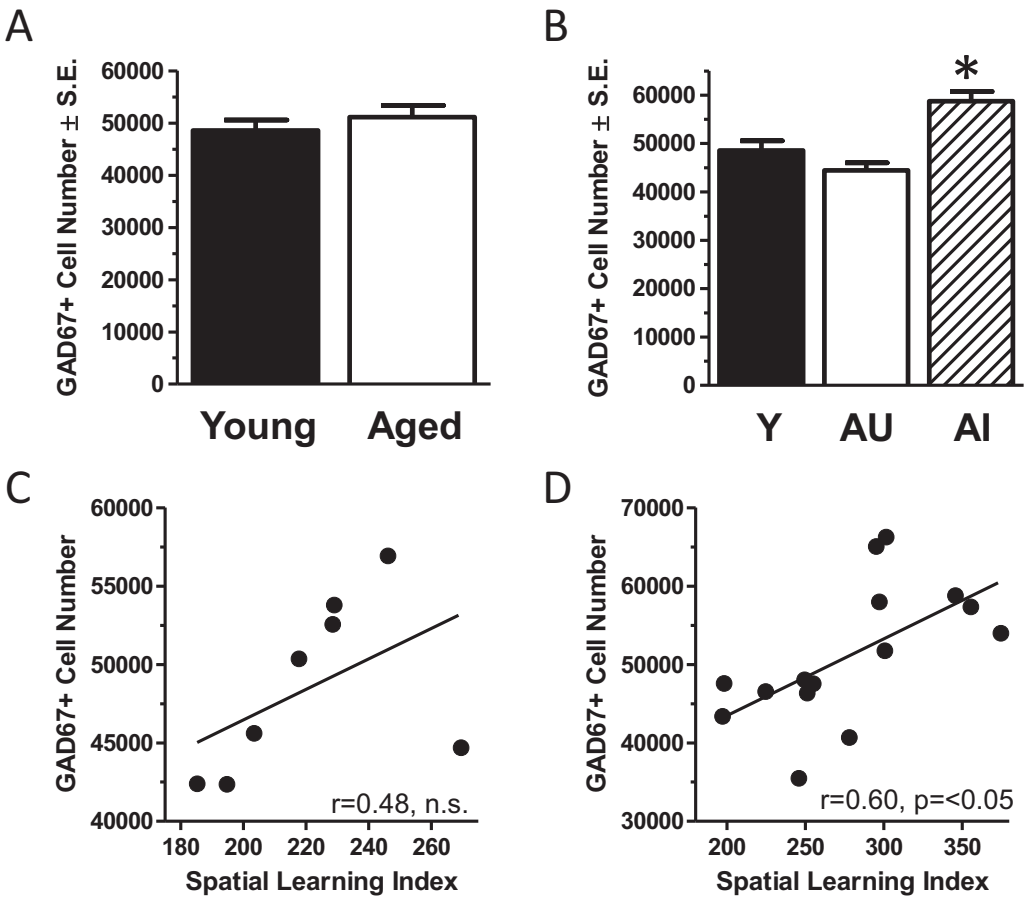

Fig. 8. GABAergic (glutamic decarboxylase [GAD]67 immunopositive) cell number in the rostral basal forebrain of young (Y) and aged rats. (A) GAD67 immunopositive cell number was numerically but not significantly greater in aged rats in comparison with young. (B) A significant difference in GAD67 immunopositive cell number was evident between cognitive age groups, such that aged spatially impaired (AI) rats exhibited a marked, reliable elevation in GAD67 immunopositive cells in comparison with both young and aged spatially unimpaired (AU) rats. (C) Scatter plot shows a trending though nonsignificant relationship between GAD67 immunopositive cell number and spatial learning index in young rats such that higher numbers were associated with worse learning. (D) Scatter plot shows that the same relationship was observed among aged rats, but in this case, greater GAD67 immunopositive cell number was significantly associated with worse spatial learning ability. See text for statistical analyses. * $p < 0.05$ relative to Y and AU rats.

ergic and GABAergic neurons that project to the hippocampus contribute to hippocampal-dependent spatial learning abilities in aged rats. Indeed, age-dependent changes were evident in both cholinergic and GABAergic neuronal populations, but the direction of these changes and their relationships to cognitive abilities were distinct. A modest but significant decline in cholinergic (ChAT immunopositive) neuron number occurred in the rostral basal forebrain with age, although this reduction was not related to spatial learning ability in aged rats. However, there was a strong relationship between cholinergic cell number and spatial learning abilities in young rats. In contrast to findings with cholinergic neurons, a robust increase in the number of GABAergic projection (GAD67 immunopositive) neurons was observed selectively in the rostral basal forebrain of aged rats with impaired spatial abilities. These phenotypically-specific alterations were not reflected in the total number of rostral basal forebrain neurons, as NeuN immunopositive cell number was stable with age and across cognitive groups.

4.1. Cholinergic (ChAT immunopositive) neurons

Cholinergic signaling in the hippocampus and neocortex has been heavily implicated in cognitive functioning, including learning, memory, and attention (Deutsch, 1971; Dunnett and Fibiger, 1993; Everitt and Robbins, 1997; Fragkouli et al., 2005; McKinney and Jacksonville, 2005; Ormerod and Beninger, 2002; Sarter and Bruno, 1997; Sarter et al., 2003; Schliebs and Arendt, 2006, 2011; Woolf, 1997). A number of cholinergic indexes decline with age, and the cholinergic system remains a primary target of drugs currently used to treat age-related cognitive impairment (Giacobini, 2004; Lane et al., 2004, 2006; Nordberg, 2006). Indeed, degeneration of cholinergic neurons in the nucleus basalis of Meynert and concomitant depletion of acetylcholine in cortical targets is a hallmark of Alzheimer's disease (Whitehouse et al., 1982), suggesting that degeneration of cholinergic neurons may be an important contrib-

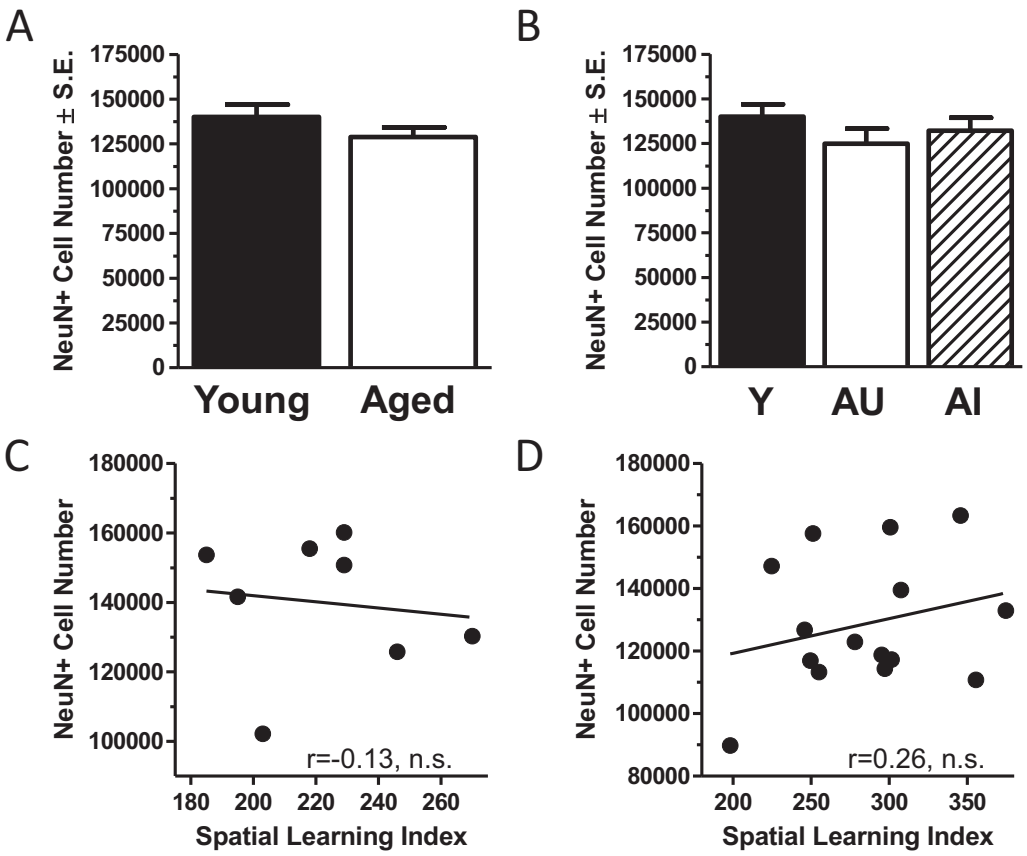

Fig. 9. Total (NeuN immunopositive) cell number in the rostral basal forebrain of young and aged rats. NeuN immunopositive cell number in the rostral basal forebrain did not differ as a function of age (A) or cognitive ability (B). Moreover, the total number of NeuN immunopositive cells did not predict spatial learning ability in either young (C) or aged (D) rats. See text for statistical analyses. AI, aged spatially impaired; AU, aged spatially unimpaired; Y, young.

utor to cognitive deficiencies observed in normal aging. However, across species, findings regarding cholinergic neuronal integrity at advanced ages have been mixed. A number of studies, employing a variety of counting techniques, report a decline in cholinergic neuron number in aging (Altavista et al., 1990; Armstrong et al., 1993; Bartus et al., 1982; Baskerville et al., 2006; Fischer et al., 1989, 1992; Lee et al., 1994; Stroessner-Johnson et al., 1992), although others report no or only a modest decline (Bigl et al., 1987; McQuail et al., 2011; Ypsilanti et al., 2008). While the current findings provide support for a significant loss of rostral basal forebrain cholinergic neurons in normal aging, it should be noted that this reduction was modest, even with the relatively large sample size used. Indeed, some of the reported differences in findings across studies comparing cholinergic neuron numbers between young and aged rats may be attributable to the numbers of subjects employed relative to the numbers necessary to achieve sufficient statistical power for detecting small-magnitude group differences.

In addition to the modest but significant decline in cholinergic neuron number with advancing age, there is considerable evidence that age-related decline in cholinergic neuronal function and signaling contributes to cognitive impairment. For example, age-associated dysregulation in calcium signaling in cholinergic neurons could affect a range of neuronal functions that include neurotransmitter release and synaptic plasticity (Disterhoft et al., 1996; Foster et al., 2001; Kumar et al., 2009). Specifically, Murchison and Griffith (1998) reported increased intracellular calcium buffering in cholinergic basal forebrain neurons in aged rats, and more recently, that age-related calcium dysregulation in basal forebrain cholinergic neurons strongly predicts impaired hippocampal-dependent cognition using the same rat model employed here (Murchison et al., 2009). Other evidence indicates that aging may disrupt cholinergic signaling at the receptor level. Specifically, muscarinic signaling in the hippocampus, a principal target of rostral basal forebrain projection neurons, is significantly altered in aged rats. Whereas muscarinic receptor-mediated phosphoinositide turnover is blunted in hippocampal CA1, CA3, and subiculum across aged rats (Nicolle et al., 2001), G protein coupling to muscarinic receptors in hippocampus is specifically associated with compromised hippocampal-dependent spatial learning (Zhang et al., 2007). Together with the current data, these findings suggest that age-related alterations in cholinergic signaling not only result from the loss of

acetylcholine-synthesizing neurons in basal forebrain but also reflect suboptimal functioning of these neurons and/or postsynaptic signaling associated with the cholinergic receptors in hippocampus and other cortical targets. This interpretation is consistent with the finding that cholinergic cell number strongly correlated with cognitive abilities in young but not aged rats (see Fig. 7). In young rats, the cellular machinery and environment is largely intact and functioning optimally, and, thus, the overall number of cholinergic neurons might provide an accurate index of acetylcholine signaling in target fields. In aged rats, however, disruption in cholinergic signaling at multiple levels might result in a functional decoupling, such that cell number is a less accurate predictor of overall cholinergic function.

4.2. GABAergic projection (GAD67 immunopositive) neurons

A major finding from the current work is the selective and significant increase in GABAergic projection neurons (indicated by greater numbers of GAD67 immunopositive cells) in the rostral basal forebrain of spatially impaired rats. Notably, the 67 isoform of GAD is preferentially expressed by GABAergic neurons in rostral basal forebrain that project to cortical targets, whereas the 65 isoform is expressed by rostral basal forebrain interneurons (Castañeda et al., 2005). Very few prior studies have quantified rostral basal forebrain GABAergic neurons in aging, and to our knowledge, no other studies have quantified this specific GAD67 immunopositive neuronal population. Using a nonisoform-specific GAD antibody, Smith and Booze (1995) reported no age-related change in GAD immunopositive neurons in caudal neocortical projecting basal forebrain nuclei (Smith and Booze, 1995). The current findings are consistent with this previous report in that no significant difference in GABAergic projection neuron number was evident in rostral basal forebrain when age groups were compared in the absence of cognitive subgrouping. Together, these data indicate that GABAergic neurons do not degenerate with advancing age, an important finding for interpreting phenotypic and functional age-related alterations in basal forebrain. For example, the calcium binding protein parvalbumin is highly coexpressed in GABAergic projection neurons and reduced parvalbumin cell number has been reported in aging (Krzywkowski et al., 1995). The present findings would support an interpretation that reduced parvalbumin cell number with age is indicative of cellular dysregulation of Ca^{2+} signaling akin to that described above for cholinergic neurons rather than overt neuronal loss (Murchison and Griffith, 1998). Additional characterization of age-related changes in the signaling properties and function of these GABAergic neurons represents an important direction of future work.

Importantly, the increase in GAD67 immunopositive cell number observed in aged spatially impaired rats did not appear due to an overall increase in total neuron number in rostral basal forebrain, as stereological estimates of NeuN immunopositive cells in adjacent sections did not differ with age or as a function of cognitive ability. Although the specific mechanisms responsible for the elevation in GABAergic cell number observed in spatially impaired aged rats remains an outstanding question, it seems plausible that this change might reflect an age-related upregulation of GAD67 protein expression in a subset of rostral basal forebrain neurons which might otherwise express only nominal levels of GAD67. Such an upregulation would increase the detectability of these neurons, which in turn would be reflected as an increase in the total number of GAD67 immunopositive neurons. Indeed, GAD67 expression can be regulated by a number of factors including activity, stress, estrous cycle, and caloric restriction (Carta et al., 2008; Cashion et al., 2004; Cheng et al., 2004; Liang et al., 1996). Moreover, altered GAD expression has been reported in a number of brain disorders, including epilepsy, Parkinson's disease, and schizophrenia (Briggs and Galanopoulou, 2011; Lanoue et al., 2010; Lewis et al., 2005).

Alternatively, it is important to consider that the neurons in rostral basal forebrain are quite heterogeneous and the full extent of distinct neuronal subtypes in these fields remains undetermined and was not exhaustively evaluated in the current study. In addition to cholinergic and GABAergic projection neurons, other cell types include GABAergic interneurons and a recently identified subpopulation of hippocampal-projecting glutamatergic neurons (Colom et al., 2005; Manseau et al., 2005). As such, it is possible that the failure to detect differences in numbers of NeuN immunopositive cells between age and cognitive groups reflects differential effects of age on multiple phenotypically distinct neuronal populations in this region. Notably, the rostral basal forebrain glutamatergic neurons share size and certain neurochemical characteristics with GABAergic projection neurons (Colom et al., 2005; Manseau et al., 2005) and some basal forebrain neurons have been identified that have the capacity to synthesize both GAD67 and phosphate-activated glutaminase, a mitochondrial enzyme used in the production of glutamate (Gritti et al., 2006). In addition, reverse transcription polymerase chain reaction studies have shown that mRNA for the glutamate vesicular transporter 2 may be coexpressed with ChAT and GAD67 messenger RNA in young and adult rats (Danik et al., 2005). These findings make it intriguing to speculate that the present results represent a functional shift from excitatory to inhibitory signaling in a subpopulation of hippocampal-projecting neurons as has been shown in kindling models in which stimulation induces the production of GAD67 in hippocampal granule cells (Gómez-Lira et al., 2005; Sloviter et al., 1996). It will be important in future studies to determine the effects of age on glutamatergic projections as well as on GABAergic interneuronal populations, in order to better understand how age-related changes in basal forebrain dynamics contribute to a loss of hippocampal-dependent learning and memory.

It is becoming increasingly clear that corticopetal basal forebrain GABAergic neurons influence neural transmission and cognitive functions linked to their terminal fields (Freund and Antal, 1988; Kiss et al., 1990b; Pang et al., 2001). GABAergic neurons are well-positioned to modulate cortical circuitry, both through their direct input to cortical structures and via the highly interconnected cholinergic and GABAergic neuronal networks within the rostral basal forebrain itself (Freund and Antal, 1988; Freund and Buzsáki, 1996). Neuronal tracing studies indicate that GABAergic hippocampal interneurons are a primary target of GABAergic afferents from rostral basal forebrain (Freund and Antal, 1988; Gulyás et al., 1991). As such, an increase in the inhibitory influence from rostral basal forebrain would be expected to result in enhanced excitability of the hippocampal principal neurons. Indeed, many studies have reported hyperexcitability in the hippocampus with advanced age, and such alterations have been linked to hippocampal dysfunction and a decline in hippocampal-supported cognition (Dickerson et al., 2005; Gallagher and Koh, 2011; Wilson et al., 2005; Yassa et al., 2010). The present findings support the hypothesis that age-related alterations in inhibitory signaling within septohippocampal circuitry might contribute to this reported shift in the inhibitory-excitatory dynamics of the aged hippocampal formation.

Overall, the findings from the current work add to the evidence that rostral basal forebrain systems are significantly altered in aging. In particular, these studies support a growing literature which indicates that inhibitory networks are particularly vulnerable to dysfunction in aging and that such dysfunction can profoundly impact cognition. Future work in which the present findings are extended to determine the innervation patterns of both inhibitory and excitatory afferents from rostral basal forebrain and that better characterize the intrinsic dynamics within basal forebrain of aged behaviorally characterized rats will help to further elucidate the role of this system in age-related cognitive decline.

Acknowledgements

We thank Vicky S. Kelley, Julia Chapman, Caleb J. Bostwick, George Edwards III, Brian Ambrose, Bryce Gagliani, Jennifer Graham, and Jigar Amin for their assistance in acquiring confocal image stacks and neuronal counting, and Dr. Barry Setlow for critical reading of the manuscript. Supported by NIH R01 AG029421 (JLB) and the McKnight Brain Research Foundation.

Disclosure statement

The authors report no actual or potential conflicts of interest.

The Institutional Animal Care and Use Committee approved all protocols described in this report.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2012.06.013.

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frontiers in
AGING NEUROSCIENCE

REVIEW ARTICLE
published: 12 September 2012
doi: 10.3389/fnagi.2012.00021

Characterizing cognitive aging in humans with links to animal models

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With the population of older adults expected to grow rapidly over the next two decades, it has become increasingly important to advance research efforts to elucidate the mechanisms associated with cognitive aging, with the ultimate goal of developing effective interventions and prevention therapies. Although there has been a vast research literature on the use of cognitive tests to evaluate the effects of aging and age-related neurodegenerative disease, the need for a set of standardized measures to characterize the cognitive profiles specific to healthy aging has been widely recognized. Here we present a review of selected methods and approaches that have been applied in human research studies to evaluate the effects of aging on cognition, including executive function, memory, processing speed, language, and visuospatial function. The effects of healthy aging on each of these cognitive domains are discussed with examples from cognitive/experimental and clinical/neuropsychological approaches. Further, we consider those measures that have clear conceptual and methodological links to tasks currently in use for non-human animal studies of aging, as well as those that have the potential for translation to animal aging research. Having a complementary set of measures to assess the cognitive profiles of healthy aging across species provides a unique opportunity to enhance research efforts for cross-sectional, longitudinal, and intervention studies of cognitive aging. Taking a cross-species, translational approach will help to advance cognitive aging research, leading to a greater understanding of associated neurobiological mechanisms with the potential for developing effective interventions and prevention therapies for age-related cognitive decline.

Keywords: aging, cognition, memory, executive function, processing speed, visuospatial function, language

INTRODUCTION

Preserving our cognitive abilities is an essential part of maintaining a high quality of life as we age. Although an extensive and varied literature exists in the use of cognitive measures to assess the effects of aging and age-related neurological disease, the development of a standardized and widely available set of tests to characterize the profiles associated with healthy cognitive aging has been recognized as an important direction for advancing future research (Wagster, 2009). It is well established that healthy aging is associated with declines in aspects of memory, executive function, and information processing speed, as well as other selected cognitive abilities (Zec, 1995; Lezak et al., 2012). There are, however, substantial individual differences in the ability of elders to maintain their cognitive functions during aging. This heterogeneity can be viewed as a continuum extending from "successful cognitive aging" with the ability to maintain high levels of functioning throughout the lifespan, to pathological aging, with impairment in memory and other cognitive abilities often leading to dementia (Daffner, 2010). Understanding why some individuals demonstrate successful cognitive aging and others do not may provide an essential foundation for developing effective interventions to enhance the abilities and quality of life for the rapidly growing population of community-dwelling elderly.

The opportunities to relate tests of cognitive abilities to current and emerging measures of neural system integrity and function, to genetic variability and biological risk factors, and to health and lifestyle characteristics may lead to the development of useful biomarkers of cognitive aging. The development of such biomarkers to evaluate the effects of healthy aging on cognition complements recent efforts to identify optimal markers of age-related neurodegenerative disease (e.g., Jack et al., 2008). The availability of biomarkers that are sensitive to the early cognitive effects of aging and that can help track their longitudinal progression will be important for evaluating targeted interventions and prevention therapies designed to delay or diminish declines associated with cognitive aging. Further, using tests that

can be conceptually and methodologically linked to measures used in studies of non-human animal models of aging provides a valuable and unique opportunity to advance understanding of the underlying neural mechanisms that influence cognitive aging. In this context, studies of aging in non-human primates and rodents provide an important complement to human studies with the potential for identifying the underlying neurobiological substrates of cognitive aging (see Moss et al., 2007; LaSarge and Nicolle, 2009). This translational approach to cognitive aging research may help to identify new molecular pathways and targets for the development of effective interventions and prevention therapies.

To support and advance such translational research in cognitive aging, we propose the need for the development and implementation of a set of measures to characterize cognitive profiles that are: (1) able to evaluate group differences in both cross-sectional and longitudinal studies of healthy aging; (2) sensitive to individual differences in performance within the continuum of healthy cognitive aging; (3) able to characterize the broad range of abilities across multiple cognitive domains that may be distinct from the impact of both incipient and clinically evident neurological disease; and (4) able to support cross-species translation between human and non-human animal models of aging to advance research into the underlying neural mechanisms of cognitive aging.

In this article, we present a selected review of methods and approaches used to evaluate the higher-order cognitive processes altered by healthy cognitive aging. We focus on five major cognitive domains, including executive function, memory, processing speed, language, and visuospatial function. Well-established age effects are discussed, while also considering aspects of those domains that can remain relatively preserved to provide the potential for developing a characteristic multi-domain profile of cognitive aging. For each domain, perspectives from both cognitive/experimental and clinical/neuropsychological approaches are presented. Further, we consider those measures that have linkages to tasks currently in use in studies of animal models of aging, as well as those that have the promise of being readily translated for research with non-human primates and rodent studies of aging. Our goal is not to provide a comprehensive description of tests for animal models, but rather to suggest human tests for studies of cognitive aging with unique potential for linkages to animal paradigms. Many of these tasks are also described in more detail in the companion papers in this issue. Together with important contributions by the NIH Toolbox for Assessment of Neurological and Behavioral Function (www.nihtoolbox.org) and other related initiatives, such measures may provide an array of complementary methodological tools that will help to greatly advance cognitive aging research, with the ultimate goal of identifying effective interventions and prevention therapies for age-related cognitive decline.

EXECUTIVE FUNCTION

Numerous approaches and models have attempted to characterize executive function in humans. Many suggest that there may be some overarching unitary function that captures what we mean by executive function, such as attentional control (e.g., Kane et al., 2007) or goal maintenance (Braver and West, 2008). Subsumed under this general rubric, however, is a diversity of processes that are engaged differentially depending on task demands. Based on converging evidence from the cognitive/experimental, clinical/neuropsychological, and neuroimaging literatures, we identified three sub-processes of executive function—task-switching, updating, and inhibition—that have been associated with different brain regions in prefrontal cortex and appear to be negatively affected by normal and pathological aging. In this section, we discuss the supporting evidence for the existence of these specific executive processes and propose a set of tests to measure them. We also include working memory, focusing on its executive components.

EVIDENCE FROM COGNITIVE/EXPERIMENTAL STUDIES

The most influential model of executive function emerging from the cognitive/experimental approach is a model proposed by Miyake et al. (2000) based on confirmatory factor analysis (CFA) and structural equation modeling (SEM). Miyake et al.'s model, based on 137 young adults, identified three latent factors that they called task or set shifting, updating and monitoring of working memory, and inhibition of prepotent responses, with three tasks loading on each factor. The model suggests that these three latent factors tap separable components of executive function, but they are also correlated with each other suggesting they may share common variance as well. A subsequent SEM looked at the extent to which these latent variables predicted performance on more complex executive function tasks. This analysis indicated that shifting uniquely predicted perseverative errors on the Wisconsin Card Sorting Test, updating predicted performance on Operation Span, and inhibition predicted performance on the Tower of Hanoi task. Both updating and inhibition predicted performance on Random Number Generation (RNG).

Fisk and Sharp (2004), in a subsequent principal components analysis of a subset of the complex executive function tasks (WCST, span tasks, and RNG) in a group of 95 adults aged 20–81, obtained a similar factor structure across all ages, plus a fourth factor, which they called efficiency of lexical access (measured by verbal fluency). Vaughan and Giovanello (2010) in a study of 95 older adults, aged 60–90 years old, confirmed the same three-factor structure as obtained by Miyake et al. (see also Latzman and Markon, 2010) and further showed that the executive function latent variables, particularly task switching, predicted performance on instrumental activities of daily living (IADLs; see also Bell-McGinty et al., 2002). Two other studies with older adults found two-factor structures: Hedden and Yoon (2006) found a combined shifting/updating factor and a separate inhibition of proactive interference factor, whereas Hull et al. (2008) found separate shifting and updating factors but no inhibition factor.

Inhibition in particular appears not to be a single construct but may tap different underlying inhibitory functions dependent on the tasks. Friedman and Miyake (2004) in a follow-up study of the inhibition factor found two separate inhibitory functions—inhibition of prepotent responses (and distractors), and resistance to proactive interference. Resistance to proactive interference has also been hypothesized to be a key problem in working memory tasks (e.g., May et al., 1999), and indeed Friedman and

Miyake (2004) found that resistance to proactive interference predicted reading span. It is therefore likely that successful updating requires the ability to overcome proactive interference.

EVIDENCE FROM FOCAL FRONTAL LESION PATIENTS

Task-switching, updating and inhibition have also been identified in the clinical literature as frontal-based processes that are impaired differentially in patients with lesions to different regions of prefrontal cortex. Furthermore, although tasks requiring switching of task sets such as those used in the Miyake model are much simpler than tasks such as WCST, studies of individuals with focal frontal lesions suggest that even these simple tasks may require several executive processes depending on the precise demands of the task and the measures that one derives from them (e.g., Shallice et al., 2008; Stuss, 2011). Thus, correlations among latent variables in the statistical models may occur not because of some common overriding executive process but because even simple tasks are not process pure. Task-switching, for example, may require updating, monitoring, and inhibition as well as task-setting and switching.

Nevertheless, it is possible to separate some of these processes in focal lesion patients. In a review of the assessment methods for frontal lobe dysfunction, Stuss and Levine (2002) reported that dorsolateral prefrontal (DLPFC) lesions were preferentially associated with the set-shifting aspect of the WCST, whereas loss of set was evident in patients with ventrolateral prefrontal cortex (VLPFC) lesions, perhaps attributable to greater susceptibility to interference. In two more recent papers, Stuss (2011) and Stuss and Alexander (2007) identified two domain-general executive processes that are impaired in patients with dorsolateral frontal lesions—task-setting, which was defined as setting of stimulus-response contingencies in any task (left DLPFC), and monitoring of ongoing performance (right DLPFC). Although these processes do not map exactly onto the latent variable constructs from the factor analytic models, they nevertheless seem broadly consistent with or related to the processes of task-shifting, monitoring, and control of interference that were identified in those models. Stuss et al. also identified a process referred to as energization (which they did not describe as an executive process), which involved the initiation and sustaining of a response and was associated with superior medial prefrontal cortex. They found that patients with lesions in this region showed impairments in the incongruent condition of the Stroop task (and other reaction time (RT) tasks), which they attributed to a failure to sustain activation of the intended response.

EVIDENCE FROM NEUROIMAGING

Neuroimaging studies have also found evidence consistent with multiple executive functions. For example, DLPFC has been associated with complex span tasks that require updating and monitoring of working memory (e.g., D'Esposito et al., 1999), whereas VLPFC has been associated with control of proactive interference and inhibition of prepotent responses (Jonides et al., 1998; D'Esposito et al., 2000). Task switching has been associated primarily with DLPFC, but VLPFC and parietal cortex have also been implicated (Braver et al., 2003), consistent with the idea that executive control functions involve a frontoparietal network. Sylvester et al. (2003) noted common areas of activation in several regions of prefrontal and parietal cortices for task switching and inhibition, as well as unique areas for each construct. Kim et al. (2011), in a recent study of cognitive flexibility, identified a domain-general switching mechanism that was localized to the inferior frontal junction (BA 44/6/9) and posterior parietal cortex (BA 7/40), and several domain-specific switching regions in medial and lateral prefrontal cortex (PFC) that varied as a function of the type of switching (cognitive set, stimulus or response). Finally, in a meta-analysis of neuroimaging studies of the WCST, task-switching and a go/no-go task, Buchsbaum et al. (2005) similarly reported a common frontal parietal network with specific sub-areas of PFC associated with task-switching (bilateral VLPFC, BA 47) and response inhibition (right DLPFC, BA 44/45/46). These findings, although not entirely consistent on the specific localization of different functions, nevertheless are fairly consistent in revealing distinctions between the constructs of shifting, updating and monitoring, and inhibition.

EVIDENCE FROM AGING

Similar constructs have been examined in the aging literature but not all of them have been found to be age-sensitive. For example, age effects in task-switching have tended to be found in global switching but not in local switching (e.g., Verhaeghen and Cerella, 2002). Global switching costs reflect the increased time taken to complete a block of trials in which two tasks have to be performed in alternating or random order, compared to blocks of trials in which each of the tasks are performed separately. Local switching costs are measured as within-block time differences between switch trials and non-switch trials. Although both switches result in increased reaction times, the global cost seems to be most affected by age. This finding may be partly attributable to the extra costs of maintaining the two task sets in working memory.

Working memory tasks have generally shown substantial age effects particularly on the more complex span tasks, such as reading and operation span, which require updating and monitoring. These tasks also predict higher cognitive functions such as reasoning and problem solving (Kyllonen and Christal, 1990; Engle et al., 1999), episodic memory (Park et al., 1996), and fluid intelligence (see Kane et al., 2007) in both older and younger people. Age effects in measures of inhibition have not been found reliably in older adults, particularly on one of the most commonly used tests of inhibitory function, the Stroop (1935), although some studies have shown age-related deficits (e.g., Davidson et al., 2003). However, a recent study (Clark et al., 2012) reported that scores on a color-word interference test from the Delis-Kaplan Executive Function System (D-KEFS) were predictive of cognitive decline on the Dementia Rating Scale (Mattis, 1988) a year later, suggesting this test might be sensitive particularly to pathological aging (see also, Hutchison et al., 2010; Bayard et al., 2011). Older adults do show age-related deficits on tasks that require control of proactive interference (see, Hasher et al., 2007), and on stop-signal and go/no-go tasks as well as anti-saccade tasks (e.g., Nielson et al., 2002; Hasher et al., 2007), which have been associated with inhibition.

EXECUTIVE FUNCTION TESTS

Although there is still considerable inconsistency in the literature, there is some consensus that there are at least three separable functions or processes, similar to those identified in Miyake et al. (2000)—task or set shifting, updating, and monitoring, and inhibition—which seem to depend partly on different regions of prefrontal cortex. In addition, inhibition may be further divided into two sub-components: inhibition of prepotent responses or distractors, and resistance to proactive interference. Complex tasks may require more than one of these processes. We therefore recommend that the executive function tasks used in cognitive aging be selected so as to tap into one of these identified functions, and that they be age-sensitive. We also suggest that one or more of the complex tasks that have been commonly reported in the literature be included for comparisons to previous studies and to potentially allow for a de-construction of the component processes underlying these tasks.

For each of the component processes, three tests that are related to the underlying process of interest should be selected. Ideally these tests should differ in other aspects so that the composite is most likely to reflect the targeted executive component. The following is a list of tests that have been associated with each specific function and appear to be sensitive to normal and/or pathological aging. Further details on the methodologies can be found in the papers referenced.

Shifting

- *Plus-minus task*: In this task, people see three separate lists of 30 two-digit numbers. For List1, they add 3 to each number; for List 2, they subtract 3 from each number; for List 3, they alternate addition and subtraction by 3. Shift cost is measured by the difference between time to complete List 3 and the average time for Lists 1 and 2 (from Miyake et al., 2000). This version of the task measures global shift costs. It may also be important that no external cues are provided (i.e., no + or − signs appear). Internally generated shifts appear to be more sensitive to aging than externally cued shifts.
- *Number-letter task*: In this task (Rogers and Monsell, 1995; Miyake et al., 2000; Gamboz et al., 2009), a number-letter pair (e.g., 8*F*) is presented in one of four quadrants. If the stimulus appears in the top two quadrants, participants make an odd/even judgment about the number; if it appears in the bottom two quadrants, they judge whether the letter is a consonant or vowel. In the first block of trials, stimuli are all on the top; in the second block they are all on the bottom, and in the third block stimuli shift in a clockwise fashion such that half of stimuli appear on the top, and half are on the bottom and a shift is required on half the trials. Both global and local shift costs can be calculated.
- *Global-local task*: In this task, people view a figure in which the lines of a global figure (e.g., a square) are constructed of smaller local figures (e.g., triangles). As in the previous tasks, participants are required to shift between the global and local figures, reporting how many lines make up the figure. Depending on how the task is constructed, one can measure global or local shift costs as described previously (Miyake et al., 2000).

Shifting tasks in humans may tap processes similar to those used in delayed alternation tasks in rodents, which show age deficits. In addition, the ability to shift attention from one perceptual dimension to another perceptual dimension of the same stimulus (i.e., an extra-dimensional shift) has been assessed with a variety of tasks in both rodents and non-human primates (see Bizon et al., 2012; also **Table 1**). In rodents, these tasks are dependent upon the rodent homologue of primate DLPFC and are analogous in design to the WCST.

Updating/resistance to proactive interference

- *Letter memory/consonant updating*: In this task (Morris and Jones, 1990; Miyake et al., 2000; Vaughan and Giovanello, 2010), single letters are presented visually on a computer screen one at a time for 2 s each, and participants are required to repeat the last four letters out load, continually updating the working memory set, and then recall the last four consonants at the end of the list. List lengths vary randomly (5, 7, 9, and 11).
- *Keep track task*: Participants are first familiarized with category labels and instances of six categories (see Miyake et al., 2000). On each subsequent trial block, a subset of the category labels is shown at the bottom of the computer screen and remains visible throughout the trial block. Two to three instances from each of the six categories appear one at a time on the screen for 1500 ms, and participants monitor for the last word of each of the target categories. Miyake et al. (2000) used 15-item lists with 3 blocks of 4 and 5 target categories. In the modified version used by Hull et al. (2008) with older adults, four blocks of 10 trials were presented with only two target categories. We recommend using 3 blocks of 2, 3 and 4 categories with 15-item lists.
- *Operation span*: Operation span, although more complex than the other measures, is a classic working memory task developed by Turner and Engle (1989) in which people are asked to verify a solution for simple arithmetic problems [e.g., $(2 \times 4) - 3 = 3$] and retain a following word in memory (e.g., mouse). Set size varies from 2 to 5 problem-word pairs with three trials of each set size. After each set of problem-word pairs is presented, people recall the words in any order. Miyake et al. reported that his latent updating variable predicted performance on operation span (see also, Fisk and Sharp, 2004). Complex

Table 1 | Executive processes and associated tests for use in humans and animal models.

Executive process	Human tests	Animal tests
Shifting	Plus-minus Letter-number Global-local	Delayed alternation Extra-dimensional shift
Updating/resistance to proactive interference	Consonant updating Keep track Operation span	Delayed matching to sample Delayed match-to-place
Inhibition of prepotent responses	Stroop Simon Go/NoGo	5-choice serial reaction time Stop signal

span tasks, including operation span, are sensitive not only to normal aging, but also in increasing fashion to mild cognitive impairment (MCI) and AD (Gagnon and Belleville, 2011).

Updating tasks in humans, which require resistance to proactive interference, appear similar to tasks described in Bizon et al. (2012; see also **Table 1**) that involve delayed matching to sample. For example, using a delayed-match-to-place version of the Morris water maze task, in which rats must learn a new platform location each day, older rats showed poorer delayed retention of the trial-unique stimuli, arguably because of greater interference from previous trials. Their performance on this task was unrelated to their performance on a spatial reference memory task that required them to remember the same platform location each day.

Inhibition of prepotent responses

- *Stroop task* (Stroop, 1935): There are several versions of the Stroop Color-Word Task. We recommend using a version that does not require task shifting, namely one in which the participants are always required to name the color of the ink. The key comparison is between the incongruent color-word and a neutral condition, which most often involves naming the color of asterisks or blocks of color. In some cases, a neutral word is used as an intermediate case. Both reaction time and errors should be recorded; the latter appear to be more sensitive to pathological aging (Hutchison et al., 2010). The measure commonly reported is the difference in RT between the incongruent and neutral conditions (Miyake et al., 2000), although a ratio of incongruent to neutral RTs has also been recommended to account for general slowing (e.g., Bayard et al., 2011).
- *Simon task* (Simon, 1990): In the Simon task, people are instructed to press the left button when a left-pointing arrow appears and the right button when a right-pointing arrow appears. The critical conditions occur when a right-pointing arrow occurs on the left of the screen or a left-pointing arrow occurs on the right of the screen, creating a mismatch between the location of the stimulus and the location of the response. Evidence suggests that the stimulus/response spatial match is the more primitive response, and has to be inhibited in order for a directional response to be made. The dependent measure is the RT difference between the congruent and the incongruent mapping. Castel et al. (2007) have found that this task is sensitive to normal aging and also distinguishes normal aging from mild AD.
- *Go/NoGo task*: Several versions of Go/NoGo tasks have appeared in the literature. We propose a task used by Nielson et al. (2002), which minimizes the working memory/updating component and emphasizes the inhibition component. The task involves continuous presentation every 500 ms of various letters of the alphabet with targets X and Y. Participants are required to respond with a button press to the occurrence of X and Y when they alternate in the serial sequence but not when they repeat consecutively (i.e., respond to X when it follows Y but not when it follows X). The alternating rule is added after two practice runs of "go" trials to establish the prepotent response. Letters are presented continuously at a 500 ms rate with a ratio of go to no go responses of between 3:1 and 6:1. The dependent measure is the percentage of successful inhibitory responses.

Several tasks (see **Table 1**) have been used in rodents to assess the ability to inhibit prepotent responses, including the five-choice serial reaction time task and the stop signal reaction time task (see Robbins, 2002; Eagle et al., 2008; Bizon et al., 2012). These tasks were developed to be analogous to tests of response inhibition in humans, and are sensitive to both damage to prefrontal cortex and advanced age (Winstanley et al., 2006; Harati et al., 2011).

Complex tasks

Even though it likely involves multiple executive processes, the WCST remains the gold standard of executive function tests. Although a measure of perseverative errors allows for a broad range of scores, not all studies have found perseverative errors to be age-sensitive. Additional measures—number of categories achieved, total errors, number of trials needed to achieve a category—should also be recorded and may reflect different component processes. The WCST has most often been associated with switching but as noted above, it may well involve other executive function processes as well.

MEMORY

EVALUATING MEMORY IN OLDER ADULTS

Memory problems are one of the most common complaints among older adults. An extensive literature exists demonstrating that older adults are indeed impaired relative to young adults on some, but not all, types of memory. Most relevant to the present discussion of aging is the distinction between semantic and episodic memory (Tulving, 1972, 2002). There is ample evidence that episodic memory (memory for specific personal events that includes event-specific spatial and temporal context) declines over the adult lifespan while semantic memory (our general store of knowledge including words, concepts and facts about the world and ourselves) remains relatively stable across the adult lifespan (e.g., Nilsson et al., 2002). Those semantic tasks that decline are generally ones that require the rapid retrieval of information, such as category fluency (Nyberg et al., 2003), suggesting that older adults have problems related to efficient retrieval, rather than a deficit in semantic representations.

Several theoretical accounts of age-related memory changes have been proposed. Theoretical views have emphasized age-related declines in speed of mental processing (e.g., Park et al., 1996; Salthouse, 1996), decreases in general resources necessary for effortful processing (e.g., Craik, 1986; Craik and Rose, 2012), the inability to bind new associations among elements of an event (e.g., Chalfonte and Johnson, 1996; Naveh-Benjamin, 2000), decreases in inhibitory processing or executive functions (e.g., Hasher et al., 1999), limitations in working memory capacity (e.g., Cowan, 2010) and sensory-perceptual inefficiency (e.g., Baltes and Lindenberger, 1997). These views are not mutually exclusive, and they likely share a common outcome—inefficiency of processing, which leads to greater resources being expended during both encoding and retrieval, memory representations that

are therefore less robust, and retrieval processes that are more prone to failure (Park and Reuter-Lorenz, 2009).

Memory deficits associated with aging are thought to be mediated primarily by two brain regions, medial temporal lobes and prefrontal cortex, which play different but interactive roles in memory, depending upon the specifics of the memory task. Although age-associated volume changes in the medial temporal lobes have been demonstrated (Small et al., 2002; Raz et al., 2005), aging disproportionately affects the prefrontal cortex compared with other brain regions (Raz and Rodrigue, 2006). Other age-related changes include the loss of integrity of white matter as measured by diffusion weighted imaging in both prefrontal and temporal regions (Ryan et al., 2011), reductions in dopamine production and dopamine receptors that are especially prominent in prefrontal cortex as well as medial temporal lobe regions (Giorgio et al., 2010), and amyloid deposition (Sperling et al., 2009).

Importantly, age-related memory impairments are not ubiquitous. There are considerable individual differences across older adults, and the source of these individual differences is of considerable interest to researchers. Likely they are determined by some combination of genetics, participant characteristics (e.g., verbal ability, education level, and domain expertise), developmental and environmental factors, health status (e.g., physical fitness, weight, and hypertension) and social and emotional variables (e.g., positive emotion and interpersonal goals).

The number and types of tests that have been used to assess memory in older adults are truly staggering, and include both traditional clinical neuropsychological tests and an extensive set of experimental measures. Here, we describe a small subset of these tasks that are well suited to assessing age-related memory functions, with sufficient sensitivity to capture not only age-related memory decline but individual differences in memory function among older adults. We will focus on four memory domains that have received considerable empirical attention: encoding and retrieval processes, associative, source, and prospective memory. Importantly, these processes and kinds of memory have all been shown to be modifiable in older adults by provision of environmental support or appropriate strategies, making them good targets for intervention.

MEMORY DOMAINS

Encoding and retrieval

An influential view of aging and memory is the notion that the processing resources necessary for successful learning and remembering are available to a lesser degree in older adults compared to young adults (Craik, 1986, 2002). Craik (e.g., Craik and Byrd, 1982) originally conceptualized this impairment as a lack of available "mental energy," which results in a failure to carry out self-initiated mental operations at both encoding and retrieval leading to poor memory. Thus, tasks that inherently require more self-initiated processing will be more likely to show age-related memory impairment. For example, lists of unrelated words are more difficult for some older adults to learn compared to stories or pictures. Similarly, tests with minimal retrieval cues such as free recall are more likely to show greater age-related decline than cued-recall and recognition (Craik and McDowd, 1987; Nyberg et al., 2003). This difference in performance has been attributed to older adults' dependence on familiarity-based rather than recollective retrieval processes (i.e., the dual-process model of recollection). Recall tasks, which are heavily dependent upon recollection, show greater age-related changes than familiarity-based tasks, such as recognition (Jennings and Jacoby, 1997; Bastin and Van der Linden, 2003), although there are considerable individual differences. For example, Davidson and Glisky (2002) showed that older adults with poor frontal function relied to a greater degree on familiarity and were therefore more prone to recognition errors.

Associative memory

One component of episodic memory decline in older adults is the degree to which they retain the ability to bind together the components of an episode (Mitchell et al., 2000; Naveh-Benjamin, 2000) or to associate items with their contextual features (Chalfonte and Johnson, 1996). Most commonly these tasks present previously unrelated pairs of items followed by independent tests of memory for the items and for the associations between the items. The consistent outcome of these experiments is a greater age deficit for associations relative to items. This pattern has been observed with word (Castel and Craik, 2003), word-spatial location (Mitchell et al., 2000), word-font (Naveh-Benjamin et al., 2003), name face (Naveh-Benjamin et al., 2004; Miller et al., 2008) and face-face pairs (Bastin and Van der Linden, 2003). Age-related impairment in paired associate learning has been related to reductions in hippocampal activity as well as dorsolateral prefrontal activity (Dennis and Cabeza, 2008). It appears that some portion of the associative deficit is likely mediated by an inability to produce appropriate strategies for learning and can be overcome to a great degree by providing older adults with specific strategies for remembering (Glisky et al., 2001; Naveh-Benjamin et al., 2007).

Source memory

Source memory refers to the ability to remember the conditions surrounding the encoding of a particular episodic memory that may include information that specifies the source of the experience (e.g., Did you read it in the newspaper or in a magazine) as well as various aspects of the encoding context, including perceptual, spatio-temporal, affective, and other features (Johnson et al., 1993; Glisky and Kong, 2008). Source memory is particularly impaired in older adults relative to young adults (Chalfonte and Johnson, 1996; Mitchell et al., 2000; Glisky et al., 2001) and to a greater degree than item memory (Spencer and Raz, 1995). These deficits have often been associated with poor performance on frontally-mediated, executive function tests (see **Figure 1**; Glisky et al., 1995; Glisky and Kong, 2008) and less commonly with medial temporal lobe dysfunction (Schwerdt and Dopkins, 2001; Gold et al., 2006). Older adults also show decreases in fMRI activation relative to young adults in left ventrolateral prefrontal regions during short term source memory tasks, suggesting they may have specific problems comparing and evaluating information (Mitchell et al., 2006; for discussion see Glisky and Kong, 2008). One hypothesis is that reductions in frontal dopaminergic pathways may reduce the brain's ability to modulate incoming stimuli with respect to their specific contexts

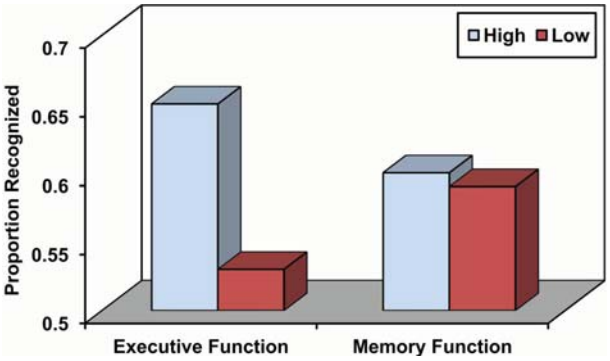

FIGURE 1 | Source memory in older adults as a function of high and low executive function and memory function. Adapted from Glisky et al. (1995).

of occurrence, thereby resulting in less distinctive cortical representations of events that are difficult to differentiate from one another (Bäckman et al., 2000; Li et al., 2001).

Prospective memory

Remembering to perform an action at some future point is a memory skill that we use daily. In laboratory tasks of prospective memory (or "remembering to remember"), participants are engaged in some ongoing activity and need to monitor the environment for the presence of a cue. Upon recognizing the cue, they must recall its associated intention without any prompting and then interrupt their ongoing activity in order to successfully complete the intended action. In this way, prospective memory places a heavy burden on self-initiated processing. Two different types of prospective memory tasks have been studied. Event-based tasks refer to an intention that is associated with a particular cue (e.g., when you see the bank, deposit the check). Time-based tasks have no specific external cue, but rely instead on monitoring time (e.g., in 10 min from now, turn off the stove). A growing literature demonstrates that prospective memory is impaired in older adults, particularly those with poor executive function as measured by neuropsychological tests, for both event based tasks (McDaniel et al., 1999; McFarland and Glisky, 2011) and time based tasks (Martin et al., 2003; McFarland and Glisky, 2009).

When prospective memory fails, it can have tremendously negative consequences for older adults, particularly for those individuals who, for example, take multiple medications daily (Park et al., 1992; Insel et al., 2006). Prospective memory is an area that warrants additional research not only because of its theoretical implications, but because of the immediate and important application to quality of daily life.

MEMORY MEASURES

The majority of empirical studies on age-related memory impairment have utilized paradigms that are specific to a single laboratory, and often, to a single empirical study. The lack of standardized tasks makes direct comparisons across studies difficult, and combining data across sites impossible. The field would benefit greatly from the development of standardized versions of some of these paradigms. Some of the existing paradigms, particularly in the visual memory domain, have excellent analogs to standard tests utilized in animal models which are highlighted below.

Verbal encoding and retrieval

This is one area where well-developed neuropsychological measures of episodic memory are widely available. In the verbal domain, we favor the inclusion of memory tests that include both a list learning task and a story recall task in order to assess the differences between verbal materials that are unrelated and verbal materials that are embedded in a richly elaborated conceptual context (i.e., a story). Together, they assess memory for unrelated and related materials, the influence of external cues through comparisons of recall and recognition performance, and the differences between immediate recall and retention of information over a delay of 20–30 min.

- *Word lists*: List learning tasks involve the presentation of a list of words over multiple learning trials. Free recall is tested after each list presentation, and an overall learning score can be computed. Delayed free recall of the word list is typically obtained 20–35 min later, followed by yes–no recognition. The two most commonly used list learning tasks are the ***California Verbal Learning Test-2*** *(CVLT-2)* and the ***Rey Auditory Verbal Learning Test*** *(RAVLT)*. The CVLT-2 includes 16 words that belong to four different semantic categories, allowing measures of semantic organization during free and cued recall. The RAVLT, a list of 15 unrelated words, may provide a purer measure of encoding ability since good performance requires the participant to engage in elaborative and self-initiated processing.
- *Story memory* (Wechsler, 1987, 1997): Memory for semantically meaningful verbal material is generally assessed using short stories that are presented orally. Because of the structured nature of stories, there is minimal demand for self-initiated organization to link elements together. The most common clinical test with extensive age-related normative data is the Logical Memory subtest from the Wechsler Memory Scale (WMS). Generally, two different stories are read to the participant and an immediate free recall test is given for each. Delayed free recall for each story takes place approximately 30 min later followed by a multiple choice recognition test. Although the WMS is now in its fourth version and the stories and details of administration have changed, for studies with older adults, the WMS-R version (Wechsler, 1987) is ideal because of the simplicity of the design and administration, and norms available for all age groups. Alternative stories that are similar in characteristics and norms to the original stories are also available, greatly enhancing the ability to engage in longitudinal memory assessments (Schnabel, 2012).

Visual-spatial encoding and retrieval

While visual information is often tested with recognition (e.g., memory for faces), other visual memory tasks require drawing designs or geometric figures from memory after a brief exposure. The latter is somewhat problematic because it can be difficult to separate visual memory problems from constructional difficulties. Visual tasks that rely on recognition alone, some of which are described here, are direct analogs to those used in animal studies

and are good candidates for cross-species studies of visual memory (see **Table 2**). The most common of these tests, including the delayed non-match to sample (Mishkin and Delacour, 1975) and spontaneous object recognition (Murray and Bussey, 1999) are reviewed in Burke et al. (2012).

- *Memory for faces* (WMS-III; Wechsler, 1997): A series of target faces are shown one at a time and people are asked to remember them. Both immediate and delayed memory is tested by showing targets intermixed with novel faces in a yes–no recognition task.
- *Visual reproductions* (WMS-III; Wechsler, 1997): Participants are shown novel designs and are asked to draw them immediately from memory after viewing them for 10 s. Delayed recall memory is tested after a 20–30 min delay.
- *Rey-Osterrieth complex figure* (Meyers and Meyers, 1995): This difficult visual spatial task requires that participants first copy a complicated geometric line drawing, and then draw the design from memory immediately after viewing it or after a brief 3 min delay. A delayed recall drawing is also obtained after 20–30 min. For the memory portions of the test, many different cognitive abilities are required for optimal performance including visual spatial ability, memory, attention, planning, and working memory (Duleya et al., 1993). Participants are not told beforehand that they will be asked to draw the figure from memory; the immediate recall condition is therefore incidental learning.
- *Doors and people*: Doors and People, normed for ages 18–80 (Baddeley et al., 1994), assesses visual recall and recognition using two types of stimuli that are difficult to encode verbally. The doors subtest assesses visual recognition by showing the participant a variety of different colored doors which they must remember and later recognize from a selection of similar doors. The shapes subtest assesses visual recall by asking the participant to copy four different patterns and then draw them from memory.

Table 2 | Human memory tests and promising analog tests currently utilized in animal models of memory.

Executive process	Human tests	Animal tests
Visual recognition	Memory for faces, doors and people recognition	Spontaneous recognition Delayed nonmatch-to-sample
Spatial memory	Field and virtual reality spatial navigation tasks	Morris water maze Radial 8-arm maze
Associative memory	Visual paired associates, doors and people name-face pairs	Object-in-location recognition Contextual fear conditioning
Source memory	Visual task e.g., chairs in rooms	Delayed nonmatch-to-position
Prospective memory	Event-based and time-based	Not yet developed

Although standardized tests of spatial memory and spatial navigation for humans are not available, human paradigms derived from non-human visual spatial memory methods have been employed in the cognitive aging literature and are promising candidates for further cross-species research (reviewed in Foster et al., 2012). For example, Newman and Kaszniak (2000) developed a human analog of the Morris water maze (Morris et al., 1982) using a tent-like enclosure and identified age differences in performance of a spatial memory task. Similarly, Bohbot et al. (2002) reported a series of real-world spatial memory tests designed to be similar to tasks used in rats. One test was an analog of the Morris water maze; human subjects located a sensor (hidden under carpeting) while moving about a room. Another task resembled the 8-arm radial maze commonly used in rodent experiments (Bizon et al., 2012). Several computerized virtual-environment versions of the water maze have also been developed (Jacobs et al., 1997; Iaria et al., 2003) that are sensitive to age-related changes in performance (Moffat and Resnick, 2002; Jansen et al., 2010) and show strong correlations between real-space and virtual space versions of the same task (Nedelska et al., 2012).

Associative memory tasks

While associative memory tasks abound in the experimental cognitive aging literature, few standardized and normed associative memory tasks are available. The most common tasks used to assess associative memory in animals are contextual fear conditioning tasks (reviewed in Foster et al., 2012). Versions of objects-in-location recognition tasks (Bizon et al., 2012; Burke et al., 2012) may be most similar to human visual paired associate tasks, and are likely candidates for developing human-animal analogs.

- *Verbal paired associates learning* (WMS-III; Wechsler, 1997): Eight pairs of unrelated words (e.g., "trunk-arrow") are presented to participants followed by cued recall. The list is repeated four times, with a final cued-recall test after a 30 min delay. Yes/no recognition is also assessed after the delay period for intact pairs interspersed with completely novel pairs. No recombined pairs are included as recognition lures.
- *Visual paired associates learning* (CANTAB[1]): This subtest assesses cued recall for pattern-location pairs. Boxes are displayed on a computer screen and are opened in a randomized order. One or more of them will contain a pattern. The patterns are then displayed in the middle of the screen, one at a time, and the participant must touch the box where the pattern was originally located. If the participant makes an error, the locations are repeated. Difficulty increases through the test by increasing the number of pattern-location pairs from one to eight.
- *Doors and People*: The battery, described earlier, includes a face-name paired associate learning test. This is a cued-recall

[1]The Cambridge Neuropsychological Test Automated Battery (CANTAB; Cambridge Cognition, http://www.cambridgecognition.com) was developed to provide human versions of tests that were widely used to test animal models of memory, making them particularly well-suited to cross-species studies of memory and aging (Robbins et al., 1994).

task, where participants must remember and then subsequently recall the names of four different people, both immediately and after a delay.

Source memory tasks

Given the wealth of experimental information in this domain, this could be a particularly fruitful area for new standardized test development. Source tasks could also prove useful as a bridge between human memory and context-dependent memory studies in animals, particularly delayed nonmatch-to-position tasks (reviewed in Burke et al., 2012).

Generally, source memory experiments utilize a one-to-many mapping, that is, multiple words, objects, or sentences are experienced in one of two different contexts. The "many" refer to the to-be-remembered items while source refers to the association between the item and its context. Most often, item memory and source memory are tested with separate sets of materials, counterbalanced for order of testing. Importantly, both item and source are tested using a two-alternative choice recognition paradigm, in order to keep the memory task demands as similar as possible.

- *Verbal source test*: Glisky et al. (1995) presented participants with multiple sentences spoken aloud by two different readers, and asked them to judge how likely they were to hear the sentence on the radio. Source memory was tested in a two-alternative forced choice recognition test. Each of the sentences was spoken by both of the voices, and participants judged which voice had spoken the sentence during the study phase.
- *Visual source test*: In a later study, Glisky et al. (2001) created a visual analog to the sentence/voice source test. They presented participants with pictures of various office chairs that were photographed in two distinctive settings—a laboratory office cubicle and the department lounge. The same general procedure was followed. Two separate study lists were presented, one followed by a two-alternative choice recognition test for the chairs (distractors were novel chairs in the same setting), and the other list followed by a recognition test for the source (the same chair presented in both rooms).

Prospective memory tasks

As is the case with source memory, prospective memory tests have not been standardized. Here we describe two types of tests, both used in the laboratory setting, that could conceivably be developed for cross-species studies with humans and animals.

- *Event based prospective memory task*: A typical example of an event based task is found in McFarland and Glisky (2011). Young and older adults engaged in a multiple choice trivia game, presented on the computer screen, which tested their general world knowledge. At the same time, they were given a secondary task that required them to press a key whenever they saw the word "state" appear on the computer screen. Half the participants were given the typical read-only instructions (when you see the word "state," press the key). Other participants were given implementation intention instructions (when I see the word "state," I will press the key). The measures in this study were the number of times a participant accurately pressed the key when the target word appeared on the screen.
- *Time based prospective memory task*: McFarland and Glisky (2009) used a similar multiple choice trivia game as the primary task, presented on the computer screen with touch screen technology to record responses. For the secondary task, a clock icon was visible in the upper right hand corner of the screen, with two boxes just below it that were numbered "1" and "2." Participants were instructed that every 5 min, they were to touch the 1 and 2 boxes in alternating order. To monitor time, they could touch the clock icon and the time elapsed since their last button press would appear. This study measured not only the number of prospective memory tasks completed, but also the proximity of the box presses to the elapsed time, as well as the pattern of clock monitoring. For example, one of the findings in this study was that young adults and older adults with high executive functioning (measured by neuropsychological tests) monitored the clock more often in the 1 min interval prior to their next scheduled button press, while low executive functioning older adults did not significantly increase their clock-monitoring in the final minute.

PROCESSING SPEED

The measurement of processing speed during the performance of mental tasks has been an important part of the longstanding effort to identify the origins of the observed differences in cognitive abilities over the lifespan (Deary et al., 2010). Tasks designed to measure mental speed typically involve the assessment of an individual's efficiency in completing a series of test items that require relatively low cognitive demands (e.g., perceptual matching). Tests of information processing speed have been widely used as measures sensitive to the cognitive effects of multiple neurological and psychiatric disorders, such as Alzheimer's disease, multiple sclerosis, traumatic brain injury, and depression (e.g., Alexander et al., 1994; Kail, 1998; Comijs et al., 2001; Bashore and Ridderinkhof, 2002; Denney et al., 2004). Information processing speed has been consistently shown to be an important correlate of age in cross-sectional studies and has also been identified as a major source of variance in the relation of other cognitive measures with age (Salthouse, 1993, 2000; Kail and Salthouse, 1994).

There is growing interest in characterizing the neural substrates of information processing speed and the neurobiological pathways that lead to age-related cognitive slowing with emerging support for the importance of the integrity of white matter tracts and the connectivity of regionally distributed brain networks (Rabbitt et al., 2007; Deary et al., 2009; Kennedy and Raz, 2009; Bartzokis et al., 2010; Penke et al., 2010; Burgmans et al., 2011; Eckert, 2011; Takeuchi et al., 2011; Lee et al., 2012).

Research is ongoing and new measures of information processing speed continue to emerge in the literature (e.g., Wiig et al., 2007) from both clinical/neuropsychological and cognitive/experimental approaches. The differences in their methods of acquisition, levels of stimulus complexity, and assessment of response timing suggest a potential complementary value for including tests represented by both classes of information

processing measurement within a comprehensive battery for cognitive aging.

CLINICAL/NEUROPSYCHOLOGICAL MEASURES

The neuropsychological approach often requires decisions following the recognition, matching, or discrimination of perceptual stimuli, with accuracy providing a measure of performance within a specified time limit. The Coding and Symbol Search subtests of the Wechsler Adult Intelligence Scales – IV are two common examples of this class of processing speed measures that can be combined to form a composite score for a Processing Speed Index (Wechsler, 2008).

- *Coding (a.k.a. Digit-Symbol)*: Participants select and draw a visual symbol below a corresponding number derived from a digit-symbol code key presented at the top of the page. The score is based on the number of correct digit-symbol matches within the time limit.
- *Symbol search*: Participants perform multiple trials in which they place a line through two target symbols presented within a series of five possible choices. If no match is found, a line is drawn through a "No box." The score reflects the number of correct responses across all test trials within a specified time.
- *Trail making test, Part A* (Trails A; Reitan, 1958): This test measures the time required to draw lines connecting a series of numbers within circles arranged non-contiguously on a page.
- *Letter and pattern comparison tests* (Salthouse and Meinz, 1995): These tests were developed specifically for studies of cognitive aging. Participants are required to make same versus different judgments on a series of letter strings or line patterns that contain three to nine line segments presented on pages with a specified time limit. The total number of correct responses minus the number of incorrect responses during the test time is calculated.
- *Finger-tapping* (Reitan and Wolfson, 1993): Participants are required to use their index finger to repeatedly press a key counter as quickly as they can in 10 s with their dominant and non-dominant hands. The total number of key presses with each hand during the time limit provides the finger tapping scores. An electronic version of the finger tapping test has been recently applied in a study of cognitive aging to provide a measure of simple motor processing speed that has been associated with neural function and myelin integrity (Bartzokis et al., 2010).

COGNITIVE/EXPERIMENTAL MEASURES

The cognitive or experimental approach to measuring information processing speed has typically relied on computerized administration of tasks that assess component processes reflecting simple (SRT) and choice (CRT) reaction times. Although these tasks are varied in the types of stimuli and their complexity, they tend to present relatively simpler visual stimuli compared to the psychometric tests and measure average or median response times for individual task trials, rather than measures of performance integrated over a specified test time limit.

One example of a SRT task used in studies of cognitive aging requires subjects to press a button when a zero is presented on a screen using a variable 1–3 s inter-trial interval presented on a stand-alone, rectangular stimulus presentation/response box (Deary et al., 2001, 2010; Der and Deary, 2006; Penke et al., 2010). Response times are averaged for correct responses over the 20 test trials to obtain a measure of test performance. A corresponding four-choice reaction time task has been used in conjunction with this SRT task in which subjects are instructed to press one of four keys when the numbers 1, 2, 3, or 4 appear on the screen. The same 1–3 s variable inter-trial interval is used in this CRT task and test performance is measured by averaging response times for correct responses across 40 test trials. A freely available, computerized version of the SRT and CRT reaction time test has been recently developed, the Deary-Liewald reaction time task (Deary et al., 2011), that has shown high correlations with the established response-box SRT and CRT tasks.

Another example of an SRT test has been developed and applied in a recent study of aging in which subjects are instructed to touch a computer-screen, using a stylus, when they see a yellow square appear. The single visual stimulus is repeatedly presented using 1, 2, or 4-s inter-trial intervals in random order with 18 total test trials. A CRT version of this SRT task simultaneously presents subjects with two squares and requires them to touch the upper square if the two presented squares are the same color. They are required to touch the bottom square if the two presented squares are a different color. The dual visual stimuli are presented using the same 1, 2, or 4-s random inter-trial intervals with a total of 20 trials presented. Performance in both tasks is measured as the median hit response times over test trials (Lee et al., 2012).

An alternative experimental method to test processing efficiency has been proposed using a psychophysical approach (Deary et al., 2004). In this method, measures of visual inspection time are obtained as participants make discriminations between two parallel lines of different lengths presented on a computer screen by pressing one of two keys if the longer line is on the left or right side. Importantly, in this task, the subjects respond at their own pace with no response time recorded. The line pairs are each shown 10 times using 15 different durations extending from 6 to 200 ms. Since mean responses per duration length can range from chance level performance at the shortest presentations to nearly 100% accuracy at the longest presentations, the total number of correct responses provides an index of visual processing efficiency without measuring motor response speed (Deary et al., 2004). In a recent comparative study of several types of processing speed measures, the visual inspection time task was found to be least dependent on early life measures of overall intellectual ability, supporting its potential value as a complementary cognitive marker of processing speed during aging (Deary et al., 2010). This measure, however, may be more sensitive to administration differences, influences of ambient lighting, and degrees of intact or corrected peripheral visual processing.

Of the processing speed measures commonly used in human studies, the SRT and CRT tasks lend themselves particularly well to cross-species studies of aging and processing efficiency. For example, animal studies have examined reaction time using tasks analogous to SRT and CRT. Very little change in reaction time is observed between young adult (4–6 months) and aged (2 year old) rats on simple stimulus-response operant

tasks (Menich and Baron, 1984; Burwell and Gallagher, 1993). However, an overall slowing of reaction time latencies is observed for animals older than 2 years. Moreover, there is considerable individual variability suggesting differences in biological aging.

For choice reaction time, a five-choice serial reaction time (5-CSRT) task has been employed (Harati et al., 2008). In this task the presentation of a light in one of five response openings signals where the animal needs to make a response (nosepoke) to receive a food reward. In addition, the stimulus duration can be varied (2–0.2 s). The latency to make a correct response to the light stimulus is a measure of decision-making speed, and latency between a correct response and food collection is used as a measure of motor function. An examination of age-related differences under standard conditions (0.5 s visual stimulus duration) revealed that decision-making speed was reduced in aged animals (25 months), compared to young adult and middle-aged rats. Decision-making speed continued to be reduced when a longer stimulus (2 s) was employed. Impaired processing efficiency, as measured by total number of correct responses, was reduced in aged animals when the stimulus was shortened (0.2 ms). The results suggest that a decline in simple and choice reaction time does not appear until late in life, and it is likely to be variable, depending on preceding life history, including cognitive and sensory-motor stimulation through environmental enrichment. For further details, see Bizon et al. (2012).

LANGUAGE

A widely accepted dictum is that vocabulary and other language-related skills remain relatively preserved during the course of normal aging. In this section, components of the language system (i.e., semantics, phonologic-orthographic) will be briefly described along with current data suggesting that while some aspects of language are *relatively* impervious to the effects of aging, others are not. Even those language components that remain relatively stable with age can become susceptible to other co-occurring cognitive changes outside the language system (i.e., working memory). We will briefly discuss views about "core vs peripheral" neural substrates of language, and findings over the past decade that neural networks supporting language differ in younger and older adults. Finally, an outline will be provided of commonly used language measures in clinical assessments along with a rationale for screening older adults who participate in studies of healthy cognitive aging.

COMPONENTS OF LANGUAGE

The language system can be fractionated in multiple ways (Coltheart, 1987; Caplan, 1993; Caramazza and Mahon, 2006) but the fundamental components of language include a lexicon or vocabulary (words denoting objects, actions, and their modifiers), syntax (rules specifying relationships among words) and a phonologic-orthographic system (sounds and written symbols that constitute the actual spoken or written word). The term *lexico-semantic* typically refers to semantic meaning or knowledge that is conveyed by words, phrases, and syntax. *Naming* involves knowing what the object is (accessing semantic representation) and then accessing its phonologic representation in order to articulate that word. There are numerous cognitive information models that describe this process quite elegantly, and a variety of views on the breakdown of naming (Patterson and Shewell, 1987). A higher level aspect of language is pragmatics, which refers to how situational and contextual information can alter meaning of linguistic communication (i.e., inferred intent of speaker).

Another important distinction, drawing from the aphasia literature, is *comprehension* versus *speech production* (Goodglass and Kaplan, 1983). Speech output, or fluency, refers to ease and effort of articulation as well as quantity of output, and is associated with brain lesions anterior to the central sulcus (i.e., frontal) and can often involve Broca's area. Auditory comprehension deficits in aphasias are typically associated with posterior perisylvian lesions.

AGE-RELATED CONSTRAINTS ON LANGUAGE

At least three types of age-related phenomena influence language processing (Wingfield, 1996; Wingfield and Grossman, 2006). First, cognitive slowing results in older adults having more difficulty understanding rapidly presented speech (Wingfield et al., 1999). Second, reduced working memory capacity constrains the comprehension of syntactically complex sentences and results in less frequent use of complex syntax by older adults (Caplan et al., 2011). Kemper et al. (2001) analyzed a longitudinal corpus of speech samples obtained from older adults and found a marked decline in the grammatical complexity of the older adult's speech, which was directly related to working memory capacity. Third, the increased incidence of age-related hearing loss (particularly high frequency sounds) also affects comprehension of spoken language (Morrell et al., 1996). This can cause older adults to miss critical words during conversation, leading in some situations to reduced comprehension.

LANGUAGE SKILLS AFFECTED BY HEALTHY COGNITIVE AGING

With aging, semantic aspects of language are generally well-maintained including vocabulary, language comprehension, and conversational discourse. Cross-sectional studies suggest that vocabulary increases with age until the 60s and 70s and remains stable thereafter (Verhaeghen, 2003; Salthouse, 2009). However, a somewhat different pattern emerges from at least two longitudinal studies that show age-related declines in vocabulary and other verbal skills starting around age 60 and continuing to decline more precipitously after age 74 (Berlin Aging Study, Baltes and Lindenberger, 1997; Seattle Longitudinal Study, Schaie, 2005). Even so, these language declines are less dramatic than those in other cognitive domains, such as episodic memory, and do not appear to undermine the functional adequacy of healthy older adults.

One of the most commonly experienced language-related problems across all ages is the inability to produce a well-known word on demand. These word retrieval difficulties become more frequent with age, and are evident on tasks of confrontation naming of object pictures (Kaplan et al., 1983; Burke et al., 1991; Au et al., 1995; Ivnik et al., 1996; Welch et al., 1996; Connor et al., 2004; Zec et al., 2005; Goral et al., 2007; Kave et al., 2010; see Feyerisen, 1997 for a meta-analysis). Similar age-related word retrieval problems are present on word generation tasks (verbal fluency) where individuals are required to rapidly produce words

beginning with a target letter or from a semantic category (Troyer et al., 1997). One question is the extent to which these verbal fluency problems represent changes to language function, executive functions, or processing speed (McDowd et al., 2011). Another question relates to differential role of frontal and temporal lobe mechanisms in letter vs. category fluency tasks and how neural substrates for this distinction differ in young vs. old adults (Meinzer et al., 2009).

One prominent interpretation of age-related word finding difficulties is a decline in efficiency of lexical access, rather than degradation of verbal knowledge. Older adults produce more ambiguous references, have more filled pauses (um, er, etc.), and reformulate words more often than younger adults (Kemper et al., 2001; Burke and Shafto, 2004). These behaviors represent tactics for bypassing word retrieval difficulties. Access difficulties might stem from weakening of connections between a word's semantic and phonologic representations (Burke et al., 1991). It might also relate to age-associated changes in brain functions that affect frontally mediated search mechanisms (Weirenga et al., 2008; Meinzer et al., 2009), supported by functional MRI studies, diffusion tensor imaging, and connectivity analyses (Obler et al., 2010; Stamatakis et al., 2011).

CORE VS. SUPPORT LANGUAGE NETWORKS

The classic aphasia literature identifies the left perisylvian cortical region as critical for language (Goodglass and Kaplan, 1983; Stemmer and Whitaker, 2008), including Wernicke's region within the temporal lobe, Broca's area within the frontal lobe, and connections between these two regions. More recent neuroimaging approaches have found that additional brain regions outside the perisylvian region also become activated during language processing—particularly anterior frontal regions as well as the homologous right hemisphere regions. Wingfield and Grossman (2006) draw a distinction between a core perisylvian language network that is necessary for language tasks, and additional regions beyond the core language network that become activated when healthy adults are engaged in language tasks. In line with this view, neuroimaging evidence indicates that older adults who successfully perform tasks such as naming and text processing recruit substantially more brain regions than poor performers or younger adults (Weirenga et al., 2008; Obler et al., 2010), including right hemisphere perisylvian and midfrontal regions, in conjunction with traditional left perisylvian regions (Obler et al., 2010). Those who are relatively poor namers do not recruit these support regions. This recruitment likely reflects compensation, which has been posited to account for findings of decreased laterality on various language-mediated cognitive tasks (Cabeza, 2002; Reuter-Lorenz and Park, 2010). One important implication of this line of research is that similar levels of performance accuracy on language tasks by younger and older adults may be mediated by different (though overlapping) neural circuitries.

TRANSLATIONAL CHALLENGES

Although there is little disagreement that animals have unique communication systems, considerable controversy exists regarding whether propositional language is exhibited by non-human primates or other animal species. While this controversy is beyond the scope of the current paper, suffice to say that few animal models of language currently exist. One line of animal research has focused on the basic neuroanatomic substrates of language, and whether non-human primates exhibit language-related brain asymmetries similar to those observed in humans. Geschwind and Levitsky (1968) were the first to document that the planum temporale, a region well known for its role in language comprehension, was larger in the left hemisphere than the right in humans. This asymmetry has been systematically replicated in postmortem and structural imaging studies (Steinmetz et al., 1989; Good et al., 2001; Eckert et al., 2006). Chimpanzees show a similar leftward asymmetry in the size of planum temporale, whereas lower monkey species (rhesus, vervet, and bonnet macaques) do not (Lyn et al., 2011).

ASSESSMENT OF LANGUAGE IN OLDER ADULTS: RATIONALE AND RECOMMENDATION

Relative to other cognitive domains (executive function, processing speed, and episodic memory), language is less sensitive to the influence of underlying age-associated neural changes (Raz et al., 2010). Even so, commonly occurring neurodegenerative conditions, such as Alzheimer's disease, typically disrupt aspects of language, particularly word retrieval and naming. These changes occur secondary to alteration of temporo-parietal networks involved in semantics. In fact, impairments on relatively simple tasks of confrontation naming and word generation, especially semantic fluency, are commonly observed in individuals with early Alzheimer's disease. Subtle deficits can even be seen in individuals with the amnestic variant of MCI, who are at greater risk for transitioning to dementia. Thus, language screening is particularly important if one's goal is to study memory or executive function in healthy older adults in the absence of co-occurring neurodegenerative disease.

Table 3 depicts an overview of commonly used measures in the clinical assessment of language in older adults, ranging from tasks of vocabulary knowledge, word retrieval (confrontation naming) and verbal fluency to those examining discourse and pragmatics. This table does not include batteries for assessing individuals with acquired aphasia that is sometimes associated with focal strokes but also observed in certain neurodegenerative conditions (i.e., semantic dementia, progressive non-fluent aphasia). Certain measures, particularly confrontation naming and speeded verbal fluency tasks, can be helpful for identifying subsets of older adults who may already be experiencing neurodegenerative changes. For this reason, many centers and large-scale studies routinely include a confrontation naming task, such as the Boston Naming Test (Kaplan et al., 1983), as one of several screening tools for the purpose of excluding (or including) individuals with early signs of dementia. The Boston Naming Test is relatively brief, consisting of 60 line drawings of items that an individual "names." Short forms are available (30 and 15 item versions) and can be used rather easily for screening purposes.

VISUOSPATIAL FUNCTIONS

Visuospatial performance generally declines with age in both humans and other species (Studzinski et al., 2006). A good

Table 3 | Commonly used language tasks in clinical assessment of non-aphasic older adults.

Domain	Tasks	Older adult norms
Semantics		
Knowledge	Expressive vocabulary (WAIS, WASI)	Yes
	Receptive vocabulary (PPVT)	Yes
Word retrieval	Visual confrontation naming (Boston naming test)	Heaton and MOANS
	Auditory confrontation naming	
	Action naming	
Directed fluency	Letter fluency (COWA)	Heaton and MOANS
	Category fluency (animals, fruits-vegetables, etc.)	Heaton and MOANS
	Fluency tasks from DKEFS	Yes
Syntax comprehension	Token test (multilingual aphasia exam)	MOANS
Discourse	Oral description of complex pictures (i.e., cookie theft picture from BDAE, kite picture from the WAB)	
	Open ended script questions	

Task Abbreviations: WAIS, Wechsler Adult Intelligence Scale; WASI, Wechsler Abbreviated Scale of Intelligence; PPVT, Peabody Picture Vocabulary Test; COWA, Controlled Oral Word Association Test; DKEFS, Delis-Kaplan Executive Function System; BDAE, Boston Diagnostic Aphasia Exam; WAB, Western Aphasia Battery.

Norms Abbreviation: Heaton refers to norms published in Heaton et al. (2004); MOANS refers to Mayo Older Adult Norms (MOANS), in Ivnik et al. (1996). See Lezak et al. (2012) for detailed description of individual test measures.

working definition of visuospatial dysfunction is attributed to Boller et al. (1984):

> "Difficulty in appreciating the position of stimulus-objects in space, difficulty in integrating those objects into a coherent spatial framework, and difficulty in performing mental operations involving spatial concepts."

There are many approaches intended to test human visuospatial performance. Most have grown out of clinical neuropsychology research with humans, rather than from the perspectives of experimental approaches with either humans or in animal models (but see a discussion of recent human and animal research on object discrimination in Burke et al., 2012). The field is further complicated by ongoing reconsideration of the basic organization of the neural and cognitive psychology of systems involved in responding to visual stimuli (Kravitz et al., 2011).

A major challenge to systematic study of this construct lies in the wide variety of cognitive functions subsumed under "visuospatial function." Visuospatial tests might preferentially weight spatial perception and localization, spatial memory, and organization of cognitive and motor responses to spatial information. Aspects of visual perception affect performance on many visuospatial tasks and vary by instrument and presentation medium.

Aspects of the testing procedures related to other cognitive functions, rather than either visual or spatial processing losses, are significant contributors to age-related decline in visuospatial performance (Libon et al., 1994). For instance, when Kemps and Newsom (2006) used the "Doors and People test" to assess age-related changes in visual and verbal memory, they found that working memory and executive function made the greatest contributions to age related declines, while processing speed and perceptual issues were lesser contributors. Many of the tests considered to assess visuospatial abilities more closely map on to memory processes. In general these tasks demonstrate low reliability and limited construct validity (Moye, 1997). Even tests that clearly map to similar visuoperceptual processes such as the Gollin Incomplete Figures test and the Mooney test of Incomplete Faces Perception, show important variability in test samples, such as gender based differences in performance (Foreman, 1991). Overall, older adults appear to be vulnerable to the effects of reduced stimulus redundancy, decreased signal-to-noise, and limited processing speed. Offsetting those vulnerabilities by varying the testing procedure (e.g., altering stimulus exposure time or signal-to-noise ratio) attenuates, but does not eliminate age differences on relatively "pure" tests of visuospatial integration such as incomplete figure identification (Kennedy et al., 2009). Nonetheless, age can influence performance on several of these tests.

- The *Rey-Osterreith complex figure* test typically consists of three test conditions: Copy, Immediate Recall and Delayed Recall. Whereas the memory aspects of the test have been discussed previously, the copy portion tests graphomotor spatial functions; the subject is asked to draw a complex geometric line drawing. Age norms have been published, based on a sample of 211 adults ranging from 30 to 85, for the standard administration as well as supplemental matching trials (Fastenau et al., 1999). Matching may be more easily translated to studies of non-human species.
- The *Gollin incomplete figures* test requires subjects to identify a series of fragmented line drawings over successive stimulus presentations that increase the "completeness" of the drawings with age-decade cohorts (50–99, 60–69, and 70–79) showing declining performance (Read, 1988).
- The *Poppelreuter-Ghent* figures assess visual perceptual functions by asking subjects to identify overlapping line-drawing images. Performance of healthy controls is influenced by age and education, but not sex and normative values adjusted for age and education are available (Della Sala et al., 1995).
- The *Visual object and space perception battery* is a proprietary battery consisting of eight untimed tests, each designed to assess a particular aspect of object or space perception, while minimizing the involvement of other cognitive skills. Four subtests measure visual object perception (Incomplete Letters, Silhouettes, Object Decision, and Progressive Silhouettes). The other four measure visual space perception (Dot Counting, Position Discrimination, Number Location, and Cube Analysis). Herrera-Guzmán et al. (2004) reported significant age-cohort effects in 5 of the 8 subtests in a Spanish language sample. For the object perception tasks, age

effects were significant for 3 of 4 subtests. For the space perception tasks, significant age effects were identified for 2 subtests. This confirms an earlier pattern seen in an American sample (Bonello et al., 1997). However, longitudinal data among elderly subjects has not been reported.

A major problem in aligning visuospatial assessment in humans and non-human species is that human tests typically require verbal output or higher-order cognitive tasks like manual drawing, whereas non-human species respond with whole body positions (e.g., moving to a target location) or simple manual responses (e.g., lever press). Also, the non-human testing is heavily biased toward learning and memory of visuospatial material, rather than action based on contemporaneously available visual information (see Burke et al., 2012).

SUMMARY

In this review, we presented a selected set of methods and approaches that have been applied in evaluating the effects of healthy cognitive aging on higher-order cognitive processes. We focused on five major cognitive domains, including executive function, memory, processing speed, language, and visuospatial function, each showing support for well-established age effects, while also presenting aspects of those domains that can remain relatively preserved with age. Examples from cognitive/experimental and clinical/neuropsychological approaches were presented, with a consideration of the measures that have clear linkages to tasks currently in use in animal studies of aging, as well as those that have the potential, with further development, to be translated for non-human animal aging research.

Using multiple measures both within and across cognitive domains can provide both task-specific and composite scores to identify the characteristic profiles associated with healthy cognitive aging. Such profiles may advance cross-sectional, longitudinal, and intervention studies with the potential for applications across species in the study of cognitive aging. As part of a targeted, rational approach to identifying effective interventions and prevention therapies, cognitive measures that can translate across species offers a unique opportunity to efficiently identify the most promising interventions for the effects of cognitive aging in rodents or in other non-human animal models prior to initiating much larger and more expensive human clinical trials. Further research is needed to expand the availability of a wide variety of complementary methodological tools to help advance cognitive aging research. Such research efforts may lead to a greater understanding of the underlying neural mechanisms with the potential for developing effective interventions and prevention therapies to slow, delay, or diminish the effects of age-related cognitive decline.

ACKNOWLEDGMENTS

We thank Cortney Jessup, M.A. and Kristina Irwin for technical assistance in the preparation of this manuscript. The authors would like to acknowledge support from the National Institute on Aging (AG025526, AG19610, AG029421, AG14979, AG037984, and AG036800), the state of Arizona and Arizona Department of Health Services, Arizona Advanced Research Institute for Biomedical Imaging, and the McKnight Brain Research Foundation.

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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.

Received: 19 March 2012; accepted: 15 July 2012; published online: 12 September 2012.

*Citation: Alexander GE, Ryan L, Bowers D, Foster TC, Bizon JL, Geldmacher DS and Glisky EL (2012) Characterizing cognitive aging in humans with links to animal models. Front. Ag. Neurosci. **4**:21. doi: 10.3389/fnagi.2012.00021*

Progress in Neurobiology 96 (2012) 283–303

Contents lists available at SciVerse ScienceDirect

Progress in Neurobiology

journal homepage: www.elsevier.com/locate/pneurobio

Dissecting the age-related decline on spatial learning and memory tasks in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca^{2+} channels in senescent synaptic plasticity

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ARTICLE INFO

Article history:
Received 29 August 2011
Received in revised form 9 January 2012
Accepted 19 January 2012
Available online 28 January 2012

Keywords:
Episodic memory
Incremental learning
Long-term potentiation
Aging

ABSTRACT

In humans, heterogeneity in the decline of hippocampal-dependent episodic memory is observed during aging. Rodents have been employed as models of age-related cognitive decline and the spatial water maze has been used to show variability in the emergence and extent of impaired hippocampal-dependent memory. Impairment in the consolidation of intermediate-term memory for rapidly acquired and flexible spatial information emerges early, in middle-age. As aging proceeds, deficits may broaden to include impaired incremental learning of a spatial reference memory. The extent and time course of impairment has been be linked to senescence of calcium (Ca^{2+}) regulation and Ca^{2+}-dependent synaptic plasticity mechanisms in region CA1. Specifically, aging is associated with altered function of N-methyl-D-aspartate receptors (NMDARs), voltage-dependent Ca^{2+} channels (VDCCs), and ryanodine receptors (RyRs) linked to intracellular Ca^{2+} stores (ICS). In young animals, NMDAR activation induces long-term potentiation of synaptic transmission (NMDAR-LTP), which is thought to mediate the rapid consolidation of intermediate-term memory. Oxidative stress, starting in middle-age, reduces NMDAR function. In addition, VDCCs and ICS can actively inhibit NMDAR-dependent LTP and oxidative stress enhances the role of VDCC and RyR-ICS in regulating synaptic plasticity. Blockade of L-type VDCCs promotes NMDAR-LTP and memory in older animals. Interestingly, pharmacological or genetic manipulations to reduce hippocampal NMDAR function readily impair memory consolidation or rapid learning, generally leaving incremental learning intact. Finally, evidence is mounting to indicate a role for VDCC-dependent synaptic plasticity in associative learning and the consolidation of remote memories. Thus, VDCC-dependent synaptic plasticity and extrahippocampal systems may contribute to incremental learning deficits observed with advanced aging.

Contents

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CaN, calcineurin; Ca^{2+}, calcium; CaMKII, calmodulin kinase II; IP3R, inositol trisphosphate receptor; ICS, intracellular calcium stores; F344, Fisher 344; F344BN, Fisher 344 Brown Norway crossed; HFS, high frequency stimulation; LFS, low frequency stimulation; LTP, long-term potentiation; LTD, long-term depression; mGluR, metabotropic glutamate receptor; NMDAR, N-methyl-D-aspartate receptors; PP-LFS, paired-pulse low frequency stimulation; K^+, potassium; PKA, protein kinase A; PKC, protein kinase C; PP1, protein phosphatae 1; ROS, reactive oxygen species; RyR, ryanodine receptor; sAHP, slow afterhyperpolarization; TBS, theta burst stimulation; VDCC, voltage-dependent calcium channel.

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doi:10.1016/j.pneurobio.2012.01.007

1. Introduction

Animal models of age-related cognitive decline are employed to mimic features of human senescence in order to assist in identifying the molecular mechanisms and development of potential therapeutics. For each model, the appropriateness will depend on a number of factors including translational relevance, background genetics, availability of aged animals, and type of experimental manipulations involved. In order to enhance the translational relevance, it is important that the model has face validity, exhibiting similar symptoms relative to the human condition. Aging in humans is associated with a weakening of working memory, executive function, and processing speed; however, the most notable decline is observed as impaired episodic memory, including spatial memory (Kukolja et al., 2009; Plancher et al., 2010; Uttl and Graf, 1993). The impairment can be identified as a mild deficit in the rapid acquisition of flexible information and an increase in the rate of forgetting (Davis et al., 2003; Hogge et al., 2008; Huppert and Kopelman, 1989; Kral, 1962; Macdonald et al., 2006; Mitchell et al., 1990; Park et al., 1988; Rajah et al., 2010).

A second important aspect of age-related cognitive decline is that chronological age alone does not predict cognitive health. There is enormous variability in cognitive function across the life span from "successful" aging with little memory decline, through memory deficits associated with "unsuccessful" aging, to cognitive changes linked to dementia and neurodegeneration (Foster, 2006; Glisky, 2007). Notably, the deterioration of memory function in humans is progressive (Christensen et al., 1999; Colsher and Wallace, 1991; Mungas et al., 2010; Schonknecht et al., 2005; Zelinski and Burnight, 1997) and an increase in forgetting may be an early sign of impaired synaptic transmission or an evolving neurodegeneration (Dierckx et al., 2010; Gagnon and Belleville, 2010). Indeed, episodic memory deficits are symptomatic of impaired hippocampal function and the progressive decline in episodic memory is associated with a decrease in hippocampal volume (Kramer et al., 2007; Mueller et al., 2007; Mungas et al., 2005; Reuter-Lorenz and Park, 2010; Sexton et al., 2010; Stoub et al., 2008). The question remains as to what mechanisms underlie different trajectories for memory decline and the heterogeneity in memory function in older individuals.

Rodents offer several benefits as models for investigation of the mechanisms and potential treatment of age-related cognitive decline. Similar to humans, information that requires hippocampal processing is particularly vulnerable to age. Furthermore, the memory deficit can be observed as a delay-dependent increase in the rate of forgetting (Dunnett et al., 1988; Eichenbaum et al., 2010; Forster and Lal, 1992; Lal et al., 1973; Mabry et al., 1996; Martinez et al., 1988; Winocur, 1988; Zornetzer et al., 1982). Importantly, rodents are not subject to neurodegenerative diseases, such that cognitive decline is thought to result from aging of physiological processes rather than cell death (Baxter and Gallagher, 1996; Foster, 2006; Rapp and Amaral, 1992). Finally, like humans, rodents exhibit wide-ranging heterogeneity in the extent of memory decline. This variability can be used to better define the process of cognitive senescence and investigate age-related changes in biological mechanisms critical to episodic memory.

In order to take full advantage of the translational relevance of animal models, consideration of the behavioral tasks employed is critical in characterizing the cognitive processes under consideration and limiting confounding influences or alternative hypotheses. In the absence of dementia, aged humans and rodents exhibit a preservation of non-hippocampal processes including incremental stimulus–response learning and procedural memory (Churchill et al., 2003), as well as intact remote memories (Winocur et al., 2008). Most studies in animals describe the probability of cognitive impairment with advancing age. However, it is unclear whether cognitive decline is progressive in rodents and there is a lack of longitudinal studies tracking the course of altered cognitive function. Nevertheless, the existing literature provides a rich source from which to draw information for understanding the trajectory and extent of deficits in episodic memory. This literature provides evidence to indicate that the onset of memory decline emerges in middle-age. Furthermore, cognitive decline appears to be progressive, such that impairment in delay-dependent memory for rapidly acquired and flexible spatial information may advance to more severe deficits observed as impaired short-term memory and an inability to acquire a spatial reference memory through incremental learning.

The extent of age-related cognitive decline is highly variable. Therefore, behavioral tasks should be sensitive enough to detect mild deficits, identify the emergence of memory impairments in middle-age, and distinguish the severity of impairment in order to examine biological mechanisms. The sensitivity of the tasks for identifying age-related memory impairments will depend on the parameters of the task. Several hippocampal-dependent tasks can be designed in a manner that will permit the identification of memory deficits in middle-aged animals. Furthermore, many of these tasks suggest sex differences in the extent or the onset of cognitive decline. A short list of tasks would include olfactory memory (Roman et al., 1996; Taylor et al., 1999), contextual memory during fear conditioning (Kaczorowski and Disterhoft, 2009; Moyer and Brown, 2006), spatial working memory examined on the radial arm maze (Dellu-Hagedorn et al., 2004; Granholm et al., 2008; Sabolek et al., 2004) however see (Dellu et al., 1997; Jacobson et al., 2008; Oler and Markus, 1998), performance on various spatial mazes (Fouquet et al., 2009; Ingram, 1988; Kametani et al., 1989), and retention of inhibitory/passive avoidance (Benice et al., 2006; Moretti et al., 2011; Paris et al., 2010; Samorajski et al., 1985). Tests that examine recognition of novel objects or object locations have reported mixed results (Benice et al., 2006; Blalock et al., 2003; Fahlstrom et al., 2009; Paris et al., 2010), possibly due to procedures that render the task more or less hippocampal-dependent (Broadbent et al., 2009; Hammond et al., 2004). By far the most widely used task is the spatial version of the water escape task. Importantly, this task can be designed to identify deficits that emerge in middle-age (~12–14 months) and increase with advancing age (Adams et al., 2008;

Bizon et al., 2009; Blalock et al., 2003; Davis et al., 1993; Driscoll et al., 2006; Foster et al., 2003; Francia et al., 2006; Granholm et al., 2008; Lindner, 1997).

2. Sensitivity of the water maze task

2.1. Procedures

Several reviews have discussed the proper procedures for employing the Morris water maze (Brandeis et al., 1989; Vorhees and Williams, 2006) and factors that must be considered when examining aged animals including stress, fatigue, and sensory-motor deficits (Foster, 1999; van der Staay, 2002). Some measures, such as latency to find the platform, are poor indicators of cognitive function since latency can be influenced by an age-related decline in swim speed (Foster et al., 2001; Norris and Foster, 1999). Normally, a cue discrimination task is used in order to identify sensory-motor or motivational deficits which would impede acquisition of a spatial search strategy. In addition to identifying animals with sensory-motor deficits, training on the cue discrimination task can insure that animals learn the procedural aspects of the task, including how to swim and the fact that the pool wall is not a route of escape (Vorhees and Williams, 2006). Consequently, animals may perform better on the spatial version of the task when they are first trained on the cue task. Similarly, when animals are initially trained on the spatial version of the task, they may perform relatively poorly due to a number of factors, but exhibit superior performance on subsequent cue training (Gerlai, 2001). Accordingly, extensive training on the spatial swim maze may mask differences in procedural learning. Alternatively, a correspondence between performance on the spatial swim task and cue discrimination task may indicate more global impairments and correlations between the tasks have been used as an indication of the extent of pathology in mouse models of neurodegenerative disease (Arendash and King, 2002; Leighty et al., 2004).

Probe trials are employed to evaluate the acquisition and retention of a spatial search strategy. For the probe trial, the platform is removed from the pool and the animal is allowed to freely explore the maze for a set time (e.g. 60 s). During the probe trial, the distance from the previous platform location is averaged or summed over the trial to obtain a proximity score (Carter et al., 2009; Gallagher et al., 1993; Maei et al., 2009). In some cases, the number of times the animal crosses the previous location of the platform (platform crossings) is reported. However, this measure may be flawed for comparing across groups. A decrease in the number of platform crossings can result from differences in motor ability and young animals make quick sharp turns, while aged animals make more sweeping turns resulting in a reduction in the number of crossings and reduced variability of the measure (Clayton et al., 2002; Devan et al., 1996; Foster et al., 2001).

Measures of the percent time searching the goal quadrant provide evidence for the use of a spatial search strategy. A probe trial delivered shortly after training is used to determine whether an animal has acquired information on the location of the escape platform, and a subsequent probe trial can be used to access retention (e.g. 24 h after the acquisition probe trial) (Foster and Kumar, 2007; Foster et al., 1991, 2003, 2001; Norris and Foster, 1999). Animals are considered to have acquired or retained the spatial information if they spend greater than 25% (i.e. chance) of the time searching the goal quadrant. A discrimination index (DI score) can be calculated for probe trials using the time spent searching the goal and opposite quadrants according to the formula DI = (time in goal − time in opposite)/(time in goal + time in opposite). Since the DI score is dependent on discriminating two quadrants, it is less susceptible to influence of motor function. A larger DI score indicates better performance and a score near 0 indicates chance performance. Finally, it is possible that the animals change their search strategy within a probe trial. For example, after initially searching where the platform should be, the animals may then search other quadrants. Alternatively, during retention testing, it may take some time for the animal find their directional bearings and begin to search the correct quadrant. In this case, the probe trial can be broken down in to smaller time segments. Thus, one may want to separately examine the first and second 30 s of a 60 s probe trial.

2.2. Sensitivity of the water maze to hippocampal function

In considering the sensitivity of the spatial swim task in detecting impaired hippocampal function, it is important to note that the degree of impairment observed during aging is generally less severe than that observed following hippocampal lesions (Foster, 1999). Animals with damage to hippocampal circuits exhibit impairments in the retention of rapidly acquired spatial information (Martin and Clark, 2007; Morris et al., 1990b; Ordy et al., 1988; Steele and Morris, 1999). In contrast to hippocampal damage, most aged animals can acquire a spatial search strategy during one or a few training sessions and deficits surface as retention delays increase (Bizon et al., 2009; Blalock et al., 2003; Driscoll et al., 2006; Foster et al., 1991, 2003; Foster and Kumar, 2007; Norris and Foster, 1999). Thus, memory deficits probably result from more subtle changes in memory processes such as storage or maintenance mechanisms (Foster, 1999).

Deficits in incremental learning of a spatial reference memory and impaired short-term memory are more common in the oldest animals (Bizon et al., 2009; Frick et al., 1995; Haberman et al., 2009; Schulz et al., 2002). The impairment in incremental learning in older animals may relate to aging of other brain regions. Animals impaired in incremental acquisition of a spatial reference memory are more reactive to novel stimuli (Collier et al., 2004; Gallagher and Burwell, 1989; Rowe et al., 1998) and exhibit impaired olfactory learning (Frick et al., 2000; LaSarge et al., 2007; Matzel et al., 2008; Zyzak et al., 1995) suggesting involvement of neocortical systems and neuromodulatory input from the locus coeruleus. Older animals exhibit a sever decrease in catecholamine release (Del Arco et al., 2001; Porras et al., 1997) and a decline in cholinergic responsiveness (Ayyagari et al., 1998; Narang et al., 1996; Schwarz et al., 1990). A decrease in neuromodulatory input, particularly to the prefrontal cortex, is associated with learning and memory deficits (Dunnett, 1990; Tanila et al., 1994; Zhang et al., 2007). However, age-related impairment of prefrontal function is linked to impaired skill or source information related to the task rather than increased forgetting of specific information (Eichenbaum et al., 2010; Winocur and Moscovitch, 1990).

The use of multiple tasks, including cue discrimination, can provide important clues to the possible contribution of extra-hippocampal regions. In one study examining Wistar rats, no relationship was observed between incremental acquisition of a spatial reference memory and other hippocampal-dependent cognitive tasks; however, rats impaired for incremental learning exhibited increased thigmotaxis on the cue discrimination swim task, indicating impaired acquisition of the procedural aspects of the task (Bergado et al., 2011). Similarly, for mouse models of Alzheimer's disease, the incremental acquisition of a spatial reference memory was correlated with performance on the cue discrimination task (Arendash and King, 2002; Leighty et al., 2004). Furthermore, performance was correlated with beta-amyloid deposition throughout the brain, indicating that deficits likely involved multiple systems.

It might be expected that individual differences would correlate across hippocampal-dependent tasks. However, there are several difficulties in relating performance across tasks including

differential influences of sensory-motor processes or sensitivity of the task for cognitive processes linked to learning or memory. Even for tasks that appear to measure the same cognitive process, little relationship may be observed. For example, the ability to acquire a spatial reference memory is sensitive to age for both for the water maze and the Barnes maze; however, there is little predictability between tasks for individual animals (Arendash and King, 2002; Gallagher and Burwell, 1989; Leighty et al., 2004; Markowska et al., 1989).

The lack of correlation between hippocampal-dependent tasks can result from differences in the sensitivity of the tasks and the degree of impairment. In this case the problem of sensitivity is compounded since it requires that both tasks are similarly sensitive to the extent of cognitive decline. There is evidence for a correspondence across tasks, when both tasks focus on retention or memory consolidation (Benice et al., 2006; Foster and Kumar, 2007; Gower and Lamberty, 1993; Paris et al., 2010). Inhibitory avoidance can be designed to be sensitive to the emergence of memory decline during aging (Gold et al., 1981; Martinez et al., 1988). In Lewis (24 month) and Sprague-Dawley (22–24 month) rats, no relationship was observed between impaired retention of inhibitory avoidance and incremental learning on the water maze (Blokland and Raaijmakers, 1993; Markowska et al., 1989). In contrast, aged (26 month) Wistar rats exhibited deficits for retention of inhibitory avoidance and retention of a spatial reference memory, when retention was examined 6 days following the end of training (Miettinen et al., 1993). As detailed below, the sensitivity of the water maze task to retention deficits can be increased by providing a single training session. Retention of inhibitory avoidance was related to retention of spatial memory examined 24 h following a single training session on the water maze in aged (18–23 month) Fisher 344 (F344) rats (Foster and Kumar, 2007). For this study, retention on the inhibitory avoidance task was not correlated with acquisition of a spatial search strategy or performance on the cue task. In middle-age (14 month) and aged (24 month) F344 rats, impaired retention 24 h following a single day of water maze training was related to the 24 h retention performance on a novel object recognition task (Blalock et al., 2003). Retention impairments may emerge earlier in females. In a study of middle-age (12 month) Long-Evans female rats, declining reproductive function was associated with poor retention for the water maze, inhibitory avoidance, and object recognition (Paris et al., 2010). In C57BL/6J mice, impairment on the water maze was related to age and gender, such that impairment for a probe trial delivered 1 h after training, was greater for aged females (Benice et al., 2006). In addition, this group of aged female mice also exhibited poorer retention of inhibitory avoidance. Again, it must be emphasized that in these cases, the inhibitory avoidance task is sensitive to milder deficits in aging, namely delay-dependent forgetting, and if designed correctly, can detect the emergence of impaired retention in middle-aged animals. However, the utility of the inhibitory avoidance task in detecting individual differences is limited due to ceiling effects and it is not as amendable to repeated training and testing. To differentiate early deficits in intermediate memory from deficits that develop over the course of aging requires a certain level of sophistication in terms of behavioral testing on the water maze.

2.3. Sensitivity and training schedules

2.3.1. Distributed training

Training schedules are important in determining the sensitivity of the swim task for detection of acquisition and retention deficits (Table 1). In general, training is massed into a single session or distributed across several days in order to examine rapid or incremental acquisition of a spatial search strategy, respectively (Fig. 1). In mice the incremental learning paradigm is generally employed to examine age effects. However, recently researchers have developed versions of the water maze that focus on rapid acquisition in order to characterize more subtle changes associated with aging, age-related disease, the function of hippocampal subregions, and synaptic plasticity mechanisms (Gulinello et al., 2009; Magnusson et al., 2003; Malleret et al., 2010; Nakashiba et al., 2008).

Training distributed across several days may not be as sensitive to the emergence of deficits in middle-aged rats (Bizon et al., 2009; Harati et al., 2009; Jacobson et al., 2008; Luparini et al., 2000; Oliveira et al., 2010; Wu et al., 2004). For example, little or no difference in acquisition was observed between 6 and 25 month old F344 Brown Norway crossed (F344BN) rats for training distributed across multiple days (Hebda-Bauer et al., 1999; Wu et al., 2004); however, training within a single day was sensitive, revealing learning deficits between 12 and 25 month old F344BN rats (Carter et al., 2009). One study employed distributed training for young, middle-aged, and aged F344 rats and probe trials, delivered at the end of training on alternate days, were used to determine the extent of learning (Bizon et al., 2009). While a significant difference was observed for probe trial measures between young and middle-aged animals, this difference was mainly due to a subgroup of young animals with highly superior performance. If a cut-off was set using the performance in young animals, the vast majority of middle-aged animals exhibited learning measures within the range of young animals and these animals could be considered unimpaired (Fig. 1D).

The number of days of training is an important factor in determining the sensitivity of distributed training (Fig. 1C). In many cases, older animals can acquire a spatial reference memory following distributed training (Clayton et al., 2002; Miyagawa et al., 1998; Nyffeler et al., 2010; Rapp et al., 1987; van Groen et al., 2002), such that acquisition deficits, that are apparent during the first couple of days of training, disappear after several days of repetitive training to the same location (Clayton et al., 2002; Jacobson et al., 2008; Luparini et al., 2000). For aged (~24 months) Wistar rats, subgroups of impaired and unimpaired rats could be differentiated over the initial two to three days of training (2–6 trials/day) (Fontana et al., 1995; Miyagawa et al., 1998). However, differences were diminished following further training. The diminished age differences with extended or repetitive training may be due to compensation, such that the animals may engage mechanisms involved in slower incremental acquisition of spatial information. Animals with hippocampal lesions can acquire a spatial reference memory following training distributed across several days, indicating extrahippocampal mechanisms involved in incremental learning of a spatial reference memory (Gerlai et al., 2002; Hannesson and Skelton, 1998; Martin and Clark, 2007; Morris et al., 1990b; Steele and Morris, 1999; Stoelzel et al., 2002; Whishaw et al., 1995). Furthermore, these other systems may be more resistant to aging.

The deficits observed early in distributed training may represent impaired memory consolidation processes mediated by the hippocampus (Fig. 1C). We have noted that memory consolidation deficits could explain the characteristic "saw-toothed" pattern of behavior observed for aged rats (Foster, 1999). In this case, older rats exhibit improved performance across trials within a day and impairments are observed for the first trial on the following day (Aitken and Meaney, 1989; Diana et al., 1995; Driscoll et al., 2006; Gage et al., 1984; Mabry et al., 1996; Rapp et al., 1987). This pattern has also been reported to emerge in middle-age (12 month) Long-Evans rats (Aitken and Meaney, 1989), and a similar pattern may be observed for aged male mice (Benice et al., 2006). However, the saw-toothed pattern of memory deficits disappears with extended training, as aged animals incrementally acquire a spatial reference memory (Fig. 1C).

Several labs have characterized a subgroup of aged rats that exhibit impaired incremental acquisition of a spatial reference memory. The impairment is particularly evident in the oldest animals (28–30 month) or when training consists of one to two trials per day (Bergado et al., 2011; Bizon et al., 2009; Collier et al., 2004; Frick et al., 1995; Gallagher and Burwell, 1989; Ivy et al., 1994; Lindner, 1997; Rowe et al., 1998; Schulz et al., 2002; Yamazaki et al., 1995). The ability to detect memory deficits during aging is enhanced by using a single training event or minimal training massed into a single session (Lal et al., 1973; Vasquez et al., 1983). As such, the sensitivity of distributed training in detecting age differences can be increased by reducing the number of training trials per day. When the number of trials was reduced to one trial per day for 10 days, the majority of F344 rats, 12 months or older, exhibited impaired acquisition, relative to 2 month old animals (Lindner, 1997). In contrast, when the training consisted of 6 trials per day for 5 days, F344 rats 4–17 months of age performed similarly after 5 days of training and deficits were only readily apparent for 24 month old rats (Frick et al., 1995). Studies that have employed multiple training trials (usually 4–8 trials per day) have provided evidence that the age at which incremental learning deficits emerge is strain sensitive. For Sprague-Dawley and F344 rats, the proportion of animals classified as impaired increases with advancing age and group differences were readily apparent by ~22–24 months of age (Bizon et al., 2009; Fischer et al., 1992). In contrast, F344BN rats exhibit either minimal impairment or a subgroup exhibit impaired incremental learning at ~24 months and notable deficits are not observed until ~31 months (Barrientos et al., 2006; Hebda-Bauer et al., 1999; Markowska and Savonenko, 2002; Wu et al., 2004). The appearance of deficits at divergent ages across rat strains may be related to differences in life span, which varies from ~25 months for F344 and ~34 months for F344BN rats. Thus, impairment in the ability to gradually acquire spatial knowledge becomes prominent when animals reach ~90% of their average life span (LaSarge and Nicolle, 2009).

As noted above, animals that exhibit impaired incremental learning may exhibit other behavioral differences including reactivity to novel stimuli (Collier et al., 2004; Gallagher and Burwell, 1989; Rowe et al., 1998) and impaired associative learning (Bergado et al., 2011; Frick et al., 2000; LaSarge et al., 2007; Matzel et al., 2008; Schulz et al., 2002; Zyzak et al., 1995) suggesting involvement of extrahippocampal systems. Certainly, for mouse models of Alzheimer's disease, impaired incremental acquisition of a spatial reference memory is correlated with impairment in several behavioral domains as well as the extent of brain beta-amyloid deposition (Arendash and King, 2002; Leighty et al., 2004). The results suggest that the inability to acquire a spatial reference memory following extensive training may portend robust hippocampal dysfunction and/or more global changes which preclude incremental learning (Cho and Jaffard, 1995; Moffat et al., 2007). As such, impaired incremental acquisition of a spatial reference memory following distributed training may be a good model of severe cognitive deficits associated with dementia.

2.3.2. Massed training

Due to the fact that learning and/or memory deficits on the water maze are more pronounced during the initial training period, some studies have massed training into a single session in order to increase the sensitivity of the task (Table 1). Memory following massed training is not as strong as memory acquired following distributed training (Commins et al., 2003; Dash et al., 2002; Spreng et al., 2002) and depends more on the hippocampus (Bouffard and Jarrard, 1988). Furthermore, massed training schedules make use of distinct molecular memory mechanisms that are sensitive to aging (Foster et al., 2001; Genoux et al., 2002; Malleret et al., 2001). Again, aged animals may exhibit slower learning during training (Fig. 1E). Acquisition of a spatial search strategy following a single training session can be confirmed by a probe trial and calculating the discrimination index or measuring the portion of time spent searching in the quadrant that originally held the escape platform (Fig. 1F). This probe trial can be used to detect age-related impairment in the acquisition of a spatial search strategy. Once it is clear that an animal has acquired a spatial search strategy, a subsequent probe trial may be delivered at more distant time points in order to evaluate retention (Fig. 1F). Work from our lab and others indicates that a subset of middle-age animals exhibit memory deficits examined 24 h after acquisition and the probability of memory impairment increases with advancing age (Aitken and Meaney, 1989; Blalock et al., 2003; Driscoll et al., 2006; Foster et al., 1991, 2003; Foster and Kumar, 2007; Mabry et al., 1996; Norris and Foster, 1999).

Virtual environments, including versions of the water maze, have been used to examine spatial learning and memory in amnesic and elderly humans. In general, training is massed into a single session and the research confirms that damage to the hippocampus results in impaired spatial navigation (Astur et al., 2002; Bartsch et al., 2010; Bohbot et al., 2004; Goodrich-Hunsaker et al., 2010; Skelton et al., 2000). Furthermore, elderly individuals exhibit deficits involving increased path length during acquisition and impaired performance on probe trials (Driscoll et al., 2003; Moffat et al., 2007, 2001; Moffat and Resnick, 2002). While all age groups exhibit acquisition on a virtual environment maze, middle-aged and aged subjects were slower to learn and exhibited increased spatial memory errors (Driscoll et al., 2003, 2005; Jansen et al., 2010; Moffat et al., 2001; Thomas et al., 1999). Similar to animal studies, deficits were apparent following a single training session, and with continued training, older subjects performed in a manner that was not different from younger individuals

Table 1
Training methods commonly employed for examining spatial learning and memory on the water maze. Each method focuses on distinct memory components or cognitive processes and is associated with different advantages and disadvantages. Further, the sensitivity of the task in detecting the age of onset for impaired memory can vary according to the training methods employed.

Method	Cognitive processes	Advantage	Disadvantage	Age of onset (months)
Distributed training	Incremental learning, spatial reference memory	Sensitive to incremental learning deficits, may better mimic global deficits of dementia	Lack of sensitivity for early detection of memory deficits, deficits may disappear with extended training, may result in chronic stress	18–24
Massed training	Rapid acquisition and intermediate memory	Sensitive to early detection of acquisition and memory deficits	May induce an acute stress	Acquisition 12–18 Memory 12–14
Repeated acquisition	Rapid, flexible acquisition	Sensitive to early detection of acquisition deficits for rapidly acquired information	May result in a chronic stress with repeated training	18–24
Delayed match-to-sample	Intermediate memory	Sensitive to early detection of memory deficits for rapidly acquired information	May result in a chronic stress with repeated training	Retention duration decreases with age

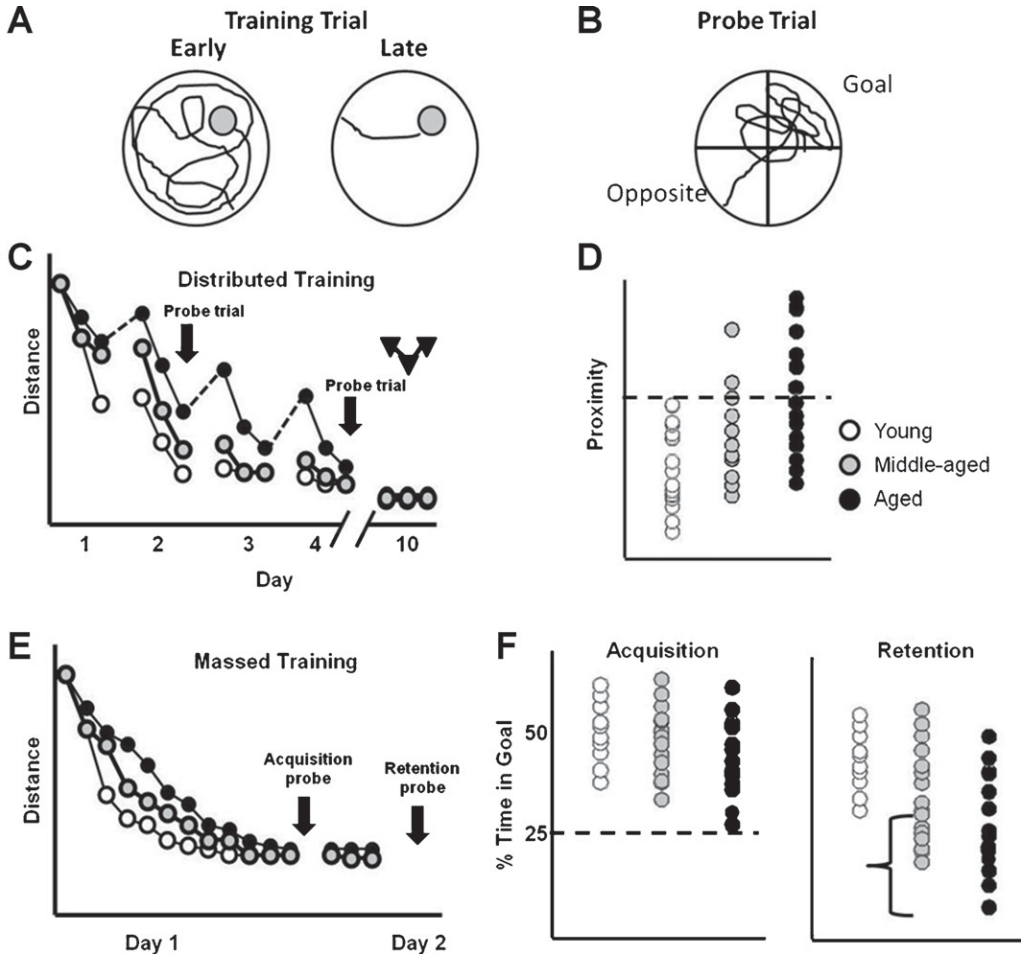

Fig. 1. Performance differences during aging for distributed or massed training on the water maze. (A) Schematic illustration of the pool, with an escape platform (grey circle) in the upper right hand quadrant. The line indicates the path length to find the platform, which decreases from the earlier to later training trials. (B) During a probe trial, the platform is removed and the search pattern is focused on the area (i.e. goal quadrant) in which the platform was located. (C) Hypothetical performance curves of age-related differences in performance for distributed training, modeled from Aitken and Meaney (1989), Bizon et al. (2009), Clayton et al. (2002), Diana et al. (1995), Driscoll et al. (2006), Gage et al. (1984), Mabry et al. (1996), Miyagawa et al. (1998), Nyffeler et al. (2010), Rapp et al. (1987) and van Groen et al. (2002). Older animals (filled circles) exhibit an initial impairment compared to middle-aged (grey circles) and young (open circles). However, most aged animals can acquire a spatial reference memory following extended distributed training. The saw-tooth pattern of performance in aged animals may be due to forgetting across days (dashed lines). A subset of aged animals (filled triangles) may exhibit profound learning deficits such that they will not be able to acquire a spatial reference memory with repeated training to the same location across several days. (D) A probe trial, delivered on alternate days, can be substituted for the last trial of that day. The average distance from the platform (proximity) is recorded and those animals that exhibit distances greater than young (dashed line) are classified as learning impaired. Most middle-aged animals would not be classified as learning impaired. (E) Age-related differences in performance for massed training, adapted from Blalock et al. (2003), Foster et al. (1991, 2003), Foster and Kumar (2007), Mabry et al. (1996), Norris and Foster (1999). Aged animals exhibit a slower rate of learning when training is massed into a single training session. The acquisition probe trial is delivered near the end of training, followed by three refresher training trials, and a retention probe trial, delivered 24 h later. (F) Measures of the percent time searching the goal quadrant for the initial probe trial (Acquisition) indicate that most animals have acquired a search strategy, focusing their search in the goal quadrant relative to chance (dashed line). The retention probe trial reveals a subgroup of memory impaired aged and middle aged animals (bracket) that exhibit goal search times that are less than that observed for young.

(Jansen et al., 2010). Again, the initial learning and asymptotic level of performance in virtual environments may depend on different brain systems (Bohbot et al., 2007; Etchamendy and Bohbot, 2007; Etchamendy et al., 2011; Hartley and Burgess, 2005). The results suggest that training massed into a single session is sensitive in detecting the emergence of deficits in intermediate-term memory for rapidly acquired spatial information during middle-age in rodents and humans.

2.3.3. *Repeated acquisition/matching-to-sample training*

Repeated acquisition training provides another version of massed training, which is designed to specifically examine rapid flexible learning and can be adapted to examine retention or forgetting (Table 1). For this task, the escape location is changed across sessions such that the animal must repeatedly learn new escape locations (Fig. 2A). This task usually involves only one or two days of training to each location. Deficits in the rapid acquisition of a spatial memory emerge by ~12 months of age in spontaneously hypertensive rats; however, most rat strains exhibit deficits by 18 months of age (Driscoll et al., 2006; Markowska and Savonenko, 2002; van der Staay and de Jonge, 1993; Wyss et al., 2000) (Fig. 2B). Moreover, studies that directly compare incremental learning using distributed training and repeated acquisition in which the escape platform is changed each day indicate that repeated acquisition testing is more sensitive in detecting the emergence of age-related cognitive decline (Bizon et al., 2009; Markowska and Savonenko, 2002; Miyagawa et al., 1998; Nyffeler et al., 2010). In these cases, animals that exhibited impairments on the repeated acquisition version of the task could still acquire a spatial reference memory with training distributed across several days, consistent with the idea that impairments in rapid acquisition precede impaired incremental learning.

Memory deficits can be characterized using the repeated acquisition training and delayed matching-to-sample testing to

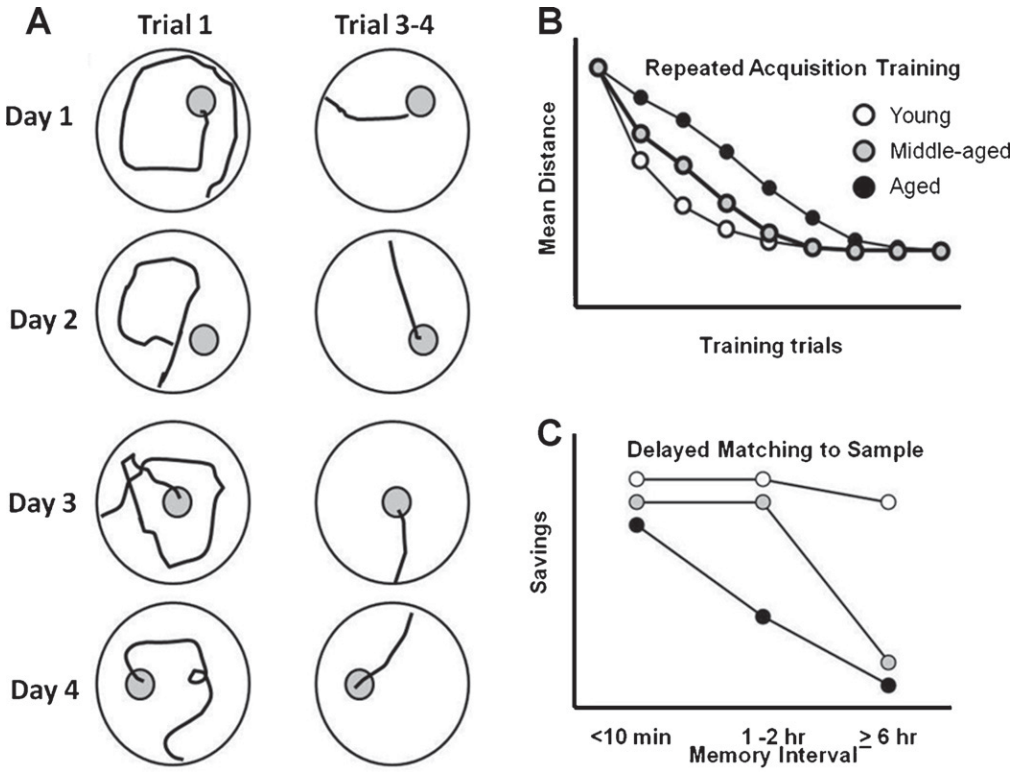

Fig. 2. Performance differences related to aging for repeated acquisition training. (A) Schematic illustration of the pool, with an escape platform (grey circle) shifted to a new location each day such that the animal must repeatedly learn new escape locations. (B) Hypothetical performance curves of the age-related decline in the rate of acquisition, modeled from Driscoll et al. (2006), Markowska and Savonenko (2002), Miyagawa et al. (1998), Nyffeler et al. (2010), van der Staay and de Jonge (1993), Wyss et al. (2000). The distance for each trial is averaged across days of training. The rate of learning decreases with age (aged: filled circles; middle-aged: grey circles; young: open circles). (C) For delayed matching-to-sample, a variable delay (minutes to hours) is inserted between the first two trials. The savings (i.e. decrease in path length to find the platform on trial two compared to the first trial) is used as an indication of memory. An age by delay interaction is observed such that similar performance is observed for short delays and age-related memory impairments are seen with increasing delays.

examining the savings from trial to trial. In this case, deficits are observed as a decrease in savings as the inter-trial interval is increased (Fig. 2C). Interestingly, there is evidence to indicate that the extent of the memory impairment increases with advancing age. Compared to young rats, middle-aged animals exhibit a similar level of performance during acquisition training (8 trials/day) and impaired retention is observed on the first trial examined on day 2, 24 h after the initial training (Driscoll et al., 2006). Furthermore, the extent of impairment increases with advancing age (Driscoll et al., 2006). Indeed, following a single training trial, young rats exhibit retention for up to 6 h; middle-aged animals begin to exhibit retention deficits as the delay is increased to 2 h and aged animals exhibit deficits for delays greater than ~1 h (Bizon et al., 2009; Means and Kennard, 1991) (Fig. 2C).

2.4. Reliability

One distinguishing feature of the swim task is that it is amendable to multiple testing sessions in order to examine the reliability of acquisition and retention deficits, as well as the success of treatments (Fontana et al., 1995; Markowska et al., 1994). However, it is important to recognize that there are carry-over effects of multiple testing sessions. Furthermore, testing on the water maze increases the release of stress hormones (Engelmann et al., 2006) and the stress of extended water maze training can influence hippocampal neurogenesis (Namestkova et al., 2005) and may have enduring affects on hippocampal function, particularly in learning impaired animals in which water maze training may be analogous to a being exposed to an uncontrollable swim stress (Foster and Kumar, 2007). On the other hand, training may act as cognitive stimulation and promote skills needed to solve the water maze. Animals that are trained on the maze during young adulthood or middle-age exhibit an advantage in re-learning when tested during aging (Hansalik et al., 2006; Vallee et al., 1999; van Groen et al., 2002; Vicens et al., 2002). As noted above, procedural memory is relatively intact during aging such that animals likely acquire a procedural strategy for how to find a hidden platform across multiple testing sessions. However, individual differences are observed such that heterogeneity in performance is consistently observed between individual subjects. Tests of repeated acquisition in the same environment indicate a slower rate of acquisition for aged animals, while performance of middle-aged animals is similar to young (Driscoll et al., 2006; Frick et al., 1995; Wyss et al., 2000). Nevertheless, middle-aged animals continue to exhibit retention deficits relative to young (Bizon et al., 2009; Driscoll et al., 2006; Means and Kennard, 1991). Similarly, for incremental learning across days of repetitive training, some aged animals consistently perform more poorly than young (Fontana et al., 1995; Lindner, 1997). When the acquisition of a spatial reference memory is retested using a new environment, carry-over effects are observed such that young and aged animals exhibited more rapid learning relative to the initial environment; however, aged animals that exhibited the poorest initial performance also exhibited poorer performance in the new environment (Colombo and Gallagher, 2002).

2.5. Aging of hippocampal subregions and the trajectory of cognitive decline

Little is known concerning the trajectory of cognitive decline during aging in rodents. The preceding review of the literature, focused on the water maze, supports the idea that cognitive decline

is progressive and can be tracked using different water maze training procedures. Middle-age is associated with impairment of rapid spatial learning/memory and intact gradual acquisition of spatial knowledge. Tests involving a single training session or delayed matching-to-sample provide sensitive measures for deficits in intermediated-term memory as an early marker of cognitive decline. Repeated acquisition training can detect impairments in rapid, flexible spatial learning as animals move from middle-age to old age. Furthermore, the propensity for acquisition and memory deficits increases with advanced age. Finally, a subset of the oldest animals will exhibit profound learning deficits such that they will not be able to acquire a spatial reference memory, even with distributed training to the same location.

The cognitive changes observed with advancing age suggest a progression in the senescence of biological mechanisms of memory. Cognitive deficits are not associated with obvious hippocampal pathology involving cell death (Gallagher et al., 1996). Rather, memory deficits probably result from more subtle changes in memory processes for storage or maintenance mechanisms (Foster, 1999). All hippocampal subregions; dentate gyrus, CA3, and CA1, show some form of age-related change, which is likely to contribute to impaired cognition (Burke and Barnes, 2011; Rosenzweig and Barnes, 2003; Small et al., 2011; Wilson et al., 2006). Furthermore, these subregions are differentially susceptible to diseases of age including effects of stress, Alzheimer's disease, and cell death due to ischemia.

Importantly, the subregions differentially contribute to different phases or aspects of episodic memory (Daumas et al., 2005; Kesner and Hunsaker, 2010; Kesner et al., 2004), suggesting that aging of subregions could uniquely modify performance on the water maze. As such, aging of different subregions or senescence of different mechanisms may account for early deficits in memory consolidation relative to later deficits in rapid acquisition, and finally impaired incremental learning. For example, the dentate gyrus is involved in spatial pattern separation (Gilbert et al., 2001; McHugh et al., 2007). Pattern separation declines during aging in humans and is associated with altered activity in the dentate gyrus (Small et al., 2011; Yassa et al., 2011). Neurogenesis is a notable form of neuroplasticity observed in the dentate gyrus, which declines during aging (Cameron and McKay, 1999; Kempermann et al., 1998; Kuhn et al., 1996; Nacher et al., 2003). The exact role of neurogenesis in memory function is currently a subject of intense research. However, it is interesting to note that variability in neurogenesis in aged animals is correlated with the rapid acquisition of spatial information observed during the early phases of spatial learning (Drapeau et al., 2003, 2007; Driscoll et al., 2006) and is not associated with impaired incremental learning (Bizon and Gallagher, 2003; Bizon et al., 2004; Merrill et al., 2003).

Region CA3 is thought to be involved in rapid encoding and pattern completion for spatial information and the modifiability of CA3 cell activity is reduced with age (Wilson et al., 2005). Behavioral stress induces dendritic structural changes in region CA3 and susceptibility to stress effects increases with age (Christian et al., 2011; Cohen et al., 2011; Gartside et al., 2003; McEwen, 2001; Shoji and Mizoguchi, 2010). Gene changes in this region during aging indicate that preservation of CA3 function is maintained by neurotrophic mechanisms that permit adaptation to stress (Zeier et al., 2010) and failure to enlist these adaptation mechanisms is associated with impaired learning (Haberman et al., 2009). N-methyl-D-aspartate receptors (NMDARs) contribute to CA3 function in rapid encoding and mutant mice with a deletion of NMDARs, specific to CA3, exhibit normal incremental learning and impaired performance on repeated acquisition training (McHugh et al., 2007; Nakazawa et al., 2002). In addition, the stress mediated dendrite changes require activation of CA3 NMDARs (Christian et al., 2011). Aging and learning impairment are associated with changes in CA3 synaptic markers, including NMDARs (Adams et al., 2001; Smith et al., 2000). However, the decline in CA3 NMDAR markers have been associated with learning deficits (Adams et al., 2001), no change in learning (Nicolle et al., 1996), or better performance (Le Jeune et al., 1996), suggesting that loss of NMDAR may mediate deficits or may act as a compensatory mechanism to protect cells from stress.

Region CA1 of aged animals exhibits gene changes related to increased susceptibility to inflammation, oxidative stress, Ca^{2+} dysregulation, and a decline in neurotrophic support (Blalock et al., 2003; Jackson and Foster, 2009; Jackson et al., 2010; Wang and Michaelis, 2010; Zeier et al., 2010). In turn, changes in these processes are thought to contribute to the propensity for region CA1 to exhibit increased susceptibility to Alzheimer's disease and increased cell loss following ischemia, as well as impaired memory and synaptic plasticity during aging (Blalock et al., 2003; Zeier et al., 2010). Region CA1 is involved in consolidation of an intermediate or long-term form of memory (Dudai, 2004; Kesner and Hunsaker, 2010; Kesner et al., 2004; Lee and Kesner, 2002; Morris et al., 2003; Rawlins and Tsaltas, 1983), and as discussed above, disruption of memory consolidation is an early behavioral marker of cognitive aging. As such, the age-related disruption in Ca^{2+} regulation and synaptic plasticity is thought to contribute to the emergence of memory consolidation deficits (Foster, 2007; Foster and Norris, 1997; Kumar et al., 2009).

3. NMDAR-dependent and VDCC-dependent plasticity during aging

The general view is that synaptic plasticity mechanisms represent the biological substrate for memory and learning and the weight of evidence indicates a link between synaptic plasticity and spatial memory (McNaughton et al., 1986; Morris et al., 1986; Moser et al., 1998). Considering the prominence of synaptic plasticity as a model of memory storage, it is not surprising that a number of studies have examined age-related changes in two major forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD). LTP is an increase in synaptic transmission, induced by pattern stimulation of afferent fibers and is proposed as a cellular mechanism for the kind of rapid and flexible intermediate-term memory that is disrupted early in the course of cognitive senescence (Dudai, 2004; Morris et al., 2003). LTD is a decrease in synaptic strength, which may contribute to loss of synaptic contacts and increased forgetting during aging (Foster, 1999, 2007; Shinoda et al., 2005; Zhou et al., 2004). Age-related changes in LTP and LTD suggest the functional significance of altered synaptic plasticity for cognitive function (Foster, 1999, 2002; Foster and Norris, 1997).

While age-related changes in synaptic plasticity have been reported for all regions of the hippocampus, the majority of work has focused on perforant path to dentate gyrus and CA3 to CA1 synapses. For the dentate gyrus, the evidence indicates that impaired induction of LTP during aging results from neuroinflammatory changes, oxidative stress, and a decrease in NMDAR function (Lynch, 2009; Rosenzweig and Barnes, 2003). Undoubtedly, synaptic contacts between CA3 and CA1 pyramidal cells have received the most attention in terms of age-related changes in synaptic plasticity and this synapse is the focus of the current review. In general, no difference is observed in the expression mechanisms or the asymptotic amplitude of LTP and LTD, rather there is a shift in the mechanisms that regulate the threshold for induction of synaptic plasticity (for a review see Foster, 1999). Thus, age differences are likely to be observed for a given stimulation paradigm, particularly if the pattern of stimulation

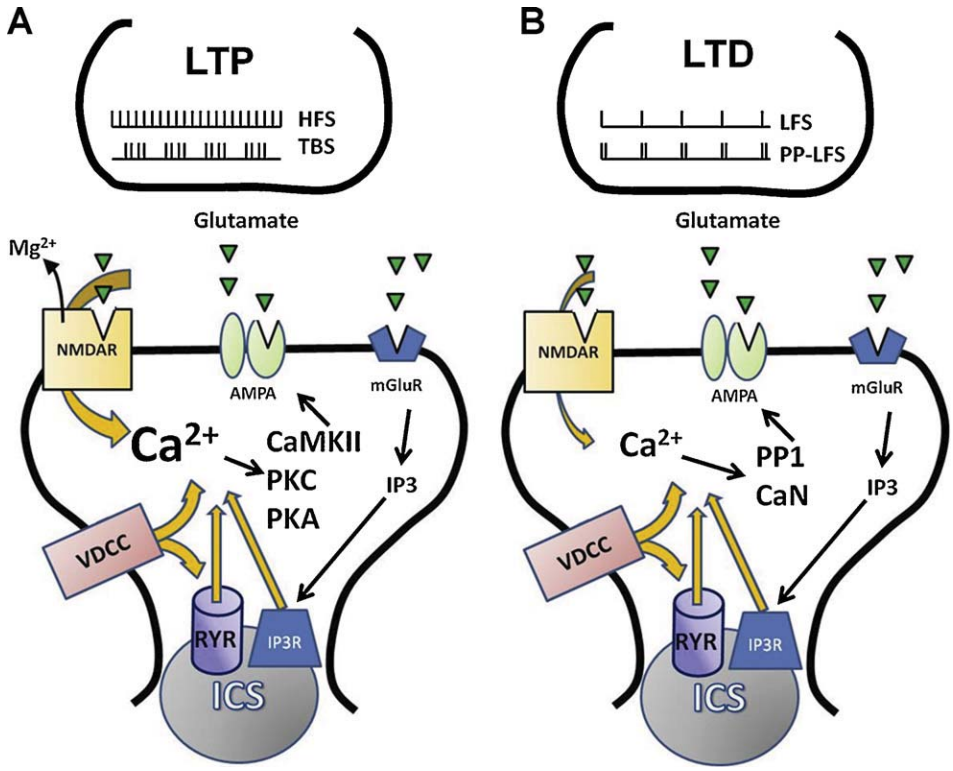

Fig. 3. Synaptic plasticity induced by pattern stimulation. (A) LTP is an increase in synaptic transmission, induced by HFS or TBS activation of presynaptic fibers to release glutamate, depolarizing the postsynaptic side to release the Mg^{2+} block of the NMDAR, and provoke a brief, large magnitude rise in postsynaptic Ca^{2+} from NMDARs, VDCCs and ICS. In addition, glutamate acting on mGluRs can increase the release of Ca^{2+} from ICS through IP3R activation. The large rise in Ca^{2+} activates kinases (CaMKII, PKC, PKA) to increase the AMPA receptor component of synaptic transmission. (B) LTD is a decrease in synaptic strength induced by LFS or PP-LFS to produce a modest and prolonged rise in intracellular Ca^{2+}, activating phosphatases (CaN, PP1) to decrease the AMPA receptor component of synaptic transmission.

is close to the threshold for induction of synaptic plasticity. The threshold for induction of LTP increases with age such that higher stimulation frequencies or more induction sessions are required in older animals in order to achieve the same level of LTP. Similarly, the threshold for induction of LTD is lowered in aged animals, facilitating induction of LTD. Thus, an important question concerns differences in the mechanisms that regulate the induction of synaptic plasticity.

Induction of LTP involves released of glutamate from the presynaptic terminals. The glutamate binds to postsynaptic NMDARs and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Fig. 3). The activation of AMPA receptors depolarizes of the postsynaptic membrane. The depolarization is critical in removing the Mg^{2+} block of the NMDAR channel and provoking a brief, large magnitude rise in postsynaptic Ca^{2+}. The large rise in intracellular Ca^{2+} activates kinases including protein kinase C (PKC), Ca^{2+}-calmodulin kinase II (CaMKII), and protein kinase A (PKA). The kinases phosphorylate proteins, increasing the function of AMPA receptors, resulting in an increase in synaptic transmission (Fig. 3A). Induction of LTD is achieved by prolonged low frequency pattern stimulation to produce a modest and prolonged rise in intracellular Ca^{2+}. A modest rise in Ca^{2+} activates phosphatase cascades, calcineurin (CaN) and protein phosphatae 1 (PP1), dephosphorylating proteins to decrease synaptic responses (Fig. 3B). Thus, both LTP and LTD depend on a rise in intracellular Ca^{2+}, and the degree and direction of altered synaptic function is determined by the level and duration of the Ca^{2+} rise.

The rise in intracellular Ca^{2+} levels is a function of the stimulation pattern, and the stimulation pattern influences the source of intracellular Ca^{2+}. There are three main Ca^{2+} sources for induction of synaptic plasticity, NMDARs, voltage-dependent Ca^{2+} channels (VDCCs), and intracellular Ca^{2+} stores (ICS) (Fig. 3). During aging, NMDAR function decreases and Ca^{2+} from VDCCs-ICS increases in region CA1. This shift in Ca^{2+} sources contributes to modifications in the threshold for induction of synaptic plasticity, leading to the proposed Ca^{2+} dysregulation hypothesis for age-related changes in synaptic plasticity (Foster, 1999; Foster and Norris, 1997).

3.1. LTP

Electrophysiological and pharmacological data indicate that at CA3-CA1 synapses, there are at least two forms of LTP that differ in terms of induction mechanisms. It should be emphasized that all Ca^{2+} sources normally contribute to LTP induction in the absence of specific antagonists. Nevertheless, depending on the stimulation pattern, LTP induction is mainly due to NMDAR activity (NMDAR-LTP) or can include NMDAR-independent and VDCC-dependent mechanisms (VDCC-LTP). In young animals, high frequency stimulation (HFS 25–100 Hz) or stimulation patterns near the threshold for LTP-induction, including brief episodes of theta burst stimulation (TBS) induce a rise in intracellular Ca^{2+} mainly through activation of NMDARs (Fig. 3A). The shift in LTP mechanisms during aging can be observed as a decrease in the LTP magnitude for weak or threshold stimulation that largely activates NMDARs (Barnes, 1979; Foster, 1999, 2002, 2007; Landfield et al., 1978) and no age-related difference is observed when strong stimulation is used (Kumar et al., 2007). Higher frequency stimulation (200 Hz) can induce LTP independent of NMDARs, due to increased Ca^{2+} from VDCCs, particularly L-channels (Cavus and Teyler, 1996; Grover and Teyler, 1990). The Ca^{2+} from L-channels binds to ryanodine receptors (RyRs) to release Ca^{2+} from ICS, resulting in a further increase in intracellular Ca^{2+} (Fig. 3). In addition, glutamate binding to metabotropic glutamate receptors (mGluRs) can

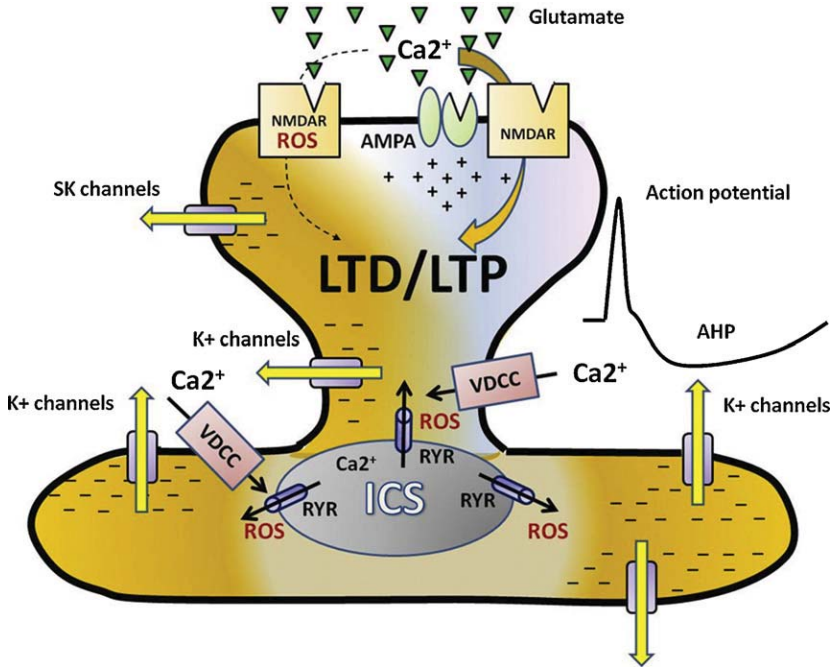

Fig. 4. The interaction of NMDARs, VDCCs, RyRs, and redox state regulates synaptic plasticity during aging. In young animals, the rise in intracellular Ca^{2+} for induction of synaptic plasticity (LTD/LTP) arises mainly from NMDARs. Activation of NMDARs requires glutamate binding and postsynaptic depolarization (+) due to AMPA glutamate receptor activity and backpropagating action potentials. The action potential will activate VDCCs to increase Ca^{2+} influx along the dendritic membrane. The influx of Ca^{2+} from VDCCs acts on RyRs to release Ca^{2+} from ICS. In turn, the rise in intracellular Ca^{2+} activates K^+ channels along the membrane, resulting in an efflux of K^+, which hyperpolarizes (−) the cell membrane. The K^+ channels include SK channels near NMDARs and channels involved in the generation of the sAHP. With advanced age, an increase in oxidative stress (ROS) shifts the function of Ca^{2+} sources, decreasing and increasing Ca^{2+} from NMDARs and RyRs, respectively. The increased Ca^{2+} from ICS results in a larger hyperpolarizing response, which further inhibits NMDAR activation, raising the threshold stimulation needed to induce LTP.

increase release of Ca^{2+} through inositol trisphosphate receptor (IP3R) activation on ICS. Unlike NMDAR-LTP, VDCC-LTP tends to be greater in older animals (Boric et al., 2008; Robillard et al., 2011; Shankar et al., 1998).

There are two mechanisms that mediate the age-related decrease in NMDAR-LTP, one involves a reduction in NMDAR function and the other involves active inhibition of NMDARs through VDCCs and ICS (Fig. 4). Electrophysiological studies consistently show a decrease in the NMDAR component of the synaptic transmission in region CA1 during aging (Barnes et al., 1997; Billard and Rouaud, 2007; Bodhinathan et al., 2010a; Tombaugh et al., 2002). The weight of evidence indicates that NMDAR binding declines in a number of brain regions, including the hippocampus (Magnusson et al., 2010). However, the level of receptor binding does not readily match the levels of protein or RNA expression, suggesting that changes in the functional state of the receptors or regulation of receptor activity contribute to the decrease in agonist/antagonist binding and the decrease in NMDAR function (Magnusson et al., 2010).

Recent work demonstrates that the decrease in NMDAR function is related to oxidative stress and a shift in the intracellular oxidation–reduction (redox) state (Bodhinathan et al., 2010a). The nervous system is highly sensitive to oxidative stress (Halliwell, 1992). Irreversible damage to lipids, DNA, and proteins results from the production of the oxygen radical, superoxide. To protect tissue from irreversible damage, superoxide dismutase converts superoxide in to the nonradical hydrogen peroxide. Hydrogen peroxide per se does not induce irreversible oxidative damage (Catala, 2010; Leutner et al., 2001; Linden et al., 2008; Shacter, 2000). The relatively milder hydrogen peroxide induces the reversible formation of disulfide bonds between pairs of cysteine residues in proteins, shifting protein structure and function, and influencing multiple signaling cascades including Ca^{2+} signaling (Foster, 2007). The redox state of cysteine residues on the extracellular portion of the NMDAR have been implicated in regulating NMDAR function in cell cultures and neonatal animals (Aizenman et al., 1990, 1989; Bernard et al., 1997; Choi and Lipton, 2000), suggesting that altered redox state of these extracellular cysteine residues could mediate the decline in NMDAR function during aging. Indeed, application of the cysteine specific reducing agent, dithiothreitol, selectively increased the NMDAR component of the synaptic response and the magnitude of LTP in hippocampal slices from older rodents (Bodhinathan et al., 2010a). The membrane-impermeable reducing agent, L-glutathione, failed to increase the NMDAR response when applied to the slice; however, an increase in the NMDAR response was observed by intracellular delivery of L-glutathione through the intracellular recording pipette. The results indicate that intracellular redox state, rather than disulfide bonds of extracellular cysteine residues, mediates the suppression of NMDAR responses and impaired LTP. The decline in NMDAR function during aging, mediated by intracellular redox state, has recently been confirmed by other labs (Robillard et al., 2011; Yang et al., 2010). Finally, the dithiothreitol mediated growth of the NMDAR response was blocked by inhibition of CaMKII, but not by inhibition of PKC, PP1, or CaN. Furthermore, CaMKII activity of aged animals could be enhanced by dithiothreitol treatment, indicating that the effect was specific to CaMKII activity (Bodhinathan et al., 2010a).

The other mechanism for decreasing NMDAR-LTP in aged animals is mediated by VDCCs and ICS. VDCCs and ICS regulate NMDAR-dependent LTP by influencing the membrane potential (Fig. 4). NMDAR activation requires depolarization of the postsynaptic membrane (Fig. 3A). Release of Ca^{2+} from ICS or influx through VDCCs activates Ca^{2+}-dependent SK potassium (K^+) channels to hyperpolarize dendrites (Faber, 2010) and inhibition of Ca^{2+} release from ICS increases the NMDAR response in older animals (Kumar and Foster, 2004), suggesting a direct connection between ICS and NMDAR function. The membrane potential is also

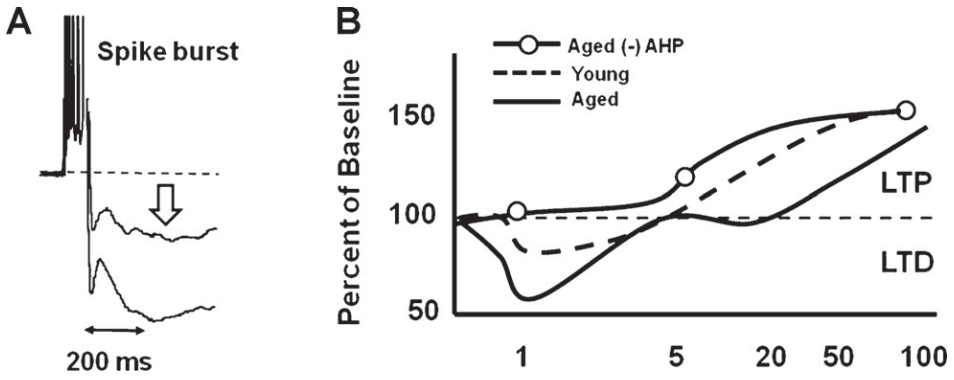

Fig. 5. The threshold for LTP is regulated by the sAHP. (A) Illustration of the sAHP. The resting membrane potential is indicated by the dashed line. Intracellular injection of depolarizing current induced a burst of five action potentials (spike burst), which is followed by the sAHP with a peak ~200 ms from the spike burst and lasting several 100 ms. The sAHP creates a window of hyperpolarization that disrupts the activation of NMDARs. The arrow indicates a reduction in the sAHP induced by pharmacological treatments to decrease Ca^{2+} from VDCC or ICS. (B) Frequency–response functions for stimulation induced synaptic plasticity. The baseline synaptic response is 100%. Pattern stimulation (900 pulses) of increasing stimulation frequency (1–100) was delivered and the synaptic response was measured at least 30 min after the end of pattern stimulation. The curves indicate the changes in synaptic transmission (LTP > 100 percent of baseline or LTD < 100 percent of baseline). Normally, LTD is observed for LFS between 1 and 3 Hz and aged animals (solid curve) exhibit increased susceptibility, exhibiting greater LTD relative to younger animals (dashed curve). The threshold for LTP is ~5 Hz in young animals. In contrast, aged animals exhibit a plateau region of no synaptic modification starting at ~5 Hz, such that the threshold stimulation frequency for LTP induction is increased. The age-related shift in synaptic plasticity is due in part to the increase in the sAHP. Pharmacological treatments that decrease the sAHP amplitude in aged animals (Aged (−) AHP, solid curve with open circles) reduce the threshold stimulation required for induction of LTP.

regulated by another well-characterized biomarker of age-related memory impairment; the Ca^{2+}-dependent, K^+ mediated slow afterhyperpolarization (sAHP). In this case, action potentials activate VDCCs, the influx of Ca^{2+} stimulates RyRs to release Ca^{2+} from ICS and the rise in intracellular Ca^{2+} opens K^+ channels to hyperpolarize the membrane (Fig. 4). In aged animals, increased Ca^{2+} from VDCCs and ICS enhance the magnitude and duration of the sAHP (Bodhinathan et al., 2010b; Kumar et al., 2009; Kumar and Foster, 2004; Oh et al., 2010; Thibault and Landfield, 1996). The sAHP represents a window of time after cell discharge activity, with a peak at ~200 ms (Fig. 5A), in which the hyperpolarization disrupts the integration of depolarizing postsynaptic potentials. This disruption is evident for stimulation patterns near the theta frequency (i.e. 5 Hz with a 200 ms interval between stimulation episodes), including TBS (Diamond et al., 1988; Fuenzalida et al., 2007; Kramar et al., 2004; Moore et al., 1993).

The effect of the larger sAHP on the threshold for induction of LTP can be observed by plotting the change in synaptic strength as a result of different stimulation frequencies (Fig. 5B). For young animals, the threshold frequency for induction of LTP is near 5 Hz and the level of LTP increases as stimulation frequency increases. The frequency–response function for CA3-CA1 synapses in older animals is marked by a plateau region in which no LTP occurs for intermediate stimulation frequencies (~5–25 Hz), resulting in an increased threshold frequency required for induction of LTP (Foster, 1999; Hsu et al., 2002; Kumar and Foster, 2007b). An important observation is that treatments that reduce the sAHP drastically decreased the LTP threshold for aged animals; unmasking NMDAR-LTP for stimulation patterns as low as 5 Hz (Fig. 5B). Treatments to reduce the sAHP and promote LTP include blockade of Ca^{2+} release from intracellular stores, blockade of SK channels, and L-channel antagonists (Bodhinathan et al., 2010a; Kumar and Foster, 2004; Norris et al., 1998). The results emphasize that VDCCs, RyRs, and ICS regulate synaptic plasticity not only by supplying intracellular Ca^{2+} for induction of VDCC-dependent synaptic plasticity, but also by regulating the membrane potential to dynamically inhibit NMDAR activation. Thus, increased Ca^{2+} from VDCCs and ICS facilitates LTP for higher frequency stimulation (i.e. 200 Hz) and actively inhibits NMDAR-LTP in older animals.

Recent work has emphasized oxidation of RyRs and increased release of Ca^{2+} from ICS in mediating age-related changes in the sAHP and synaptic plasticity. Blockade of L-channels reduces the sAHP to a similar extent (~30%) in young and aged animals (Bodhinathan et al., 2010b; Disterhoft et al., 2004; Norris et al., 1998; Power et al., 2002). In contrast, treatments to reduce the release of Ca^{2+} from ICS has greater effect in aged animals, reducing the sAHP by ~50%, such that the amplitude approximates that normally observed in young animals (Bodhinathan et al., 2010b; Kumar and Foster, 2004). RyRs are redox sensitive such that release of Ca^{2+} from ICS is increased under conditions of mild oxidative stress (Bodhinathan et al., 2010b; Hidalgo et al., 2004). Application of cysteine specific reducing agents decreased the amplitude of the sAHP specifically in aged animals (Bodhinathan et al., 2010b). The antioxidant mediated reduction in the sAHP could be blocked by depletion of Ca^{2+} from ICS or blockade of RyRs, but not through blockade of VDCCs. The results indicate that redox state of RyRs is the major contributor to the age-related increase in the sAHP. In addition, RyR involvement in the age-related growth of the sAHP appears to emerge in middle-age (Gant et al., 2006). Finally, the shift in redox state and increased release of Ca^{2+} from ICS may underlie the age-related shift from NMDAR-LTP to VDCC-LTP. As noted above, Ca^{2+} release from ICS decreases the NMDAR response in older animals (Kumar and Foster, 2004), suggesting a direct connection between ICS and NMDAR function.

The disruption of Ca^{2+} regulation and impaired learning and memory are hypothesized to result from a shift in the level of hydrogen peroxide and reversible redox state of proteins, rather than irreversible oxidative damage associated with superoxide. Application of hydrogen peroxide to hippocampal slices from young animals decreases CaMKII activity (Shetty et al., 2008), decreases NMDAR responses (Bodhinathan et al., 2010a), and impairs LTP (Kamsler and Segal, 2003; Pellmar et al., 1991). Furthermore, hydrogen peroxide can enhance VDCC-LTP, possible through increased RyR activation (Huddleston et al., 2008; Kamsler and Segal, 2003). Virus mediated over expression of individual antioxidant enzymes in the hippocampus decreased the level of lipid and DNA oxidative damage without improving learning on the water maze (Lee et al., 2011). In fact, expression of superoxide dismutase-1 in older animals enhanced impairments of learning on the water maze. The effect of superoxide dismutase-1 is thought to result from an increase in the production of hydrogen peroxide. In support of this idea, deficits due to over expression of superoxide dismutase-1, as well as age-related deficits on the water maze,

were ameliorated by co-expression of superoxide dismutase-1 and catalase. Together, the results establish a mechanism that links general theories of aging (i.e. oxidative stress and redox state) with altered Ca^{2+} homeostasis and senescent physiology, thought to under lie cognitive decline (Foster, 2007; Kumar et al., 2009).

3.2. LTD

Relative to LTP, induction of LTD requires a modest rise in Ca^{2+}, which is usually induced by 1–3 Hz low frequency stimulation (LFS) or paired-pulse LFS (PP-LFS) using a 50 ms interval between pulse pairs (Fig. 3B). In the hippocampal CA1 region, several mechanisms for induction of LTD have been characterized and these mechanisms involve different Ca^{2+} sources. Again it must be emphasized that in the absence of specific blockers, all sources are likely to contribute to the induction of LTD. The dysregulation of Ca^{2+} homeostasis during aging results in an increased susceptibility to LTD induction (Foster and Kumar, 2007; Foy et al., 2008; Hsu et al., 2002; Norris et al., 1996), in the absence of a change in the maximum LTD amplitude (Kumar et al., 2007). Due to the age-related shift in Ca^{2+} sources, NMDARs appear to contribute less to LTD with advanced age. Thus, NMDAR antagonists reduce but do not block induction of LTD in older animals (Ahmed et al., 2011; Norris et al., 1996) and there is an increased reliance on VDCCs (Norris et al., 1996) and ICS (Kumar and Foster, 2005) for LTD induction. Another form of LTD can be induced by activation of mGluRs (Oliet et al., 1997; Palmer et al., 1997) and the susceptibility to this form of LTD also increases with age (Kumar and Foster, 2007c).

The characterization of age-related modifications in Ca^{2+} homeostasis has provided a deeper level of understanding concerning the interaction of the cellular and molecular components that regulate LTP and LTD. The next section discusses the potential role of NMDARs and VDCCs in mediating deficits in consolidation of rapidly acquired memory in middle-age and impairments in incremental learning with advanced age.

4. Age-related deficits in consolidation of rapidly acquired memories: relation to NMDAR function

Consolidation of memory from a short-term form, lasting seconds, to an intermediate-term form lasting hours is thought to depend on NMDAR activity (Jerusalinsky et al., 1992; Kim et al., 1992; Newcomer and Krystal, 2001; Packard and Teather, 1997; Roberts and Shapiro, 2002; Rossato et al., 2004; Shimizu et al., 2000; Steele and Morris, 1999). Early on, it was recognized that treatment with NMDAR antagonists impaired spatial memory (Butcher et al., 1990; Handelmann et al., 1987; McLamb et al., 1990; Mondadori et al., 1989; Morris, 1989; Morris et al., 1986, 1990a). Later research provided evidence that NMDAR blockade can result in an initial disruption in the acquisition of spatial information; however, this initial impairment may not be observed with continued training (Bannerman et al., 1995; Kesner et al., 2004; Steele and Morris, 1999). Rather, a certain level of NMDAR activity is required for the rapid consolidation of flexible spatial information (Jerusalinsky et al., 1992; Lee and Kesner, 2002; Steele and Morris, 1999). Furthermore, the role of NMDARs in acquisition and retention of spatial information appears to be region specific. Blockade of CA3 NMDARs contributes to the initial impairment in spatial working memory as the environment or task demands change (Kesner et al., 2004). Importantly, improved performance is observed with continued training suggesting non-NMDAR-dependent plasticity mechanisms may compensate for loss of NMDAR function in CA3 to reorganize or store spatial information. In contrast, blockade of NMDARs in region CA1 results in impairment of intermediate-term spatial memory (Kesner et al., 2004). Unlike the recovery of the initial learning impairment, the intermediate memory deficit did not improve with continued training, indicating that other plasticity mechanism cannot compensate for a loss of CA1 NMDARs to rescue memory consolidation.

Comparison of water maze performance during NMDAR blockade and aging supports the idea that a decline in NMDAR function could mediate the memory consolidation deficits which are an early indication of cognitive decline. An effect of NMDAR blockade on retention can be demonstrated by measuring the savings in latency to find a hidden platform on the second (i.e. retention) trial during repeated acquisition training. When the inter-trial interval is short (15 sec), rats treated with the NMDAR blocker AP5 exhibit acquisition similar to saline treated animals, decreasing the latency to find a hidden platform on the second training trial. Similar to older animals (Bizon et al., 2009), this savings is reduced if the inter-trial interval is increased (Steele and Morris, 1999). It may be important to point out that, for these studies, the spatial representations were established by prior training. It appears that aging and NMDAR blockade do not influence the ability to use or reorganize established representations; rather there is impaired maintenance of newly acquired information.

Intact acquisition and impaired memory consolidation during NMDAR blockade is apparent when the initial training is massed into a single day. In this case, repeated trials with relatively short inter-trial intervals permit rats to acquire a spatial search strategy in a novel environment under NMDA receptor blockade. However, like aged animals (Blalock et al., 2003; Foster et al., 1991; Foster and Kumar, 2007; Norris and Foster, 1999), impaired retention was observed over a 24 h period after training (Ge et al., 2010; Holahan et al., 2005; Kesner and Dakis, 1995; McDonald et al., 2005). Together, the results indicate that aged animals and animals under NMDAR blockade can acquire novel spatial information; however, consolidation deficits are evident if this information needs to be maintained.

The effect of NMDAR blockade on distributed training depends on the same factors that influence performance in aged animals, including sensory-motor function, prior training, and the number of training trials per day. Prior training facilitates incremental learning in aged animals (Colombo and Gallagher, 2002; Hansalik et al., 2006; van Groen et al., 2002) and during NMDAR blockade (Bannerman et al., 1995; Saucier et al., 1996). Similar to aged animals (Lindner, 1997), animals under NMDAR blockade fail to acquire a spatial search strategy when the number of training trials is reduced to one trial per day (Bannerman et al., 1995). This deficit may be overcome to a certain extent by increasing the number of trials per day. In studies using four to six trials per day, the antagonist AP5 resulted in a dose dependent impairment in acquisition performance (Davis et al., 1992; Uekita and Okaichi, 2009). The highest doses completely blocked learning; however, higher doses also resulted in sensory-motor disturbances and thigmotaxic behavior. Similar dose dependent effects on learning and sensory-motor function are observed for other NMDAR antagonists (Ahlander et al., 1999; Saucier et al., 1996). For doses within the range that normally block the induction of LTP, some impairment in learning was observed; nevertheless, a probe trial at the end of training indicated that search behavior was focused on the goal quadrant (Davis et al., 1992). Treatment with ifenprodil, which is a specific antagonist for the NR2B subunit of the NMDAR, did not block incremental acquisition of spatial learning over eight days of training (Ma et al., 2010). However, treatment for three days following training was associated with impaired memory consolidation (Ma et al., 2010). Thus, similar to aged animals, impaired incremental learning following NMDAR blockade is associated with sensory-motor impairment, suggesting the involvement of other brain regions. For lower doses that do not impair sensory-motor function, incremental learning can occur

during NMDAR blockade; however, impaired memory consolidation is observed. Incremental spatial learning in the face of NMDAR antagonist treatment may result from incomplete receptor blockade which slows rather than blocks learning. Alternatively, NMDAR-independent mechanisms may compensate to permit acquisition of a spatial reference memory following multiple days of distributed training.

The NMDAR is composed of heteromeric subunits involving an essential NR1 subunit and four other possible subunits. Mutant mice have been developed to enable the selective inducible knockout of specific subunits and in some cases the knockout is specific to the hippocampus or hippocampal subregions. Mice with a selective knockout of the NR1 subunit in region CA3 or the dentate gyrus are able to acquire and recall a spatial reference memory during distributed training (McHugh et al., 2007; Nakazawa et al., 2002). However, deficits are observed for repeated acquisition or matching to sample training indicating impaired short-term memory for novel spatial information (Nakazawa et al., 2003). Knockout of CA1 NR1 prior to training severely impairs LTP induction and incremental learning (Shimizu et al., 2000). Furthermore, when CA1-NR1knockout was initiated at the end of incremental training a memory consolidation deficit was observed. The results are consistent with work examining region specific delivery of NMDAR antagonists (Kesner et al., 2004), pointing to CA3 NMDAR involvement in rapid acquisition and spatial working memory and CA1 NMDAR involvement in spatial information that is maintained for an intermediate period lasting several minutes or longer-term memory consolidation.

The results suggest that a reduction in NMDAR function with age could have differential effects on learning and memory depending on the regions and extent of NMDAR decline. In this regard, studies that knockout the other subunits may be enlightening since, like aging, NMDAR function is reduced, but not eliminated. For example, disruption of NR2A, limited to the hippocampus, impaired short-term spatial working memory and no deficit was observed for incremental learning on the water maze (Bannerman et al., 2008). Indeed, mice with disruption of the NR2A subunit throughout the brain exhibit only a mild impairments in LTP and the acquisition of a spatial reference memory when training is distributed across multiple days (Sakimura et al., 1995). Widespread disruption of NR2B throughout the forebrain resulted in impaired incremental spatial learning and impaired acquisition on the cue discrimination task (von Engelhardt et al., 2008). When disruption of NR2B was limited to the hippocampus, mice exhibited reduced LTP for a pairing protocol and impaired short-term spatial working memory; however, incremental acquisition of a spatial reference memory was identical to that of controls (von Engelhardt et al., 2008). Interestingly, overexpression of NR2B improved the induction of LTP, learning, and memory in aged mice (Cao et al., 2007). Finally, a study employing antisense techniques in rats, found that decreased expression of hippocampal NR2B mimicked aging effects on the incremental learning task. Specifically, the impairment was observed early in training and differences diminished following further training (Clayton et al., 2002). The results suggest that an intermediate-term memory and memory consolidation are sensitive to reduced hippocampal NMDAR function and that incremental learning impairments are only observed following a substantial loss of NMDAR function in region CA1 or a wide spread decline in NMDAR function that impairs sensory-motor function. Together the behavioral data support the idea that the onset of age-related impairment in intermediate-term memory could result from a decrease in hippocampal NMDAR function, leaving incremental learning mechanisms intact.

Is impaired NMDAR-LTP related to memory deficits? Evidence is mounting to indicate a relationship between the appearance of memory deficits, a decline in NMDAR function, and altered synaptic plasticity. Impaired induction of LTP can be observed in middle-age and LTP magnitude is related to cognitive function of middle-aged animals (Brunson et al., 2005; Fouquet et al., 2009; Rex et al., 2005). We recently observed a decrease in NMDAR function, limited to those middle-aged animals that exhibit retention deficits on the water maze following a single training session (Kumar et al., 2011a). For studies that examine aged animals, the LTP magnitude following weak stimulation that would induce NMDAR-LTP (50 pulses at 100 Hz) correlated with retention examined 24 hr after 4 days of training (Deupree et al., 1991) and treatment to enhance NMDAR function improved TBS-induced LTP and performance on the repeated acquisition version of the water maze (Burgdorf et al., 2011). Thus, the weight of evidence supports the idea that the emergence of memory consolidation deficits is associated with a decline in NMDAR function and NMDAR-LTP.

The age-related decline in NMDAR function does not appear to correlate with impairment of incremental learning and acquisition of a spatial reference memory (Boric et al., 2008). This may not be surprising since the establishment of a spatial reference memory, acquired by incremental learning, is observed for many pharmacological or genetic conditions that decrease hippocampal NMDAR function (Bannerman et al., 2008, 2006). In one study, LTP induced by 5 Hz, but not 30 or 70 Hz stimulation correlated with learning across five days of training (Tombaugh et al., 2002). While these researchers found that NMDAR synaptic responses were decreased with age, impaired incremental learning was not related to the NMDAR synaptic responses. The authors suggest that the relationship between LTP for 5 Hz stimulation and incremental learning may be due to a larger sAHP in impaired animals. Importantly, 5 Hz stimulation would result in synaptic activation arriving within a window for the peak of the sAHP from the preceding stimulation induced action potential (Fig. 5). In this case, the decrease in NMDAR activation may be enhanced for patterned stimulation that falls within the window of a larger sAHP. The sAHP amplitude is increased in aged animals that exhibit impaired incremental learning (Oh et al., 2010; Tombaugh et al., 2005) and reduction of the sAHP facilitates learning and LTP induction by 5 Hz stimulation in aged animals (Disterhoft et al., 1996; Kumar and Foster, 2004; Norris et al., 1998). Together, the results indicate that the initial memory deficits of senescence are associated with a decline in NMDAR function, including induction of NMDAR-LTP. However, incremental learning deficits observed in advance age are not directly related to the decline in NMDAR function.

5. VDCC-dependent synaptic plasticity and incremental learning

The involvement of L-type Ca^{2+} channels and VDCC-dependent synaptic plasticity in memory is complex and may depend on the brain region examined. It is important to note that L-channel antagonists have effects opposite that of NMDAR antagonists on hippocampal-dependent memory. In young animals, VDCC blockade facilitates retention of inhibitory avoidance and spatial memory, and facilitates the rate of acquisition of the radial arm maze (Batuecas et al., 1998; Kim et al., 2011; Levy et al., 1991; Quartermain et al., 2001, 1993; Quevedo et al., 1998). Furthermore, the improved retention following L-channel blockade appears to be specific for the hippocampus (Kim et al., 2011; Quevedo et al., 1998). Similar improvement in retention of hippocampal-dependent memory following L-channel blockade has also been observed for aged animals. In fact, L-channel antagonists ameliorate or prevent age-related memory decline across several species including humans (Ban et al., 1990; Deyo et al., 1989; Ingram et al., 1994; Levere and Walker, 1992; Riekkinen et al., 1997; Rose

et al., 2007; Sandin et al., 1990; Solomon et al., 1995; Trompet et al., 2008; Veng et al., 2003; Woodruff-Pak et al., 1997). The results indicate that the beneficial effects of L-channel blockade on memory are specific for hippocampal-dependent memory, possibly mediated through a reduction in the sAHP (Disterhoft et al., 1996), facilitation of NMDAR-LTP, or inhibition of LTD (Kumar and Foster, 2004; Norris et al., 1998).

In contrast to facilitation of hippocampal-dependent memory, mounting evidence indicates that blockade of L-channels impairs incremental learning and the consolidation of remote memories in the neocortex. The L-channel antagonist nifedipine, but not the NMDAR antagonist AP5, blocked incremental associative learning on an olfactory discrimination task (Zhang et al., 2010). Comparison of NMDAR and VDCC antagonists on performance of the radial 8-arm maze confirmed that NMDAR blockade impairs rapidly acquired spatial memory. In contrast, animals under VDCC blockade acquired the task to the same extent as controls, but exhibited a deficit in spatial reference memory when retested 7–10 days later (Borroni et al., 2000; Woodside et al., 2004). Furthermore, mice with knockout of L-type VDCCs exhibit intact spatial learning on the water maze; however, retention deficits were observed when examined 30 days after training, indicating disruption of processes for remote memories (McKinney and Murphy, 2006; White et al., 2008), which are thought to be processed in the neocortex (Quinn et al., 2008; Teixeira et al., 2006; Wiltgen et al., 2004). Together with the fact that young rats with hippocampal lesions continue to exhibit incremental acquisition of a spatial reference memory, the results suggest preserved incremental learning in aged rats may involve VDCC-dependent synaptic plasticity in neocortical regions.

Before discussing the relationship between VDCC-plasticity and incremental learning during aging, it is important to point out that there are several parallels between the effects of behavioral stress and aging on memory and synaptic plasticity. As such, the level of stress associated with training and the time between training and examination of synaptic plasticity is important in determining the relationship between synaptic plasticity and memory. An acute stress, including a single day of water maze training, can result in impaired induction of LTP and enhanced induction of LTD lasting hours or days (Kavushansky et al., 2006; Li et al., 2005; Shors et al., 1997; Xu et al., 1997). Stress hormones have a direct effect on NMDAR function (Ooishi et al., 2011) and the sAHP amplitude is influenced by stress hormones, which in turn can influence the induction of synaptic plasticity (Diamond et al., 1992; Joels and de Kloet, 1991; Weiss et al., 2005). Treatments such as environmental enrichment will protect memory, the sAHP, and synaptic plasticity from mild stressors and aging (Kumar and Foster, 2007a; Kumar et al., 2011b; Sierra-Mercado et al., 2008; Yang et al., 2006). Acute stressor effects can be minimized by examining synaptic plasticity several days after a single training session and results indicate no relation between acute stress for a single day of water maze training and altered synaptic plasticity during aging (Foster and Kumar, 2007). In contrast, exposure to stress for several days can impair LTP and enhance LTD for months (Artola et al., 2006; Holderbach et al., 2007; Sterlemann et al., 2010). Furthermore, chronic stress modifies L-channel function and VDCC-synaptic plasticity, (Foster and Kumar, 2007; Liebmann et al., 2008; Mamczarz et al., 1999; Ryan et al., 2010; van Gemert and Joels, 2006). Thus, stress associated with training across multiple days could have enduring effects on synaptic plasticity, particularly in learning impaired animals in which training may be analogous to a being exposed to an uncontrollable swim stress.

A handful of studies have examined the relationship between VDCC-dependent synaptic plasticity and behavior in aged animals. As detailed above, age-related changes in Ca^{2+} regulation include diminished NMDAR function and increased Ca^{2+} from VDCC-ICS. The increase VDCC-ICS component is thought to underlie the increase susceptibility to LTD induction in aged animals (Kumar and Foster, 2007c; Kumar et al., 2007; Norris et al., 1998). The increased susceptibility to LTD is correlated with forgetting examine 24 h after a single training session on the spatial version of the water maze (Foster and Kumar, 2007). Moreover, the level of LTD was not correlated with the level of stress measured as the amount of time swimming in the pool. The results suggest that the decline in intermediate-term memory is associated with a shift from NMDAR-dependent to VDCC-ICS-dependent regulation of synaptic plasticity. Other studies have examined VDCC-synaptic plasticity in animals characterized as impaired or unimpaired for incremental acquisition of a spatial reference memory following 8–9 days of distributed training. In one study, researchers induced LTP using 200 Hz stimulation (Schulz et al., 2002), which should favor VDCC-LTP in aged animals (Shankar et al., 1998). No difference in LTP was observed between aged impaired and unimpaired animals; however, a correlation was observed between LTP and behavior for aged unimpaired animals (Schulz et al., 2002). The results suggest a possible relationship between VDCC-LTP and incremental learning in animals that are able to acquire the task. The authors suggest that the lack of correlation for impaired learners may be due to the severity of impairment (Schulz et al., 2002). Thus, aging of systems involved in incremental learning may have precipitated a complete absence of learning in impaired animals. Another series of studies used AP5 to block NMDARs in order to examine VDCC-dependent synaptic plasticity (Boric et al., 2008; Lee et al., 2005). The researchers confirmed an age-related decrease LTP induced by TBS and TBS-induced LTP did not correlate with incremental learning in aged rats. In the presence of AP5, VDCC-dependent synaptic plasticity was enhanced in aged animals that exhibited intact incremental acquisition of a spatial reference memory. The authors suggest that VDCC-dependent synaptic plasticity may compensate for loss of NMDAR-dependent synaptic plasticity in order to preserve acquisition of a spatial reference memory. Together, the results indicate that VDCC-synaptic plasticity is intact in aged animals that can acquire a spatial reference memory. Further investigations using treatments to inhibit or enhance VDCC function may be able to determine whether VDCC-synaptic plasticity contributes to preservation of incremental learning in aged animals or whether increased stress associated with an inability to escape from the water underlies the correlation of VDCC-dependent synaptic plasticity and incremental learning.

As discussed above, in older animals, L-channel blockers improve the rapid acquisition and consolidation of intermediate-term memory that depends on hippocampal NMDARs. However, studies in younger animals indicate that L-channel blockers may impair the storage of remote memories. Thus, there may be a trade off for treatments designed to improve memory during aging through L-channel blockade. While not specifically examined, one study provides evidence for this idea. The study examined the influence of nimodipine treatment on water maze performance over 4 days of training (two trials per day) (Riekkinen et al., 1997). Untreated aged animals (22 months of age) were impaired relative to young (6 months). Nimodipine treatment facilitated acquisition in aged rats, consistent with the idea that L-channel blockade can improve learning and intermediate-term memory. Animals were then re-tested 30 days after drug washout. Young animals exhibited long-term memory observed as carry-over effects of previous training, with improved re-learning on the maze relative to original training. In contrast to young animals, aged animals, treated with nimodipine, which previously exhibited superior spatial learning, did not exhibit a carry-over advantage during retesting. The lack of a carry-over effect for aged animals is in contrast to other studies that indicate previous

learning facilitates acquisition on subsequent retesting (Colombo and Gallagher, 2002; Hansalik et al., 2006; van Groen et al., 2002). Rather, the absence of carry-over effects is consistent with work indicating that VDCC-dependent synaptic plasticity is necessary for the storage of long-term or remote memories (Borroni et al., 2000; Lashgari et al., 2006; Seoane et al., 2009; White et al., 2008; Woodside et al., 2004). For example, delivery of Ca^{2+} channel blockers to young rats impaired long-term reference memory in the absence of learning deficits (Borroni et al., 2000; Woodside et al., 2004). Thus, blockade of VDCCs may improve hippocampal and NMDAR-dependent memory in aged animals at the expense of long-term memory. This idea remains to be tested. If true, then our ideas about treating age-related memory decline with L-channel antagonists may need to be reevaluated.

6. Conclusion

Several advances have been made which refine ideas concerning Ca^{2+} dysregulation in mediating age-related changes in synaptic plasticity and memory (Foster, 1999; Foster and Norris, 1997). NMDAR-dependent and VDCC-dependent mechanisms are likely to interact cooperatively in young animals. Hippocampal NMDAR-dependent mechanisms mediate intermediate-term memory for rapidly acquired information. In turn, intermediate-term memory may contribute to incremental learning and consolidation of spatial reference memories involving VDCC-dependent mechanisms and the neocortex. The decline in NMDAR function and increase in Ca^{2+} from ICS in the hippocampus during aging are thought to underlie the initiation of memory decline. Thus, memory consolidation deficits, increased sAHP, and impaired NMDAR function, including impaired induction of NMDAR-LTP, provide early markers of cognitive decline. The inability to acquire a spatial reference memory through incremental learning manifests later and suggests a decline in other processes or the involvement of other brain regions. It will be important to continue to develop more sophisticated paradigms for early detection of cognitive aging, as well as documenting the mechanisms for changes in synaptic plasticity. Future studies may want to determine if cognitive deficits are progressive. Do memory consolidation impairments in middle-age predict the extent of rapid acquisition or incremental learning deficits?

The translational relevance of animal models will depend on the development of treatments for age-related memory impairments that depend on proper hippocampal function. Several recent reviews have addressed possible mechanisms which increase the vulnerability of the hippocampus to aging and cognitive decline (Burger, 2010; Foster, 2007; Kumar et al., 2009; Lynch, 2009; Magnusson et al., 2010; Oh et al., 2010; Penner et al., 2010; Potier et al., 2010; Schimanski and Barnes, 2010; Schneider et al., 2010; Wang and Michaelis, 2010). Most hypotheses include Ca^{2+} dysregulation and increased oxidative stress as a component of aging. The results from studies examining the effect of redox-active agents on neural physiology indicate that an age-related shift in redox state underlies the pattern of altered Ca^{2+} homeostasis, reducing NMDAR function and increasing Ca^{2+} from ICS, decreasing cell excitability, leading to alter synaptic plasticity processes that are critical for memory. Due to the interaction of NMDARs, RyR-ICS, and redox state in regulating synaptic plasticity (Fig. 4), treatments may want to focus on restoring intracellular redox state in order to reverse the age-related shift in Ca^{2+} sources. In this regard, it will be important to identify the source for the shift in redox state. Metabolic changes and increased inflammation during aging provide likely candidates for increased oxidative stress. Recent studies indicate that region CA1 is more susceptible to an age-related increase in inflammation and oxidative stress and exhibits reduced neurotrophic and pro-survival signaling (Jackson and Foster, 2009; Jackson et al., 2010; Zeier et al., 2010). Combined with studies demonstrating that NMDARs in region CA1 are involved in memory consolidation (Daumas et al., 2005; Kesner et al., 2004; Lee and Kesner, 2002), the increased susceptibility to oxidative stress in region CA1 and subsequent decrease in NMDAR function could explain the emergence of memory consolidation deficits in middle-age, as an early sign of age-related cognitive decline.

Acknowledgements

This work was supported by National Institutes of Aging Grant AG014979, AG037984, AG036800, and the Evelyn F. McKnight Brain Research Foundation.

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Brain, Behavior, and Immunity xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Named Series: Neurogenesis & Inflammation

Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Adult neurogenesis
Hippocampus
Running
Cytokine
Chemokine
Biomarker
Learning
Memory
Water maze
Fisher 344
Bio-Plex

ABSTRACT

We tested whether daily exercise modulates immune and neuroimmune cytokines, hippocampus-dependent behavior and hippocampal neurogenesis in aging male F344 rats (18mo upon arrival). Twelve weeks after conditioned running or control group assignment, the rats were trained and tested in a rapid water maze followed by an inhibitory avoidance task. The rats were BrdU-injected beginning 12 days after behavioral testing and killed 3 weeks later to quantify cytokines and neurogenesis. Daily exercise increased neurogenesis and improved immediate and 24 h water maze discrimination index (DI) scores and 24 h inhibitory avoidance retention latencies. Daily exercise decreased cortical VEGF, hippocampal IL-1β and serum MCP-1, GRO-KC and leptin levels but increased hippocampal GRO-KC and IL-18 concentrations. Serum leptin concentration correlated negatively with new neuron number and both DI scores while hippocampal IL-1β concentration correlated negatively with memory scores in both tasks. Cortical VEGF, serum GRO-KC and serum MCP-1 levels correlated negatively with immediate DI score and we found novel positive correlations between hippocampal IL-18 and GRO-KC levels and new neuron number. Pathway analyses revealed distinct serum, hippocampal and cortical compartment cytokine relationships. Our results suggest that daily exercise potentially improves cognition in aging rats by modulating hippocampal neurogenesis and immune and neuroimmune cytokine signaling. Our correlational data begin to provide a framework for systematically manipulating these immune and neuroimmune signaling molecules to test their effects on cognition and neurogenesis across lifespan in future experiments.
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1. Introduction

Developing novel strategies to protect cognition in our burgeoning elderly population is critical for managing the burden and cost of its care. Hippocampal neurogenesis is a form of plasticity that declines significantly with age in rodents (Bizon et al., 2004; Dupret et al., 2008; Kuhn et al., 1996), dogs (Siwak-Tapp et al., 2007) and non-human primates (Aizawa et al., 2009; Gould et al., 1999b) primarily because the neural progenitor cell (NPC) precursors of new neurons and glia become increasingly quiescent with age (Cameron and McKay, 1999). The abundance of neurons added daily to the young mammalian hippocampus (Cameron and McKay, 2001) suggests that neurogenesis contributes to hippocampal integrity and indeed, measures of neurogenesis and ability in hippocampus-dependent tasks generally relate in young mammals (Deng et al., 2010; but see Epp et al., 2011; Gould et al., 1999a). Measures of neurogenesis have been related to measures of performance in hippocampus-dependent tasks among aged dogs (Siwak-Tapp et al., 2007), aged non-human primates (Aizawa et al., 2009) and when an experimental manipulation introduces enough variability into both measures to detect the relationship in aged rats (Bizon et al., 2004; Dupret et al., 2008; Kempermann et al., 2002; Speisman et al., 2012). Combined, these data suggest that protecting hippocampal neurogenesis from the effects of age may also protect some forms of cognition.

Experimental manipulations that produce neuroimmune responses can impair hippocampal neurogenesis and cognition. For example, systemic or central bacterial lipopolysaccharide (LPS) injections activate microglia, potently block neuronal differentiation (Ekdahl et al., 2003; Monje et al., 2003) and disrupt the integration of young neurons into existing hippocampal circuitry (Belarbi et al., 2012). Of the cytokines known to be stimulated by LPS (see Erickson and Banks, 2011), only a handful have been shown to affect *in vivo* or *in vitro* neurogenesis (Ben-Hur et al., 2003; Buckwalter et al., 2006; Grotendorst et al., 1989; Liu et al., 2009; Lum et al., 2009; Monje et al., 2003; Qin et al., 2008; Turrin et al., 2001; Vallieres et al., 2002; Villeda et al., 2011). In humans, experimental

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0889-1591/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.bbi.2012.09.013

LPS impairs verbal and non-verbal memory (Reichenberg et al., 2001), but confirming its effects on neurogenesis awaits technology that permits the visualization of neurogenesis in the living brain. However little, if any, evidence of hippocampal neurogenesis is detected in the post-mortem tissue of patients who exhibited profound memory loss after γ-irradiation therapy, which also stimulates neuroimmune signaling (Coras et al., 2010; Correa et al., 2004; Crossen et al., 1994; Monje et al., 2007). The deleterious effects of LPS and γ-irradiation on hippocampal neurogenesis in rodents can be blocked by non-steroidal anti-inflammatory treatment (Monje et al., 2003; Rola et al., 2008; Tan et al., 2011), confirming a role for downstream immune and/or neuroimmune signaling cascades in mediating the effects of these treatments on neurogenesis.

In aged rodents, systemic or central LPS administration stimulates exaggerated microglial responses, cytokine levels and memory impairment (Barrientos et al., 2006; Chen et al., 2008; Godbout et al., 2005; Xu et al., 2010). In fact, the transcription of neuroimmune molecules is upregulated categorically with age but most robustly in aged rodents that exhibit impaired performances across hippocampus-dependent tasks (Blalock et al., 2003; Kohman et al., 2011a). Whole brain preparations have revealed that the concentrations of some cytokines that increase with age in rodents also associate negatively with measures of long-term potentiation and spatial ability (Felzien et al., 2001; Griffin et al., 2006; Prechel et al., 1996; Ye and Johnson, 1999). In aged and aging humans, increased circulating immune cytokine concentrations have been linked to cognitive impairments (Gimeno et al., 2008; Krabbe et al., 2009, 2004; Magaki et al., 2007; Rachal Pugh et al., 2001; Rafnsson et al., 2007; Weaver et al., 2002). In a recent study, Villeda and colleagues elegantly narrowed a list of 17 potential circulating cytokines (of 66 examined) down to 6 that related to age-impaired in neurogenesis and cognition. They then showed that increased circulating eotaxin concentrations alone compromise neurogenesis, synaptic plasticity and memory across hippocampus-dependent tasks (Villeda et al., 2011). These data highlight that the systematic testing of circulating and central cytokine biomarker correlates of neurogenesis and cognition can reveal mechanistic candidates. Importantly, these candidates can include hypoactive or senescent immune and neuroimmune cytokine signaling, particularly in aged rats (Conde and Streit, 2006; Ziv et al., 2006).

Elderly humans who exercise regularly exhibit better scores on cognitive tests and have larger hippocampal volumes relative to sedentary elderly humans (Christensen and Mackinnon, 1993; Churchill et al., 2002; Colcombe and Kramer, 2003; Erickson et al., 2010). Young and aged rodents that exercise daily on a running wheel exhibit enhanced measures of plasticity that include neurogenesis and long-term potentiation and better performances on hippocampus-dependent tasks (Brown et al., 2003; Creer et al., 2010; Kronenberg et al., 2003; Kumar et al., 2012; Lambert et al., 2005; Lugert et al., 2010; Madronal et al., 2010; Steiner et al., 2008; Suh et al., 2007; van Praag et al., 1999; van Praag et al., 2002, 2005). In young rats that run voluntarily, increased levels of neurogenesis are associated with reduced hippocampal IL-1β levels (Chennaoui et al., 2008; Farmer et al., 2004; Leasure and Decker, 2009; Stranahan et al., 2006), suggesting that physical activity may stimulate plasticity and improve cognition by modulating neuroimmune signaling pathways. There is even evidence in aged mice that cognition and immune system signaling can be modulated by physical exercise (Kohman et al., 2011a, 2011b). Therefore, we tested the effects of conditioned wheel running on the rapid acquisition and retention of a water maze hidden platform location, inhibitory avoidance acquisition and retention, hippocampal neurogenesis and 24 immune and neuroimmune cytokine concentrations in aging F344 rats. We expected that conditioned runners would exhibit better learning and memory indices and have higher rates of neurogenesis than control rats. We also expected that conditioned runners might have altered levels of immune and/or neuroimmune cytokines that may relate to measures of hippocampal integrity and/or hippocampal neurogenesis.

2. Methods

2.1. Subjects

All rat subjects were treated in accordance with University of Florida and federal policies regarding the humane care and use of laboratory animals. Upon arrival, sexually naïve male Fischer 344 rats (18 mo; $n = 12$) purchased from the National Institute of Aging colony at Harlan Sprague Dawley Laboratories (Indianapolis, IA) were housed individually in corn cob bedding-lined hanging shoebox cages located in a colony room maintained on a 12:12 h light: dark cycle at 24 ± 1 °C. The rats were given access to Harlan Teklad Rodent Diet #8604 and water *ad libitum*. All rats were weighed weekly and checked daily to ensure that they did not exhibit age-related health problems including (but not limited to) poor grooming, reduced food and water intake, excessive porphyrin secretion or weight loss.

One week after arrival, the rats were assigned randomly to the conditioned runner or control group ($n = 6$ per group). Control rats were maintained individually in standard laboratory cages with access to food and water *ad libitum* for the 18 weeks-long duration of the experiment while runners were conditioned to run for food to prevent the well-documented decreases in running behavior exhibited by aged rats across weeks of an experiment (Cui et al., 2009; Holloszy et al., 1985; Kumar et al., 2012). Therefore, runners were housed individually in a chamber containing a running wheel (model H10-38R, Coulbourn Instruments, Allentown, PA) on which they could run for unlimited food (Kumar et al., 2012). A Graphic State Notation computer program (Version 3.02, Coulbourn Instruments, Allentown, PA) recorded wheel rotations and was programmed to deliver 45 mg food pellets (Harlan Teklad Rodent Diet #8604) based upon wheel rotations. The frequency of 45 mg food pellet delivery was decreased from 1 pellet per rotation at the beginning of conditioning to 1 pellet per 3–4 m by ~4 weeks. By the 8th week of conditioning, all runners consistently ran ~ 4 km per week. If a conditioned runner lost more than 10% of the weight expected based on their pre-conditioning baseline and the weight changes of the control rats, the number of wheel rotations required for food delivery was reduced. Note that the body masses of conditioned runners (418.52 ± 5.12 g) were similar to controls (414.26 ± 5.26 g) at the beginning of the experiment ($t_{(10)} = -0.45$; $p = 0.66$) and tended to be smaller (357.97 ± 12.79 and 417.50 ± 33.41 g, respectively) at the end of the experiment ($t_{(10)} = 1.97$; $p = 0.08$). The experiment timeline is depicted in Fig. 1.

2.2. Water maze training and testing

Each rat was trained and tested in a black water maze tank (1.7 m diameter) housed in a well-lit room. The tank was filled with water (27 ± 2 °C) to a depth of 8 cm below the tank rim. A Columbus Instruments tracking system (Columbus, OH) was used to record latencies (s), pathlengths (cm), % time spent in the outer annulus of the maze and platform crossings. Rats were initially habituated to the pool on three trials during which they were released from different pool locations and allowed to climb onto a visible platform. Rats were dried with towels and warm air between blocks and before being returned to their home cages.

2.2.1. Visible platform training

Beginning the 13th week of the experiment, the rats were trained in 5 blocks of 3 60 s visible platform trials (15 min inter-block

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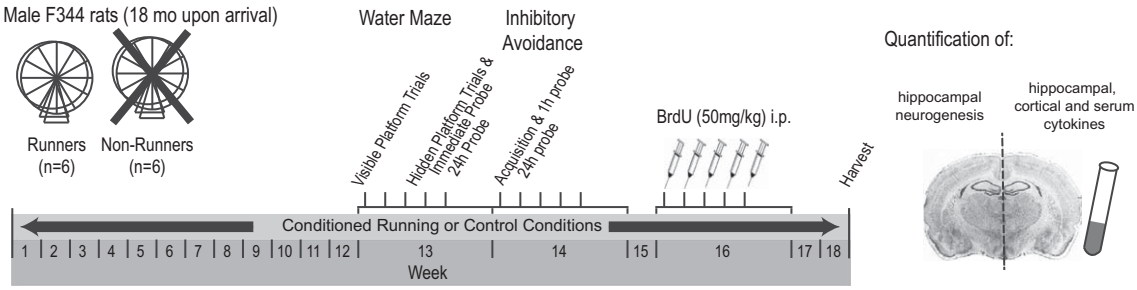

Fig. 1. Experiment timeline. Male F344 rats (18 mo) were assigned randomly to either a conditioned running group that voluntarily ran for food for the entire 18 weeks-long experiment or a sedentary control group fed *ad libitum*. All rats underwent water maze training and testing during the 13th week followed by inhibitory avoidance training and testing during the 14th week. During the 16th week, the rats were BrdU-injected (50 mg/kg/day; i.p.) daily for 5 days and then killed at the end of the 18th week to quantify 24 immune and neuroimmune cytokine simultaneously with hippocampal neurogenesis.

interval [IBI] and 20 s inter-trial interval [ITI]) that require intact procedural and sensorimotor ability (Vorhees and Williams, 2006). The flagged platform (29 cm diameter) protruded 1.5 cm from the water surface and the pool was surrounded by a black curtain to mask distal cues. The platform location and N, S, E and W release points were randomized across trials. Rats failing to locate and climb onto the platform within the allotted 60 s were guided to the platform by the experimenter. One control rat was removed from the experiment after failing to locate the visible platform on $\geqslant 2$ trials over the last 2 blocks. Latencies (s) and pathlengths (cm) served as measures of procedural and sensorimotor ability, % time spent in the outer annulus served as a measure of anxiety and swim speed (cm/s) served as a measure of locomotor ability.

2.2.2. Hidden platform training

Three days after visible platform training, the rats were trained on five blocks of 3 60 s hidden platform trials (15 min IBI and 20 s ITI) that require intact spatial ability (Vorhees and Williams, 2006). This rapid water maze training protocol is sensitive to age-related cognitive decline and the effects of differential experience on spatial ability in aged rats (Carter et al., 2009; Foster and Kumar, 2007; Foster et al., 2003; Kumar et al., 2012; Speisman et al., 2012). The platform was hidden 1.5 cm below the water surface in the center of the NE quadrant of the water maze now surrounded by highly visible distal cues. N, S, E and W release points were randomized across each trial. Rats that failed to locate and climb onto the platform within the allotted 60 s were guided to the platform by the experimenter before being removed from the maze. Latencies (s) and pathlengths (cm) served as measures of spatial ability, % time spent in the outer annulus served as a measure of anxiety and swim speed (cm/s) served as a measure of locomotor ability.

2.2.3. Immediate and delayed probe trials

The escape platform was removed from the water maze in probe trials administered immediately or 24 h after the last hidden platform training trial to test strength of learning and memory, respectively, for the platform location. In both probe trials, rats were released from the quadrant opposite to the goal quadrant for a 60 s free swim. A hidden platform trial block was administered after the first probe trial to reinforce the association between the platform localization and escape from the pool. The time (s) spent in each quadrant, platform location crossings and discrimination index (DI) scores $[(t(G) - t(O))/(t(G) + t(O))$, where $t(O)$ is time spent in the opposite quadrant and $t(G)$ is time spent in the goal quadrant] served as measures of strength of learning and memory in probe trials. DI scores take into account the quadrant to be approached (the "goal quadrant" [G]) and the quadrant to be avoided (the "opposite quadrant" [O]), and often produces a higher fidelity memory index for aged rats that frequently make wide sweeping turns while navigating by swimming.

2.3. Inhibitory avoidance training and testing

Beginning the 14th week, the rats were trained and tested in an inhibitory avoidance apparatus (Coulbourn Instruments, Allentown, PA) consisting of dark and lighted chambers with a shockable metal grid floor separated by a sliding door. During acquisition, the rat was placed in the lighted compartment for 90 s before the sliding door opened and latency to enter the dark compartment was recorded. Upon entry to the dark compartment, the door closed and a mild foot shock (0.21 mA for 3 s) was delivered 10 s later. The animal's behavioral responses (i.e. a jump or rapid movement) confirmed that they had experienced the shock. The rat was then returned its home cage before being returned to the lighted chamber for 90 s both 1 and 24 h later, and the time taken to enter the dark side after the door opened was recorded as a measure of memory. Retention latencies were set at 900 s for rats not entering the dark compartment within 15 min. Door opening, shock delivery and data acquisition was computer controlled.

2.4. Bromodeoxyuridine injections

We waited 15 weeks after the experiment onset and ~3 weeks after spatial learning before labeling dividing NPCs with the DNA synthesis marker bromodeoxyuridine (BrdU; Sigma Aldrich, St. Louis, MO) to measure the effects of long-term daily exercise on neurogenesis to minimize the well-known effects of spatial behavior on neurogenesis (Gould et al., 1999a; Epp et al., 2010). NPC proliferation is unaffected when BrdU is administered at the end of hippocampus-dependent learning (Gould et al., 1999a) and any latent effects of hippocampus-dependent behavior on new neurons produced 3 weeks earlier are possible but unexpected. Rats were injected intraperitoneally once per day over 5 days beginning 16 weeks after the experiment onset to label dividing cells. BrdU was dissolved in freshly prepared 0.9% isotonic sterile saline at a concentration of 20 mg/ml (w/v) just prior to use at a volume of 2.5 ml/kg (50 mg/kg/injection). This dose of BrdU labels dividing hippocampal NPCs safely and effectively in adult rodents (Cameron and McKay, 2001; Kolb et al., 1999).

2.5. Histology

At the end of the 18th week (21 d after the first BrdU injection), the rats were anaesthetized deeply with a ketamine (90 mg/kg)/xylazine (10 mg/kg) cocktail (Webster Veterinary Supply, Sterling, MA). Blood was collected from the left ventricle of the heart before rats were decapitated and their brains extracted rapidly.

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Hippocampi and frontal cortices were rapidly dissected from the left hemisphere, flash frozen and then stored at -86 °C until protein harvest for cytokine quantification. Although central cytokine levels in these unperfused rats could reflect circulating levels of diffusible cytokines we neither detected immune-to-brain cytokine clusters nor concentrations of individual cytokines that were affected by running similarly in the blood and brain that would validate this hypothesis. Similar masses of hippocampal ($t_{(10)} = 1.00$; $p = 0.34$) and cortical ($t_{(10)} = -0.01$; $p = 1.00$) tissue were collected from controls (79.70 ± 11.30 and 246.40 ± 21.95 mg, respectively) and conditioned runners (65.90 ± 8.05 and 246.60 ± 15.69 mg, respectively). Serum supernatant was collected from blood samples after refrigeration for 24 h at 4 °C and centrifugation at 1000*g* for 10 min at RT and then stored −86 °C until cytokine quantification. The right hemisphere of the brain was post-fixed overnight in freshly prepared 4% paraformaldehyde (Electron Microscopy Sciences; Hatfield, PA) and then equilibrated in 30% sucrose (∼4 days) at 4 °C, before being sectioned coronally through the dentate gyrus, beginning between ∼−1.72 and −1.92 mm posterior to bregma according to Paxinos and Watson (82) at 40 μm intervals on a freezing stage sledge microtome (Model 860; American Optical Corporation; IMEB Inc., San Marcos, CA). The six sets of every sixth section collected through the left side of the dentate gyrus were stored at −20 °C in a cryoprotectant solution of 30% ethylene glycol, 25% glycerin and 45% 0.1 M sodium phosphate buffer until processed immunohistochemically to quantify neurogenesis.

2.6. Protein harvest from brain tissue

Hippocampi and frontal cortices were thawed at 4 °C in 0.1 M Tris-buffered saline (TBS) containing 0.1% IGEPAL and 1 μl/ml each of 2 protease inhibitor cocktails added just prior to use. The first protease inhibitor cocktail contained 0.5 M phenylmethylsulfonyl fluoride, 5 mg pepstatin A and 1 mg chymostatin/ml DMSO and the second contained 1 M G-aminocapfroic acid, 1 M P-aminobenzidine, 1 mg leupeptin and 1 mg aprotinin/ml sterile water. Tissue was mashed manually and then sonicated using a dismembrator (ThermoFisher Scientific; Pittsburgh, PA). Tissue supernatant was collected by centrifugation (12,000 rpm for 10 min at 4 °C) and its protein concentration quantified using a Bradford protein assay and a Bio-Rad SmartSpec Plus Spectrophotometer (Hercules, CA). Similar total protein concentrations were harvested from the hippocampi ($t_{(10)} = -1.28$; $p = 0.23$) and cortices ($t_{(10)} = -0.07$; $p = 0.94$) of controls (0.93 ± 0.19 and 1.05 ± 0.06 mg/mL, respectively) and conditioned runners (1.20 ± 0.09 and 1.06 ± 0.09 mg/mL, respectively). Protein samples were stored at −86 °C until cytokine concentrations were quantified using Bio-Plex technology.

2.7. Immunohistochemistry

Hippocampal sections were stained immunohistochemically to quantify and phenotype new ($BrdU^+$) cells using methods previously described (Ormerod et al., 2003, 2004; Palmer et al., 2000; Speisman et al., 2012).

2.7.1. Enzyme substrate immunostaining

Before processing and between steps, free-floating hippocampal sections were washed repeatedly in Tris-buffered saline (TBS; pH 7.4). The sections were incubated in 0.3% H_2O_2 in TBS for 10 min at RT to quench endogenous peroxidase, rinsed in 0.9% NaCl and then incubated in 2 N HCl for 20 min at 37 °C to denature DNA. The sections were then blocked in a solution of 3% normal donkey serum (NDS) and 0.1% Triton-X in TBS (v/v) for 20 min and then incubated overnight in rat anti-BrdU (1:500; AbD Serotec, Raleigh, NC) at 4 °C. The next day, the sections were incubated in biotinylated donkey anti-rat IgG (Jackson ImmunoResearch, West Grove, PA; 1:500) for 4 h and then avidin–biotin horseradish peroxidase (PK-6100: Vector Laboratories, Burlingame, CA) for 2 h at RT. The horseradish peroxidase complex was then revealed by reaction with 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma Aldrich, St. Louis, MO) and 0.5% H_2O_2 in TBS. Sections were mounted on glass slides, dried overnight and dehydrated in an alcohol series prior to being cover-slipped under permount (Thermo Fisher Scientific, Pittsburgh, PA).

2.7.2. Fluorescent immunostaining

Sections were washed repeatedly between steps in TBS (pH 7.4). The sections were blocked in NDS solution and then incubated overnight at 4 °C in primary antibodies raised against the mature neuronal protein neuronal nuclei (mouse anti-NeuN, 1:500; Chemicon, Temecula, CA) and the immature neuronal protein doublecortin (goat anti-DCX, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA) or the oligodendrocyte precursor marker chondroitin sulfate proteoglycan (rabbit anti-NG2, 1:500; Chemicon, Temecula, CA) and the astrocyte/neural stem cell protein glial fibrillary acidic protein (chicken anti-GFAP, EnCor Biotech, Alachua, FL). The following day sections were incubated with maximally cross-adsorbed fluoroscien isothiocyanate (FITC)-conjugated anti-mouse and cyanine (Cy) 5-conjugated anti-goat secondary antibodies to reveal neurons or FITC-conjugated anti-rabbit and Cy5-conjugated anti-chicken secondary antibodies to reveal glia for 4 h at RT (all secondaries diluted at 1:500; Jackson ImmunoResearch, West Grove, PA). Sections were then fixed with 4% paraformaldehyde, rinsed in 0.9% NaCl, incubated in 2 N HCl and then incubated in rat anti-BrdU (1:500; AbD Serotec, Raleigh, NC) overnight at 4 °C followed by Cy3-conjugated anti-rat secondary for 4 h at RT. Finally, nuclei were labeled by incubation in 4′,6-diamidino-2-phenylindole (DAPI; 1:10,000; Calbiochem, San Diego, CA) for 10 min. Sections were mounted on glass slides under the anti-fading agent PVA-DABCO (2.5% diazobicyclooctane, 10% polyvinyl alcohol and 20% glycerol in TBS; Sigma Aldrich).

2.8. Cell quantification

2.8.1. Total new cell number

The total number of new ($BrdU^+$) cells was estimated on one 1-in-6 series of systematically uniform sections (spaced 240 μm apart) taken through the rostral–caudal extent of the dentate gyrus in the left hemisphere of each rat using stereological principles (Boyce et al., 2010; Cameron and McKay, 1999; Kempermann et al., 2002; Ormerod et al., 2003; West et al., 1991). We randomly selected which of the 6 collected sets of sections to process immunohistochemically to ensure that the first section in each rats' set was randomly the 1st–6th section taken from dentate gyrus. New cells produced in the hippocampal subgranular zone (SGZ) presumably migrate deeper into the granule cell layer (GCL) over the 16–21 d survival period employed. We therefore counted round or oval $BrdU^+$ cells (revealed by DAB staining) in both the SGZ and GCL on each section taken through the rostral–caudal extent of the dentate gyrus in the left hemisphere of each aged rat (∼12 sections per rat) using a Zeiss Axio Observer Z1 inverted microscope under a 40X objective. Because new cells are often situated irregularly through the SGZ and GCL, we counted $BrdU^+$ cells exhaustively on each systematically uniform series of sections per rat. The mean (±SEM) number of 131 ± 12 and 191 ± 12 $BrdU^+$ cells in the dentate gyri of control and conditioned runner groups, respectively, is considered a sufficient number of events to insure precision among stereological estimates of total events (Boyce et al., 2010).

The total number of $BrdU^+$ cells counted in the dentate gyrus of each rat was multiplied by 6 (the section interval in each set) and

by 2 (to account for the other half of the brain) to produce a stereological estimate of total number of new cells surviving in the dentate gyrus (Kempermann et al., 2002; West et al., 1991). Because age and exercise may influence vascular volumes (Fabel et al., 2003; Hattiangady and Shetty, 2008), SGZ and GCL areas (mm^2) on which new cells were counted were measured under a 20× objective using AxioVision software (Carl Zeiss, Thornwood, NY) and then GCL volumes obtained using Cavalieri's principle for calculating the volume of a truncated cone (Galea et al., 2000; Uylings et al., 1986): $Volume = \sum_{(sections)} * \frac{1}{3} I\,(h_1 + \sqrt{h_1} * \sqrt{h_2} + h_2)$, where I is the distance between sections (240 μm) and h_1 and h_2 are the two section areas between which the volume was calculated. We also confirmed that new cell densities reflected total new cell estimates because of potential changes in vascular volumes and because we quantified neurogenesis on only ½ of the hippocampus.

2.8.2. New cell phenotypes

At least 100 $BrdU^+$ cells on quadruple fluorescent-stained sections were scanned through their *x*, *y* and *z*-planes using a Zeiss LSM 710 fully spectral laser scanning confocal microscope equipped with 405 (used to excite DAPI), 488 (used to excite FITC), 510, 543 (used to excite Cy3) and 633 (used to excite Cy5) laser lines under a 40× objective (with 2.3× digital zoom) to quantify the proportion that expressed neuronal or glial proteins. $BrdU^+$ cells were considered to express neuronal or glial protein when a full "z-dimension" scan revealed that its $BrdU/DAPI^+$ nucleus clearly expressed the neuronal proteins DCX and/or NeuN, the oligodendrocyte precursor protein NG2 or the astrocyte protein GFAP. The total number of $BrdU^+$ cells was multiplied by the % of $BrdU^+$ expressing each cell phenotype to determine the total number of new neurons and glia produced in the aging brain and this number was related to water maze probe trial performance.

2.9. Multiplex quantification of cytokines

Concentrations of immune cytokines in blood serum and neuroimmune cytokine concentrations in hippocampal and cortical protein samples were quantified using a Bio-Rad Bio-Plex 2000 Suspension Array system and EMD Millipore Rat Cytokine/Chemokine kits (#RCYTO-80K-PMX; Billerica, MA) according to kit instructions. This kit detects the following concentrations of 24 analytes simultaneously in a single sample: IL-1α (6.23–20,000 pg/mL), IL-1β (2.32–20,000 pg/mL), IL-2 (3.67–20,000 pg/mL), IL-4 (2.30–20,000 pg/mL), IL-5 (2.89–20,000 pg/mL), IL-6 (9.80–20,000 pg/mL), IL-9 (12.85–20,000 pg/mL), IL-10 (5.41–20,000 pg/mL), IL-12 (4.13–20,000 pg/mL), IL-13 (23.2–20,000 pg/mL), IL-17 (1.61–20,000 pg/mL), IL-18 (4.78–20,000 pg/mL), eotaxin (3.27–20,000 pg/mL), G-CSF (1.31–20,000 pg/mL), GM-CSF (13.11–20,000 pg/mL), IP-10 (3.78–20,000 pg/mL), leptin (21.50–100,000 pg/mL), GRO/KC (2.06–20,000 pg/mL), IFN-γ (4.88–20,000 pg/mL), MCP-1 (3.81–20,000 pg/mL), TNF-α (4.44–20,000 pg/mL), MIP-1α (1.94–20,000 pg/mL), RANTES (54.42–20,000 pg/mL), VEGF (4.93–20,000 pg/mL).

All standards, controls and samples were prepared on ice and serum and tissue samples were run in separate plates. Seven standards (with expected concentrations of 20,000, 5,000, 1,250, 312.5, 78.13, 19.53 and 4.88 pg/mL of each analyte except leptin that had expected concentrations of 100,000, 25,000, 12,500, 6250, 1562.5, 390.63 and 24.41 pg/mL) were prepared by serial dilution with kit assay buffer. Serum samples were diluted 1:5 with kit assay buffer while tissue supernatant samples were kept neat and 25 μl volumes of each standard, blank, vendor-supplied known control and sample were loaded in duplicate into a 96-well filter plate (EMD Millipore; Billerica, MA). Kit serum matrix (25 μl) was added to each standard, control and sample in the serum quantification plate while kit assay buffer (25 μl) was added to each standard, control and sample in the tissue sample plates final volume of 50 μl. Approximately 100 polystyrene beads each of 24 different color addresses were added to each well and incubated for 18 h on a shaker at 4 °C. Each primary antibody raised against an analyte to be quantified was adsorbed to 1 of the 24 unique sets of color addressed beads. After several washes in kit wash buffer under vacuum filtration, the beads were incubated in biotinylated secondary antibodies for 2 h at RT and then after several washes in kit wash buffer under vacuum filtration, in streptavidin–phycoerythrin reporter for 30 min at RT before being resuspended in sheath fluid (Bio-Rad; Hercules, CA). Analytes were identified by color address and analyte concentrations were quantified by phycoerythrin emission intensity using a dual laser Bio-Rad BioPlex 2000 system with Luminex xMAP technology (Bio-Rad; Hercules, CA). Data were collected using BioPlex Manager Software version 4.1.

A standard curve for each analyte was generated using a five-parameter logistic non-linear regression model on averaged duplicate observed standard concentrations. Single standard concentrations were employed in cases that its duplicate % coefficient of variation (CV) was >10% and its % recovery (observed/expected concentration) fell outside of the accepted 70–130% range. Once the positive control concentrations were confirmed to fall within the expected ranges, sample concentrations were compared against the standard curve.

Prior to statistical analysis, duplicate sample concentrations with % CV < 10 were averaged. If the % CV for a set of duplicates was >10% and a concentration fell ±2 standard deviations from the group mean the outlying concentration was discarded. We discarded the outlying data point of one conditioned runner rat serum leptin analysis and an outlying data point from a different conditioned runner from the serum MCP-1 analysis. Cytokine concentrations below the threshold of detection were set to 0 and concentrations that exceeded the maximum expected concentration were set to 20,000 pg/ml (or 100,000 pg/ml for leptin). Data were expressed in pg/mL serum or pg/mg of hippocampal or cortical tissue.

2.10. Cytokine cluster analysis

Pathway or 'Cluster' analyses were conducted as described previously (Baron and Kenny, 1986; Erickson and Banks, 2011) to identify groups of cytokines with concentrations that may change in a coordinated fashion (i.e. in clusters) and therefore represent known or novel signaling pathways. First, cluster analyses were conducted on cytokine concentrations detected within blood, hippocampal and cortical compartments independently to both confirm and expand upon immune and neuroimmune cytokine signaling clusters in the aged rat. Second, cluster analyses were conducted on cytokine concentrations between blood and cortical compartments and between blood and hippocampal compartments to confirm and potentially reveal immune-to-brain signaling pathways in the aged rat. Third, we ran analyses on cytokine concentrations between cortical and hippocampal compartments to ask whether running modulates neuroimmune cytokines locally or regionally. Bonferroni-corrected alpha levels were set for each analysis based upon the number of analytes exceeding the threshold of detection.

Pairs of cytokines with concentrations deemed statistically related by Spearman rank correlation coefficients (*r*-values) after Bonferroni corrections were ranked and plotted in descending order connected with a solid line. If one cytokine in a pair to be plotted was already plotted in a cluster, then a decision point was reached and we employed a modification to the previously reported procedure (Baron and Kenny, 1986; Erickson and Banks, 2011). The unplotted cytokine was added to the cluster if it correlated significantly with all of the cytokines already in the

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cluster. If the unplotted cytokine was not statistically related to one or more of the already clustered cytokines, then the cytokine pair about to be plotted was plotted as a new cluster. If a cytokine pair about to be plotted was already linked through potential mediators in the already plotted cluster, then the residual of its *r*-value minus the product of the *r*-values of the plotted pairs between the cytokines about to be plotted was compared against the Bonferroni corrected *p*-value. If the residual *r*-value of the cytokine pair about to be plotted remained statistically significant, the cytokines were connected in the existing cluster with a dotted line (no mediators were detected in the current study).

2.11. Statistical analyses

All statistical analyses were conducted using STATISTICA software (Version 10; StatSoft; Tulsa, OK) and all data are represented in figures as the group average (±S.E.M.) except DI scores, which are depicted as individual scores. Student's *t*-tests were used to test the effect of the independent variable (conditioned running) on dependent measures of general health (body mass, swim speeds), strength of spatial learning and memory (probe trial discrimination index scores, number of platform crossings), neurogenesis (new cell number, total new neuron number, total new glia number) and cytokine concentration (for each of the 24 analytes). Non-parametric Mann Whitney *U* tests were used to test the effects of the independent variable (conditioned running) on categorical percentages of $BrdU^+$ cells expressing neuronal or glial phenotypes and on inhibitory avoidance acquisition and retention latencies that were set to 900 s for animals that did not enter the shock-paired side of the chamber by the end of the session. Repeated measures analyses of variance (ANOVAs) tested the effect of the independent variable (conditioned running) on dependent measures collected repeatedly, such as spatial and non-spatial acquisition of a platform location (latencies and path lengths). Newman Keuls post hoc tests were used to reveal significant differences. Spearman rank correlations were run to test the relationship between the concentration of cytokine analytes modulated by running, behavioral measures and measures of neurogenesis because some analyte concentrations fell below the threshold of detection. The α-level was set at 0.05.

3. Results

3.1. Aging rats that run daily locate a visible platform as well as controls but swim faster

Since path lengths correlated positively with latencies between visible (*all r values* $\geqslant$ 0.69; *all p values* < 0.05) and hidden (*all r values* $\geqslant$ 0.85; *all p values* < 0.01) platform trials, we report analyses on path lengths to avoid redundancy. Fig. 2A shows pathlengths across visible platform training blocks. An ANOVA exploring the effects of conditioned running and training block on visible platform path lengths revealed that conditioned runners and controls swam similar distances to the visible platform ($F_{(1,9)} = 1.38$; $p = 0.27$) across training blocks ($F_{(4,36)} = 1.56$; $p = 0.20$ and interaction effect: $F_{(4,36)} = 0.26$; $p = 0.90$). Because visible platform learning curves can be relatively shallow in aged rats (see Kumar et al., 2012; Speisman et al., 2012), planned comparisons were used to confirm that control rats and conditioned runner rats swam shorter path lengths on the 5th relative to the 1st block (*p values* < 0.05, respectively). These data suggest that both conditioned runners and controls are similarly capable of learning to locate and escape to a visible water maze platform.

The % of time spent swimming in the outer annulus of the pool by controls and conditioned runners was calculated as a measure of anxiety (Fig. 2B). An ANOVA revealed a significant effect of training block ($F_{(4,36)} = 5.32$; $p < 0.01$) but not conditioned running ($F_{(1,9)} = 0.67$; $p = 0.44$ and interaction effect: $F_{(4,36)} = 1.59$; $p = 0.20$) on this measure. Specifically, all rats spent significantly less time in the outer annulus as training commenced (Blocks 1, 2 > 3, 5 and Block 2 > 4; *all p values* < 0.05), suggesting that anxiety levels decreased in aged rats with training, regardless of exercise history.

Swim speeds exhibited across blocks by controls and conditioned runners were recorded as a measure of locomotor ability (Fig. 2C). Although an ANOVA revealed a statistically significant effect of training block ($F_{(4,36)} = 5.55$; $p < 0.01$) and a tendency for conditioned running to affect swim speed ($F_{(1,9)} = 3.84$; $p = 0.08$), these effects did not statistically significantly interact ($F_{(4,36)} = 0.37$; $p = 0.83$). Although conditioned runners tended to swim faster than controls on all blocks combined, all aged rats swam more quickly as training progressed (Blocks 1 > 3, 4 and 5 and Block 2 > 5; *all p values* < 0.05). These data suggest that daily exercise may potentiate the increased swimming proficiency or reduce the floating tendencies exhibited by aging rats across visible platform training blocks. Consistent with our previous finding (Speisman et al., 2012) and the idea that running-induced fitness rather than mild food deprivation associated with the operant delivery of food for running affects swim speeds, the body masses of conditioned runners was similar to those of the controls at the beginning of the experiment and only tended to be smaller at the end of the experiment (see Section 2.1).

3.2. Daily exercise improves spatial ability in aging rats

We compared pathlengths to the hidden platform across training blocks as a measure of spatial ability (Fig. 3A). An ANOVA revealed that pathlengths were significantly affected by conditioned running ($F_{(1,9)} = 20.89$; $p < 0.01$), training block ($F_{(4,36)} = 6.55$; $p < 0.01$) and the interaction between conditioned running and training block ($F_{(4,36)} = 4.05$; $p < 0.01$). All rats swam more directly to the hidden platform as training commenced (Blocks 1, 2 > 3, 4 and 5, *p values* < 0.05), but conditioned runners swam more directly across all blocks combined than controls ($p < 0.01$). Conditioned runners exhibited shorter pathlengths than controls on the 1st, 4th and 5th training blocks (*p values* < 0.05), indicating that they solved the spatial task more proficiently than the controls. However, their better performances on the 1st hidden platform training block could also indicate that runners better learned, remembered and/or applied procedural information obtained during visible platform training conducted first (Gerlai, 2001; Ormerod and Beninger, 2002). Therefore, we confirmed that runners (-130.22 ± 37.53 cm/block) exhibited steeper average pathlength slopes than controls (-5.36 ± 35.65 cm/block) across hidden training blocks 2–5 (gray dotted lines in Fig. 3A; $t_{(10)} = 2.29$; $p < 0.05$).

We calculated the % of time spent in the outer annulus of the maze on hidden platform trials by conditioned runners and controls to determine if anxiety was differentially affected by previous training (Fig 3B). An ANOVA revealed significant effects of conditioned running ($F_{(1,9)} = 6.45$; $p < 0.05$) and training block ($F_{(4,36)} = 3.98$; $p < 0.01$) but no interaction effect ($F_{(4,36)} = 1.62$; $p = 0.19$). Specifically, all rats combined spent less time in the outer annulus as training progressed (blocks 1, 2 > 5, *p values* < 0.05), but conditioned runners spent less time than controls on all blocks combined. These data suggest that although anxiety decreases with training in all aged rats, prior training may potentiate this effect in rats that exercise daily.

We calculated swim speeds on hidden platform training blocks as a measure of locomotor ability in aged conditioned runners and controls (Fig. 3C). Swim speeds were significantly affected by conditioned running ($F_{(1,9)} = 13.07$; $p < 0.01$), training block

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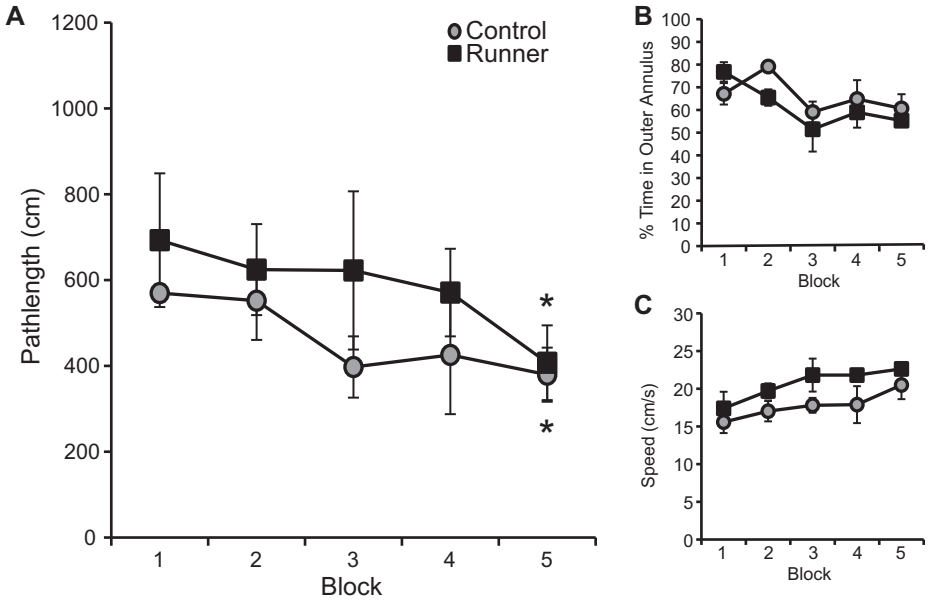

Fig. 2. Runners and controls perform similarly on the visible platform task. Data are shown as group means (±S.E.M.). Gray circles represent control values and black squares represent conditioned runner values. (A) Conditioned runners and controls located and escaped to a visible water maze platform with equal proficiency (interaction effect: $p = 0.90$). Planned comparisons confirmed decreased pathlengths on the 5th block relative to the 1st block for control rats ($^{*}p < 0.05$) and conditioned runner rats ($^{*}p < 0.05$), confirming similar sensorimotor and procedural abilities between groups. (B) Regardless of exercise history (interaction effect: $p = 0.20$), rats decreased the amount of time in a block spent swimming around the outer annulus of the water maze as training commenced (Blocks 1, 2 > 3 and 5 and Block 2 > 4; *all p values* < 0.05). (C) Although conditioned runners tended to swim more quickly on all visible platform training blocks combined ($p = 0.08$), all rats (interaction effect: $p = 0.83$) significantly decreased their swim speeds across trials ($p < 0.01$). Specifically, the aged rats swam more quickly on Block 1 versus 3, 4 and 5 and on Block 2 versus 5 (*all p values* < 0.05).

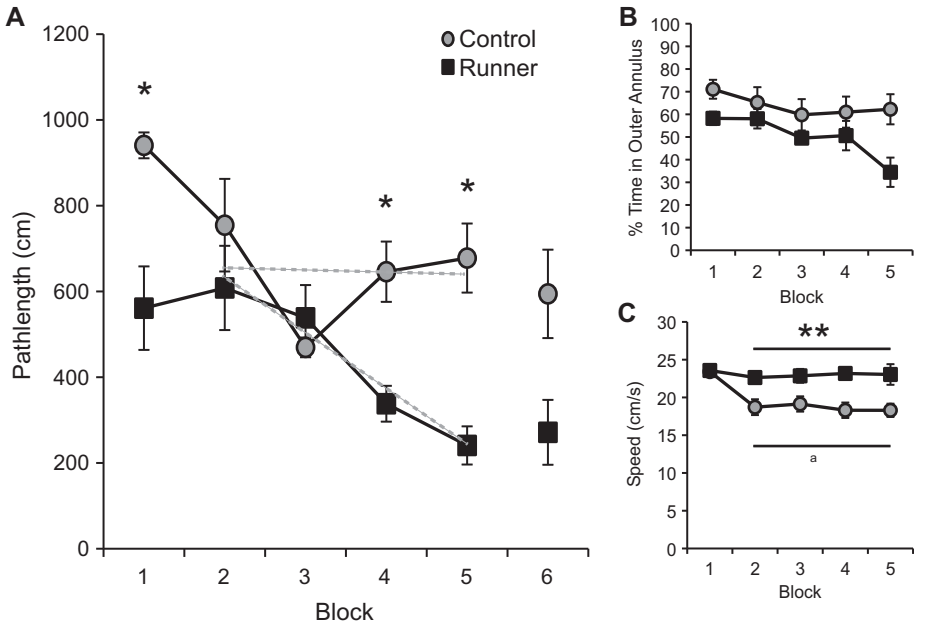

Fig. 3. Conditioned runners outperformed controls on the water maze hidden platform task. Data are shown as group means (±S.E.M.). Gray circles represent control values and black squares represent conditioned runner values. (A) All rats combined swam more directly to the hidden water maze platform as training commenced (Block 1 > 3 and 4, *all p values* < 0.05), but conditioned runners swam more directly than controls, regardless of training block ($p < 0.01$), particularly on the 1st, 4th and 5th training blocks (**all p values* < 0.05). Because runners outperformed controls on the 1st training block, we confirmed average pathlength slopes were steeper for runners (−130.22 ± 37.53 cm/block) versus controls (−5.36 ± 35.65 cm/block) across hidden training blocks 2–5 (gray dotted lines; $p < 0.05$). A paired *t*-test confirmed that the rats exhibited similar pathlengths on the 5th and a 6th training block administered after the 1 h probe trial to reinforce the association between locating the platform and escape from the pool ($p = 0.55$). (B) Although conditioned runners spent significantly less time in the outer annulus than controls on all blocks combined ($p < 0.05$), all rats combined reduced the % of time spent in the outer annulus of the maze (Blocks 1, 2 > 5; *all p values* < 0.05). (C) Conditioned runners swam significantly faster to the hidden platform than controls on all blocks combined ($p < 0.01$) and while they maintained their faster swim speeds across trials, control rats swam significantly slower than conditioned runners on Blocks 2–5 (***all p values* < 0.01) and slower than they swam on the first training block (Block 1 > 2, 3, 4 and 5; ^{a}*all p values* < 0.01).

($F_{(4,36)} = 6.27$; $p < 0.01$), and the interaction between running and training block ($F_{(4,36)} = 3.87$; $p < 0.01$). Conditioned runners swam significantly faster to the hidden platform than controls on all blocks combined ($p < 0.01$) and maintained the swim speeds that they achieved on later visible platform trials across all hidden platform training blocks (see Fig. 2c). In all rats combined, swim speeds

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decreased after the first block (*all p values* < 0.01), but this effect was because while conditioned runners maintained their speeds across blocks, control rats swam significantly slower after the first training block (Blocks 1 > 2, 3, 4 and 5; *all p values* < 0.01). These data support the notion that daily exercise can potentiate the effects of water maze training on the swimming proficiency of aging rats, potentially by improving their stamina.

3.3. Aging rats that exercise exhibit better memory for the platform location on probe trials

A 60s probe trial was conducted immediately after the final hidden platform trial (Fig. 4). An ANOVA revealed that all rats combined exhibited a significant quadrant preference ($F_{(3,27)} = 24.99$; $p < 0.0001$) and that quadrant preference significantly interacted with group ($F_{(3,27)} = 7.54$; $p < 0.001$) on the immediate probe. Specifically, conditioned runners spent significantly more time in the goal quadrant ($p = 0.0003$; Fig. 4A) and less time in the opposite quadrant ($p = 0.045$) but similar amounts of time in the left ($p = 0.32$) and the right quadrants ($p = 0.96$) relative to controls. Similarly, conditioned runners exhibited significantly better DI scores than controls ($t_{(9)} = 4.17$, $p < 0.01$; Fig. 4B) and tended to cross over the location that housed the platform on training trials significantly more frequently (4.33 ± 0.71 crossings) than controls (2.60 ± 0.51 crossings) did ($t_{(9)} = 1.90$; $p = 0.09$). A refresher block of hidden platform trials was administered after the immediate probe to minimize the probability that the association between platform localization and escape from the pool was extinguished by the immediate probe trial. A paired *t*-test on the 5th and 6th hidden platform blocks confirmed that the rats exhibited similar path lengths before and after the probe trial ($t_{(10)} = 0.29$, $p = 0.78$; see Fig. 3A).

A second probe trial was administered 24 h after the 5th hidden platform block. An ANOVA revealed that all rats combined exhibited a significant quadrant preference ($F_{(3,27)} = 3.56$; $p = 0.027$) and that quadrant preference interacted with group ($F_{(3,27)} = 5.16$; $p = 0.006$) on the 24 h probe trial (Fig. 4A). Specifically, conditioned runners spent significantly more time in the goal quadrant ($p = 0.026$), tended to spend less time in the opposite quadrant ($p = 0.052$) and spent similar amounts of time in the left ($p = 0.416$) and right quadrants ($p = 0.498$) relative to controls. Similarly, conditioned runners exhibited significantly better DI scores than controls ($t_{(9)} = 4.39$; $p < 0.01$; Fig. 4B) and crossed the location that housed the hidden platform on training trials significantly more frequently than controls (5.17 ± 0.40 versus 1.20 ± 0.20 crossings, respectively; $t_{(9)} = 8.28$, $p < 0.01$) on the delayed probe trial. These data suggest that conditioned runners both learned and remembered the hidden platform location better than controls.

Finally, a regression analysis of the distance to escape the pool on block 5 of cue discrimination training was compared to the distance to escape for block 5 of the spatial discrimination task as well as the discrimination index score obtained on the immediate probe. No association was observed indicating that acquisition of the spatial discrimination was not linked to the acquisition performance for cue discrimination.

3.4. Inhibitory avoidance scores

One week after the onset of visible platform water maze training, the rats were trained and tested in an inhibitory avoidance task (Fig. 4C). Mann–Whitney *U* tests confirmed that conditioned runners and controls entered the shock-paired dark side of the inhibitory avoidance chamber equally as quickly during the acquisition phase of the task ($U = 0.01$; $Z = -1.19$; $p = 0.92$). Although 1 h latencies were similar between groups ($U = 8.00$; $Z = -1.19$; $p = 0.24$), conditioned runners tended to have longer 24 h latencies than controls ($U = 5.00$; $Z = 1.73$; $p = 0.08$). Spearman Rank Correlation was used to compare learning and memory on the water maze discrimination index scores and 1 and 24 h inhibitory avoidance retention latencies. The results indicated a relationship between the 24 h retention scores on the water maze and inhibitory avoidance ($r = 0.63$, $p < 0.05$).

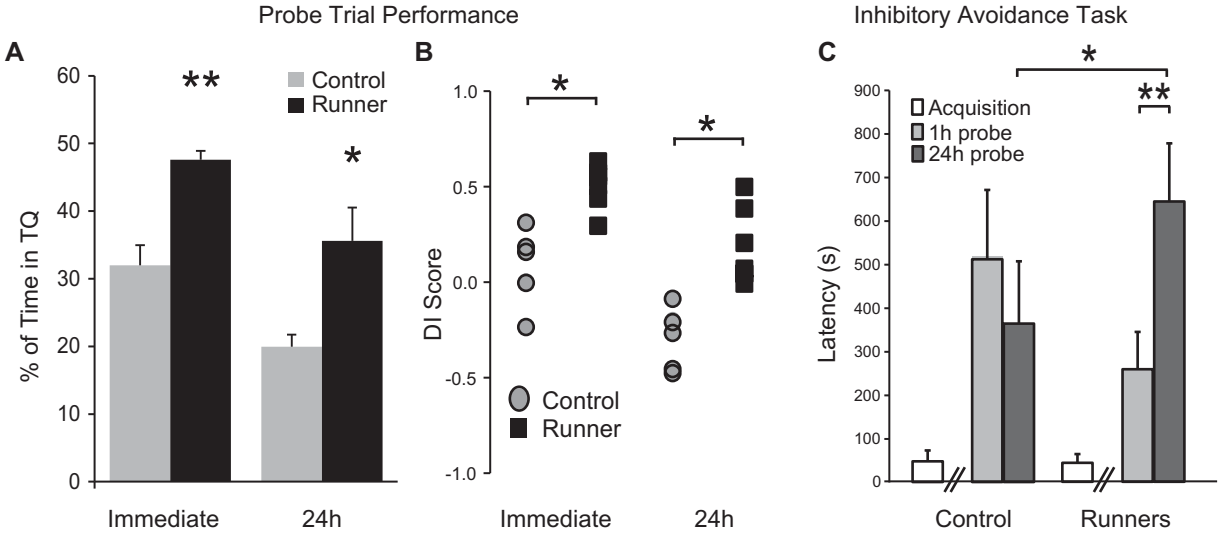

Fig. 4. Conditioned runners exhibit better memory in the water maze and on an inhibitory avoidance task. (A). A 60s probe trial was conducted immediately after or 24 h after the final hidden platform trial and mean % time spent in the goal quadrant (±S.E.M.) is depicted for the controls (gray bars) and conditioned runners (black bars). On the immediate probe (**$p = 0.0003$) and on the 24 h probe (*$p = 0.026$), conditioned runners spent significantly more time in the goal quadrant than control rats did. (B) Individual discrimination index (DI) scores were calculated for control (gray circles) and conditioned runner rats (black squares) and then plotted. Positive scores represent better goal (versus opposite) quadrant discrimination. Conditioned runners exhibited better DI scores on both the immediate (*$p < 0.01$) and 24 h (∗$p < 0.01$) water maze probe trials than control rats. (C) Finally, rats were trained (white bars) and then tested 1 h (light gray bars) and 24 h (dark gray bars) after training in an inhibitory avoidance task. Conditioned runners and controls entered the dark side of the inhibitory avoidance chamber that delivered shock equally as quickly during the acquisition phase of the task ($p = 0.90$). Although both control and conditioned runners exhibited similar 1 h retention latencies, conditioned runners took significantly longer than controls to re-enter the dark side 24 h after training ($p < 0.05$).

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3.5. Daily exercise increases neurogenesis in aged rats by increasing new cell number

The total number of new cells was estimated in the dentate gyri of all rats using stereological principles (Fig. 5A, B and E). A Student's *t*-test confirmed that the total number of BrdU$^+$ cells was higher in the dentate gyri of conditioned runners relative to controls ($t_{(9)} = 3.44$, $p < 0.01$; Fig. 5E). Although exercise could potentially increase vascular volume within the neurogenic niche, dentate gyri volumes that new cells were estimated through were similar between controls ($4.32 \pm 0.17\ mm^3$) and conditioned runners ($4.53 \pm 0.30 mm^3$; $t_{(9)} = -0.57$, $p = 0.58$). As expected from these data sets, new cell densities were also higher in conditioned runners (514.77 ± 40.95 cells/mm^3) versus controls (370.35 ± 46.14 cells/mm^3; $t_{(9)} = 2.35$, $p < 0.05$). Because the rats survived several weeks after BrdU was injected, these differences could reflect effects on NPC division and/or the survival of new cells, but are consistent with the well-known effects of physical exercise on NPC division.

We confirmed that new cell differentiation was unaffected by conditioned running by quantifying the percentage of BrdU$^+$ cells expressing immature neuronal (DCX$^+$), transitioning neuronal (DCX/NeuN$^+$), mature neuronal (NeuN$^+$), oligodendroglial (NG2$^+$), or astroglial (GFAP$^+$) phenotypes (Fig. 5C, D and F). Mann Whitney *U* tests ($n_{runner} = 6$ and $n_{control} = 5$ in all comparisons) confirmed that the percentages of BrdU$^+$ cells expressing immature neuronal ($U = 8$, $Z = 1.187$, $p = 0.024$), transitioning neuronal ($U = 14.5$, $Z = 0.0$, $p = 1.0$), mature neuronal ($U = 15.0$, $Z = 0.0$, $p = 1.00$), GFAP$^+$ ($U = 13.0$, $Z = -0.274$, $p = 0.784$) and oligodendrocyte precursor ($U = 14.5$, $Z = 0.0$, $p = 1.0$) phenotypes were similar between conditioned runner and control rats. Consistent with a 2.5–3 week long survival period after BrdU, most new cells (~70%) expressed

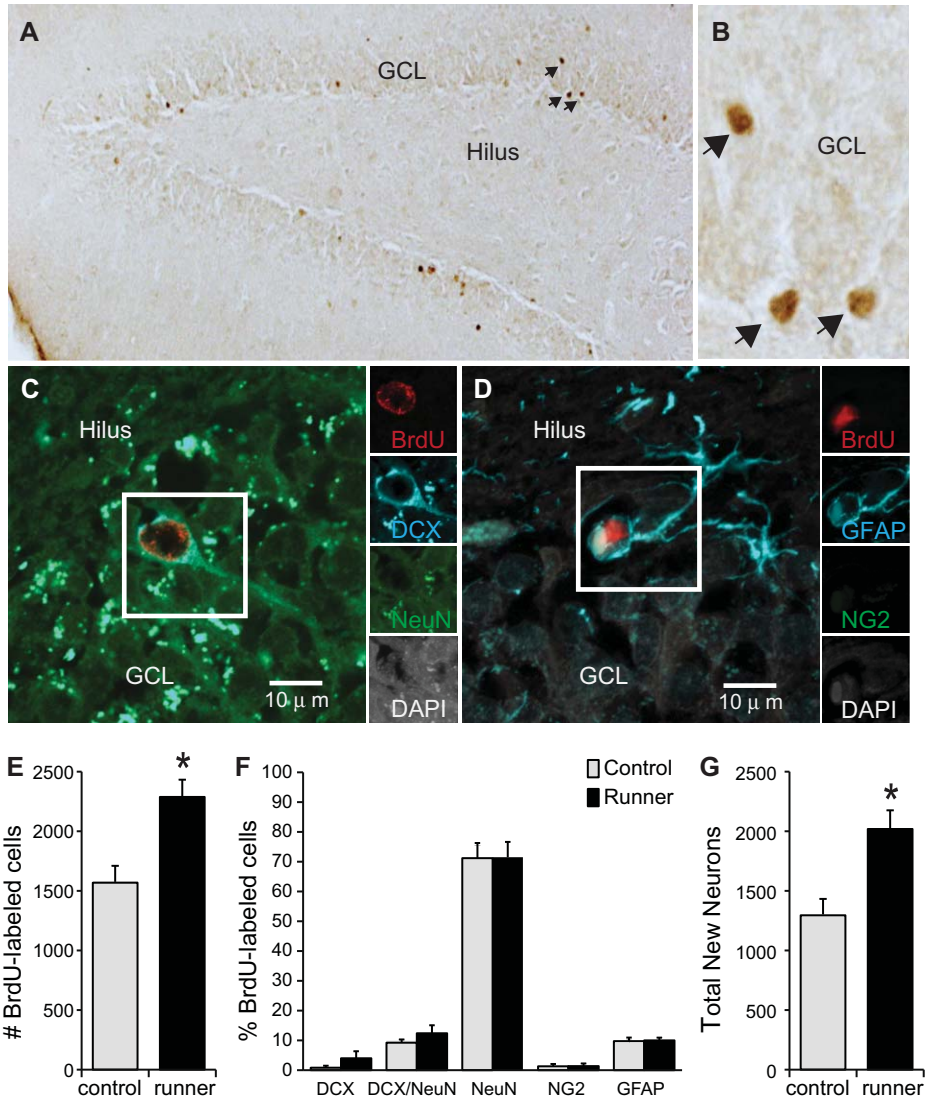

Fig. 5. Conditioned running potentiated hippocampal neurogenesis in aging rats. (A) Transmitted light micrograph showing new (BrdU$^+$; in brown) cells located through the GCL and SGZ of aged rats under a 10X objective revealed by DAB. (B) Shows a subset of BrdU$^+$ cells depicted in (A) under the 40× objective used for counting. (C and D) Confocal images of samples of new cells (BrdU/DAPI$^+$; in white and red) expressing the neuronal proteins DCX (in blue) and NeuN (in green; [C]) or the astrocyte protein GFAP (in blue; [D]). Insets show each channel independently and scale bars represent 10 μm. (A, B and E) More BrdU$^+$ cells were detected in the dentate gyri of conditioned runners vs. controls ($^*p < 0.01$). (C, D and F) Similar percentages of new cells in the dentate gyri of conditioned runners and controls expressed immature neuronal (DCX$^+$), transitioning neuronal (DCX/NeuN$^+$), mature neuronal (NeuN$^+$), oligodendroglial (NG2$^+$) or astroglial (GFAP$^+$) proteins. Consistent with the ~2 week survival period, most new cells expressed mature neuronal phenotypes, followed by astrocyte and transitioning neuronal phenotypes. (G) The total estimated new neuron number was significantly higher in conditioned runners versus controls ($^*p < 0.01$). Data are group means ± S.E.M obtained from conditioned runners (black bars) and controls (gray bars). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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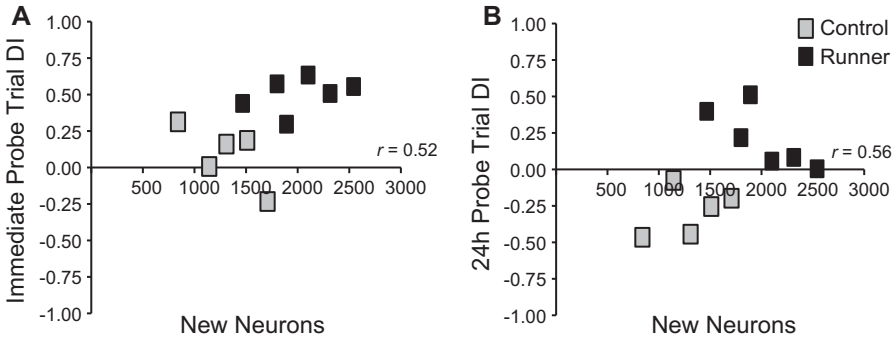

Fig. 6. Probe trials scores relate to measures of neurogenesis in aging rats. Spearman rank correlations were conducted on total new ($BrdU^+$) DCX and/or $NeuN^+$ neuron numbers and DI scores obtained from control rats (light gray squares) and conditioned runners (black squares). Total new neuron number tended to correlate with (A) DI scores obtained in the immediate probe trial ($p = 0.08$) and (B) DI scores obtained from the 24 h probe trial ($p = 0.059$).

mature neuronal phenotypes followed by astrocyte and transitioning neuronal phenotypes (~10% each). Very few new cells expressed immature neuronal or oligodendroglial phenotypes (<3%) in the dentate gyri of all rats combined (Fig. 5). Note that all of the $BrdU/GFAP^+$ cells were detected outside of the subgranular zone and exhibited an astrocyte rather than radial glial (or neural stem cell)-like morphology.

The total new cell number (Fig. 5E) was multiplied by the % of neurons (immature, transitioning and mature), oligodendrocytes or astrocytes (Fig. 5F) for each rat to estimate total numbers of each new cell phenotype (Fig. 5G). Relative to controls, conditioned runners had significantly more new neurons ($t_{(9)} = 3.26$; $p < 0.01$), tended to have more new astrocytes (157.41 ± 31.27 and 227.59 ± 22.06, respectively; $t_{(9)} = -1.88$; $p = 0.09$), and had similar numbers of new oligodendrocyte precurors (26.31 ± 16.55 and 31.43 ± 19.89, respectively; $t_{(9)} = -0.19$; $p = 0.85$). New neuron number tended to correlate positively with immediate ($p = 0.08$; Fig. 6A) and 24 h ($p = 0.059$; Fig. 6B) water maze probe discrimination index scores.

3.6. Distinct cytokine relationships were detected in serum, hippocampal and cortical compartments

Concentrations of 24 cytokines were quantified in blood serum and hippocampal and cortical protein samples of each behaviorally characterized rat that neurogenesis was also quantified in (Table 1). Note that concentrations of eotaxin, GRO-KC, IL-10, IL-13, IL-17, leptin, and RANTES were at least a magnitude higher in circulation versus the brain. IFNγ was only detected in blood serum whereas G-CSF, GM-CSF, IL1α, IL-2, IL-4, IL-5, IP-10 and TNFα were only detected in the brain. Interestingly, of the cytokines only detected in the brain, G-SCF, GM-CSF, IL-10, and IP-10 were detected in the cortex but not in the hippocampus. An ~2-fold higher concentration of IL-1β and MCP-1 was detected in the hippocampus versus cortex whereas an ~3-fold higher concentration of IL-12 and an ~2-fold higher concentration of IL-2 and IL-5 was detected in the cortex versus hippocampus. These data suggest that in aged rats, circulating cytokine concentrations do not appear to reflect central concentrations. In addition, there appear to be regional differences

Table 1
Some hippocampal (pg/mg), cortical (pg/mg) and circulating (pg/mL) cytokines are modulated by daily exercise in aging rats. Mean (±S.E.M.) values are reported.

	Serum		Hippocampus		Cortex	
	Controls	Runners	Controls	Runners	Controls	Runners
Eotaxin	55.06 ± 13.76	196.23 ± 147.02	2.37 ± 0.76	3.46 ± 0.87	1.90 ± 0.16	1.73 ± 0.27
G-CSF	0 ± 0	2.23 ± 2.23	0 ± 0	0 ± 0	0.25 ± 0.04	0.17 ± 0.07
GM-CSF	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.09 ± 0.11	0.99 ± 0.33
GRO-KC	1322.52 ± 219.98	776.89 ± 154.63†	13.36 ± 2.57	25.83 ± 5.55†	9.82 ± 0.80	10.54 ± 2.28
IFN-γ	26.41 ± 18.51	415.65 ± 398.82	0 ± 0	1.52 ± 1.29	0 ± 0	0 ± 0
IL-1α	0 ± 0	233.95 ± 233.95	2.55 ± 2.55	14.81 ± 7.03	5.60 ± 1.06	4.96 ± 1.58
IL-1β	200.54 ± 174.94	222.88 ± 182.13	40.44 ± 3.46	22.27 ± 4.38**	13.07 ± 2.38	13.56 ± 1.82
IL-2	0 ± 0	0 ± 0	8.62 ± 5.09	13.73 ± 4.28	27.50 ± 1.75	22.47 ± 4.18
IL-4	0 ± 0	47.56 ± 41.81	2.73 ± 0.90	4.88 ± 0.99	3.17 ± 0.33	2.63 ± 0.53
IL-5	0 ± 0	18.32 ± 13.88	1.46 ± 1.01	1.73 ± 0.94	4.54 ± 0.56	3.84 ± 0.91
IL-6	67.57 ± 21.94	537.54 ± 498.29	17.31 ± 8,12	30.14 ± 10.05	14.02 ± 1.82	16.28 ± 4.19
IL-9	243.99 ± 137.04	190.51 ± 111.29	491.60 ± 301.67	588.73 ± 267.64	396.14 ± 50.08	385.10 ± 86.51
IL-10	59.89 ± 59.89	59.98 ± 59.98	0 ± 0	0 ± 0	4.56 ± 0.37	4.52 ± 1.78
IL-12	38.77 ± 21.73	68.30 ± 62.32	5.13 ± 1.50	7.60 ± 2.04	19.58 ± 3.29	17.99 ± 3.55
IL-13	108.00 ± 38.54	332.52 ± 282.22	2.68 ± 2.06	9.42 ± 3.66	6.06 ± 0.25	5.13 ± 1.05
IL-17	20.47 ± 8.29	29.24 ± 19.27	0.77 ± 0.40	0.68 ± 0.38	0.57 ± 0.09	0.48 ± 0.17
IL-18	351.01 ± 189.13	789.49 ± 422.74	105.42 ± 31.38	209.72 ± 28.51*	77.38 ± 3.90	70.00 ± 10.25
IP-10	0 ± 0	53.56 ± 53.56	0 ± 0	5.72 ± 5.00	1.57 ± 0.11	1.41 ± 0.19
Leptin	10001.90 ± 850.70	5414.09 ± 743.34**	17.84 ± 5.15	19.97 ± 6.43	10.39 ± 0.51	9.52 ± 1.86
MCP-1	719.92 ± 109.32	323.09 ± 134.99*	113.92 ± 68.04	138.22 ± 49.40	51.23 ± 3.85	39.93 ± 8.78
MIP-1α	5.67 ± 2.25	9.42 ± 6.61	0.61 ± 0.25	4.05 ± 3.09	0.34 ± 0.02	0.43 ± 0.09
RANTES	18364.54 ± 1635.46	11780.8 ± 3208.50	0 ± 0	13.49 ± 6.72	17.22 ± 1.86	42.96 ± 16.84
TNF-α	0 ± 0	2.40 ± 1.52	2.44 ± 1.09	3.27 ± 1.41	2.28 ± 0.06	2.20 ± 0.61
VEGF	21.80 ± 21.80	23.38 ± 23.38	3.46 ± 1.52	6.46 ± 2.68	1.91 ± 0.25	0.98 ± 0.33†

** $p < 0.01$.
* $p < 0.05$.
† $0.05 < p < 0.10$ vs. control values.

Please cite this article in press as: Speisman, R.B., et al. Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. Brain Behav. Immun. (2012), http://dx.doi.org/10.1016/j.bbi.2012.09.013

in the basal expression of central cytokines and therefore, likely their influence.

We next analyzed cytokine relationships within and between blood serum, hippocampal and cortical compartments to further explore the ideas that circulating concentrations may predict central cytokine signaling and that differences in central cytokine expression may reflect more local signaling. Pathway analyses (see Section 2.10) revealed distinct clusters within but no clusters between compartments after Bonferroni adjustments for multiple comparisons (Table 2a–c and Fig. 7). In serum: (1) MCP-1 and GRO-KC and (2) IL-6 and IL-13 were identified as independent clusters. In the hippocampus: (1) IL-17 and VEGF, (2) IL-5 and VEGF, (3) MCP-1, IL-2 and VEGF, (4) MCP, IL-2, TNF-α, MIP-1α and IL-5, (5) IL-2 and GRO-KC, (6) eotaxin, TNFα and IL-12, (7) IL-2 and IL-4 and (8) IL-4 and IL-6 were identified as independent clusters. In the cortex: (1) IL-2 and GM-CSF, (2) GM-CSF and IL-18, (4) GM-CSF and IL-10, and (5) IL-13 and IL-4 were identified as independent clusters. Table 2 shows the *r*-values (bolded and with asterisks) of cytokine pairs that were included in these clusters because they remained statistically significant after Bonferroni corrections. While these results indicate strong relationships between cytokine concentrations within each brain region and within serum, no clusters emerged between these compartments. The lack of significant relationships between serum and brain cytokine concentrations may indicate that circulating factors neither diffuse nor are transported in detectable quantities into hippocampal and cortical regions in aging rats unchallenged by an inflammatory event (Erickson and Banks, 2011). The lack of significant relationships between hippocampal and cortical compartments suggests that basal neuroimmune signaling is a local event. Of course, the lack of significant between-compartments relationships could simply reflect the stringency inherent to Bonferroni adjustments, which increase the likelihood of type II errors.

3.7. Measures of behavior and neurogenesis relate to concentrations of cytokines modulated by running

To identify cytokine candidates linked to behavior and neurogenesis, we first identified cytokines that were modulated by exercise using Student's *t*-tests (see Table 1). Compared to controls, conditioned runners had significantly lower hippocampal IL-1β ($t_{(9)} = 3.14$; $p < 0.05$), circulating MCP-1 ($t_{(9)} = 2.28$; $p \leqslant 0.05$) and circulating leptin ($t_{(9)} = 4.06$; $p < 0.01$) but higher hippocampal IL-18 ($t_{(9)} = -2.46$, $p < 0.05$) concentrations. Concentrations of circulating GRO-KC ($t_{(9)} = 2.08$; $p = 0.07$) and cortical VEGF ($t_{(9)} = 2.16$, $p = 0.06$) tended to be lower whereas hippocampal concentrations of GRO-KC ($t_{(9)} = -1.90$, $p = 0.09$) tended to be higher in runners versus controls.

Of the cytokines with concentrations that were significantly modulated by conditioned running in aged rats, several were modulated in a correlated manner (see Fig. 7A and Table 3). For example serum MCP-1, which was decreased by conditioned running, correlated positively with serum GRO-KC ($p < 0.01$) and both concentrations tended to correlate positively with serum leptin ($p = 0.06$ and $p = 0.08$). Serum leptin was strongly decreased by conditioned running and correlated positively with hippocampal IL-1β ($p < 0.05$) but tended to correlate negatively with hippocampal IL-18 ($p = 0.06$) and hippocampal GRO-KC ($p = 0.08$). Hippocampal IL-1β was decreased by conditioned running and correlated negatively with hippocampal IL-18 ($p < 0.05$) and tended to correlate positively with cortical VEGF ($p = 0.07$). A strong positive correlation was detected between hippocampal IL-18, which was increased with running, and hippocampal GRO-KC ($p < 0.01$). These data suggest that conditioned running modulates subsets of cytokines within and between serum, hippocampal and cortical compartments.

Next we examined the strength of relationships between variables significantly affected by conditioned running (total new neuron number, probe trial discrimination index scores and 24 h inhibitory avoidance retention latencies) using Spearman rank correlations (see Table 3). Interestingly, 24 h retention latencies on the inhibitory avoidance task correlated positively with water maze 24 h probe discrimination index scores ($p < 0.05$). These data suggest that these tasks are both similarly sensitive to age-related cognitive decline and the beneficial effects of conditioned running on spatial ability in aged rats. As mentioned previously, new neuron number tended to correlate positively with immediate ($p = 0.08$) and 24 h ($p = 0.059$) water maze probe discrimination index scores (see Fig. 8B and Table 3).

Finally, we explored relationships between cytokine, behavioral and neurogenesis measures that were modulated by conditioned running (Table 3 and Fig. 8B). New neuron number, which was potentiated by running, correlated negatively with serum leptin level ($p < 0.01$) but positively with hippocampal IL-18 ($p < 0.001$) and hippocampal GRO-KC ($p < 0.01$) expression and tended to correlate negatively with hippocampal IL-1 β expression ($p = 0.06$). Immediate probe trial discrimination index scores, which increased with running, correlated negatively with cortical VEGF levels ($p < 0.05$) and circulating levels of leptin ($p < 0.05$) MCP-1 ($p < 0.01$) and GRO-KC ($p < 0.01$) and tended to correlate negatively with hippocampal IL-1β ($p = 0.06$). Twenty-four-hour discrimination index scores correlated negatively with circulating leptin levels ($p < 0.01$) and hippocampal IL-1 β expression ($p < 0.05$). Interestingly, serum leptin levels correlated negatively with immediate discrimination index scores ($p < 0.05$), 24 h discrimination index scores ($p < 0.01$) and new neuron numbers ($p < 0.01$). Serum leptin level correlated positively with hippocampal IL-1β concentrations ($p < 0.05$), and hippocampal IL-1β concentrations correlated negatively with 24 h water maze ($p < 0.05$) and 24 inhibitory retention ($p < 0.05$) performances. Cortical VEGF tended to correlate negatively with 24 h inhibitory retention latencies ($p = 0.06$).

4. Discussion

An important goal for aging research is to identify markers of biological aging that predict cognitive decline. In the current study, we measured hippocampal neurogenesis and identified potential serum and central markers in rats after rejuvenating their cognition with a behavioral treatment. Several months of food-motivated wheel running improved aging rats' abilities to rapidly learn a hidden platform location and retain or consolidate spatial/contextual information. Hippocampal neurogenesis is a well-characterized marker of brain aging that was potentiated by daily exercise in the current study along with correlated increases in hippocampal IL-18 and GRO-KC. Both central and peripheral markers of inflammation have been hypothesized to contribute to age-related decreases in cognitive function and we found that daily exercise decreased hippocampal IL-1β expression, which was correlated negatively with water maze and inhibitory avoidance memory scores. Due to the invasive nature of identifying markers in brain tissue, many researchers have focused upon identifying serum markers. In the aging rat, leptin emerged as a potential serum marker for age-related declines in cognition and plasticity because it correlated negatively with water maze performances and new neuron number. Moreover, daily exercise decreased serum leptin along with serum MCP-1 (CCL2) levels and tended to decrease serum GRO-KC (CXCL1) level. Interestingly, serum leptin, GRO-KC and MCP-1 levels along with cortical VEGF level (which tended to decrease with daily exercise) correlated negatively with the water maze learning index. Our data suggest that daily exercise

Please cite this article in press as: Speisman, R.B., et al. Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. Brain Behav. Immun. (2012), http://dx.doi.org/10.1016/j.bbi.2012.09.013

Table 2
Spearman rank correlation coefficients (r_s) between cytokine pairs detected in (A) serum, (B) hippocampal and (C) cortical compartments reveal clusters (see Fig. 7). Note that no between compartments clusters emerged after Bonferroni corrections.

	eotaxin	G-CSF	GM-CSF	GRO-KC	IFN-γ	IL-1α	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12	IL-13	IL-17	IL-18	IP-10	leptin	MCP-1	MIP-1α	RANTES	TNF-α	VEGF
(A) Serum																								
eotaxin		–	–	−0.06	0.67	–	−0.61	–	–	–	0.82	−0.12	−0.36	0.75	0.72	0.35	−0.57	–	−0.53	−0.08	−0.47	−0.09	–	−0.26
G-CSF	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GM-CSF	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GRO-KC	−0.06	–	–		0.07	–	−0.28	–	–	–	0.14	0.21	0.42	−0.04	−0.03	0.17	−0.04	–	0.56	**0.96***	0.67	0.85	–	−0.24
IFN-γ	0.67	–	–	0.07		–	−0.46	–	–	–	0.58	−0.33	0.25	0.34	0.57	0.00	−0.46	–	−0.43	0.01	−0.03	0.03	–	−0.18
IL-1α	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-1β	−0.61	–	–	−0.28	−0.46	–		–	–	–	−0.52	0.44	0.14	−0.51	−0.55	−0.36	0.83	–	0.22	−0.27	−0.01	−0.48	–	0.51
IL-2	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-4	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-5	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-6	0.82	–	–	0.14	0.58	–	−0.52	–	–	–		−0.16	−0.35	0.85	**0.88***	0.65	−0.52	–	−0.28	0.10	−0.23	0.14	–	−0.04
IL-9	−0.12	–	–	0.21	−0.33	–	0.44	–	–	–	−0.16		−0.04	−0.15	−0.47	−0.18	0.26	–	0.29	0.30	−0.11	−0.01	–	0.03
IL-10	−0.36	–	–	0.42	0.25	–	0.14	–	–	–	−0.35	−0.04		−0.60	−0.38	−0.46	0.22	–	0.02	0.41	0.69	0.40	–	−0.22
IL-12	0.75	–	–	−0.04	0.34	–	−0.51	–	–	–	0.85	−0.15	−0.60		0.85	0.69	−0.47	–	−0.15	−0.09	−0.55	−0.08	–	0.12
IL-13	0.72	–	–	−0.03	0.57	–	−0.55	–	–	–	**0.88***	−0.47	−0.38	0.85		0.55	−0.51	–	−0.17	−0.08	−0.37	0.04	–	0.04
IL-17	0.35	–	–	0.17	0.00	–	−0.36	–	–	–	0.65	−0.18	−0.46	0.69	0.55		−0.38	–	0.03	0.12	−0.05	0.21	–	−0.07
IL-18	−0.57	–	–	−0.04	−0.46	–	0.83	–	–	–	−0.52	0.26	0.22	−0.47	−0.51	−0.38		–	0.31	0.01	0.17	−0.35	–	0.61
IP-10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–
leptin	−0.53	–	–	0.56	−0.43	–	0.22	–	–	–	−0.28	0.29	0.02	−0.15	−0.17	0.03	0.31	–		0.65	0.26	0.44	–	0.29
MCP-1	−0.08	–	–	**0.96***	0.01	–	−0.27	–	–	–	0.10	0.30	0.41	−0.09	−0.08	0.12	0.01	–	0.65		0.73	0.83	–	−0.20
MIP-1α	−0.47	–	–	0.67	−0.03	–	−0.01	–	–	–	−0.23	−0.11	0.69	−0.55	−0.37	−0.05	0.17	–	0.26	0.73		0.68	–	−0.16
RANTES	−0.09	–	–	0.85	0.03	–	−0.48	–	–	–	0.14	−0.01	0.40	−0.08	0.04	0.21	−0.35	–	0.44	0.83	0.68		–	−0.50
TNF-α	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–
VEGF	−0.26	–	–	−0.24	−0.18	–	0.51	–	–	–	−0.04	0.03	−0.22	0.12	0.04	−0.07	0.61	–	0.29	−0.20	−0.16	−0.50	–	

	eotaxin	G-CSF	GM-CSF	GRO-KC	IFN-γ	IL-1α	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12	IL-13	IL-17	IL-18	IP-10	leptin	MCP-1	MIP-1α	RANTES	TNF-α	VEGF
(B) Hippocampus																								
eotaxin		–	–	0.79	–	0.77	−0.10	0.84	0.84	0.81	0.88	0.85	–	**0.89****	0.86	0.68	0.70	–	0.69	0.79	0.78	0.36	**0.89****	0.82
G-CSF	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GM-CSF	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GRO-KC	0.79	–	–		–	0.78	−0.25	**0.90****	0.86	0.72	0.88	0.70	–	0.74	0.77	0.63	0.85	–	0.42	0.82	0.75	0.79	0.80	0.87
IFN-γ	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-1α	0.77	–	–	0.78	–		−0.03	0.69	0.66	0.69	0.85	0.65	–	0.83	0.75	0.56	0.71	–	0.64	0.56	0.70	0.60	0.69	0.72
IL-1β	−0.10	–	–	−0.25	–	−0.03		−0.10	−0.30	0.13	−0.18	0.14	–	−0.05	−0.27	0.28	−0.60	–	0.01	0.06	0.04	−0.36	0.03	0.07
IL-2	0.84	–	–	**0.90****	–	0.69	−0.10		**0.90****	**0.92****	0.84	0.83	–	0.79	0.68	0.77	0.68	–	0.42	**0.95****	**0.92****	0.53	**0.94****	**0.90****
IL-4	0.84	–	–	0.86	–	0.66	−0.30	**0.90****		0.83	**0.90****	0.79	–	0.78	0.66	0.68	0.70	–	0.47	0.86	0.80	0.56	0.86	0.87
IL-5	0.81	–	–	0.72	–	0.69	0.13	**0.92****	0.83		0.77	0.88	–	0.78	0.57	0.88	0.43	–	0.44	**0.89****	**0.93****	0.33	**0.91****	**0.90****
IL-6	0.88	–	–	0.88	–	0.85	−0.18	0.84	**0.90****	0.77		0.80	–	0.87	0.70	0.59	0.75	–	0.67	0.80	0.79	0.56	0.83	0.81
IL-9	0.85	–	–	0.70	–	0.65	0.14	0.83	0.79	0.88	0.80		–	0.71	0.55	0.88	0.40	–	0.59	0.88	0.84	0.12	0.82	0.87
IL-10	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–
IL-12	**0.89****	–	–	0.74	–	0.83	−0.05	0.79	0.78	0.78	0.87	0.71	–		0.74	0.58	0.68	–	0.81	0.71	0.84	0.40	**0.91****	0.72
IL-13	0.86	–	–	0.77	–	0.75	−0.27	0.68	0.66	0.57	0.70	0.55	–	0.74		0.49	0.84	–	0.49	0.53	0.54	0.58	0.69	0.70
IL-17	0.68	–	–	0.63	–	0.56	0.28	0.77	0.68	0.88	0.59	0.88	–	0.58	0.49		0.26	–	0.33	0.82	0.80	0.08	0.75	**0.90****
IL-18	0.70	–	–	0.85	–	0.71	−0.60	0.68	0.70	0.43	0.75	0.40	–	0.68	0.84	0.26		–	0.45	0.50	0.53	0.83	0.61	0.56
IP-10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–
leptin	0.69	–	–	0.42	–	0.64	0.01	0.42	0.47	0.44	0.67	0.59	–	0.81	0.49	0.33	0.45	–		0.41	0.59	0.05	0.62	0.38
MCP-1	0.79	–	–	0.82	–	0.56	0.06	**0.95****	0.86	**0.89****	0.80	0.88	–	0.71	0.53	0.82	0.50	–	0.41		**0.89****	0.38	**0.91****	**0.90****
MIP-1α	0.78	–	–	0.75	–	0.70	0.04	**0.92****	0.80	**0.93****	0.79	0.84	–	0.84	0.54	0.80	0.53	–	0.59	**0.89****		0.31	**0.93****	0.80
RANTES	0.36	–	–	0.79	–	0.60	−0.36	0.53	0.56	0.33	0.56	0.12	–	0.40	0.58	0.08	0.83	–	0.05	0.38	0.31		0.36	0.49
TNF-α	**0.89****	–	–	0.80	–	0.69	0.03	**0.94****	0.86	**0.91****	0.83	0.82	–	**0.91****	0.69	0.75	0.61	–	0.62	**0.91****	**0.93****	0.36		0.85
VEGF	0.82	–	–	0.87	–	0.72	0.07	**0.90****	0.87	**0.90****	0.81	0.87	–	0.72	0.70	**0.90****	0.56	–	0.38	**0.90****	0.80	0.49	0.85	

	eotaxin	G-CSF	GM-CSF	GRO-KC	IFN-γ	IL-1α	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12	IL-13	IL-17	IL-18	IP-10	leptin	MCP-1	MIP-1α	RANTES	TNF-α	VEGF
(C) Cortex																								
eotaxin		0.54	0.82	0.06	–	0.55	0.25	0.70	0.80	0.64	0.47	0.68	0.82	0.54	0.88	0.52	0.70	0.68	0.63	0.12	0.37	−0.23	0.60	0.83
G-CSF	0.54		0.41	0.62	–	0.63	0.15	0.58	0.62	0.48	0.58	0.17	0.44	0.63	0.57	0.48	0.30	0.68	0.71	−0.31	0.53	−0.12	0.63	0.72
GM-CSF	0.82	0.41		0.09	–	0.71	0.19	**0.91*****	0.84	0.45	0.55	0.62	**0.93*****	0.61	0.87	0.69	**0.92*****	0.73	0.55	0.03	0.18	−0.33	0.72	0.65
GRO-KC	0.06	0.62	0.09		–	0.29	0.16	0.19	0.31	0.24	0.05	−0.15	0.05	0.42	0.27	−0.09	0.14	0.56	0.55	−0.55	0.71	0.16	0.61	0.19
IFN-γ	–	–	–	–		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
IL-1α	0.55	0.63	0.71	0.29	–		−0.25	0.80	0.87	0.66	0.73	0.47	0.67	0.82	0.75	0.74	0.62	0.52	0.38	0.14	0.10	−0.46	0.55	0.56
IL-1β	0.25	0.15	0.19	0.16	–	−0.25		0.10	−0.06	−0.28	0.03	0.19	0.35	−0.13	0.15	0.08	0.16	0.25	0.51	−0.50	0.50	−0.16	0.35	0.15
IL-2	0.70	0.58	**0.91*****	0.19	–	0.80	0.10		0.80	0.41	0.55	0.52	0.82	0.60	0.75	0.76	0.82	0.71	0.51	0.02	0.06	−0.38	0.75	0.73
IL-4	0.80	0.62	0.84	0.31	–	0.87	−0.06	0.80		0.82	0.59	0.65	0.83	0.83	**0.95*****	0.56	0.71	0.67	0.57	0.20	0.33	−0.44	0.67	0.68
IL-5	0.64	0.48	0.45	0.24	–	0.66	−0.28	0.41	0.82		0.43	0.64	0.51	0.75	0.75	0.24	0.37	0.30	0.30	0.35	0.24	−0.29	0.38	0.49
IL-6	0.47	0.58	0.55	0.05	–	0.73	0.03	0.55	0.59	0.43		0.34	0.64	0.72	0.60	0.80	0.45	0.33	0.41	−0.19	0.12	−0.29	0.32	0.38
IL-9	0.68	0.17	0.62	−0.15	–	0.47	0.19	0.52	0.65	0.64	0.34		0.77	0.32	0.63	0.51	0.43	0.25	0.38	0.39	0.12	−0.55	0.25	0.35

IL-10	0.82	0.44	0.93***	0.05	–	0.67	0.35	0.82	0.83	0.51	0.64	0.77		0.59	0.87	0.72	0.76	0.63	0.65	0.05	0.29	–0.51	0.61	0.56					
IL-12	0.54	0.63	0.61	0.42	–	0.82	–0.13	0.60	0.83	0.75	0.72	0.32	0.59		0.82	0.41	0.65	0.44	0.35	–0.09	0.24	–0.27	0.68	0.53					
IL-13	0.88	0.57	0.87	0.27	–	0.75	0.15	0.75	**0.95****	0.75	0.60	0.63	0.87	0.82		0.51	0.80	0.68	0.61	0.05	0.42	–0.36	0.74	0.71					
IL-17	0.52	0.48	0.69	–0.09	–	0.74	0.08	0.76	0.56	0.24	0.80	0.51	0.72	0.41	0.51		0.51	0.45	0.44	–0.01	–0.02	–0.40	0.30	0.44					
IL-18	0.70	0.30	**0.92****	0.14	–	0.62	0.16	0.82	0.71	0.37	0.45	0.43	0.76	0.65	0.80	0.51		0.61	0.34	–0.12	0.07	–0.15	0.80	0.60					
IP-10	0.68	0.68	0.73	0.56	–	0.52	0.25	0.71	0.67	0.30	0.33	0.25	0.63	0.44	0.68	0.45	0.61		0.85	–0.31	0.64	0.06	0.73	0.63					
leptin	0.63	0.71	0.55	0.55	–	0.38	0.51	0.51	0.57	0.30	0.41	0.38	0.65	0.35	0.61	0.44	0.34	0.85		–0.37	0.83	–0.09	0.55	0.46					
MCP-1	0.12	–0.31	0.03	–0.55	–	0.14	–0.50	0.02	0.20	0.35	–0.19	0.39	0.05	–0.09	0.05	–0.01	–0.12	–0.31	–0.37		–0.45	–0.51	–0.36	0.08					
MIP-1α	0.37	0.53	0.18	0.71	–	0.10	0.50	0.06	0.33	0.24	0.12	0.12	0.29	0.24	0.42	–0.02	0.07	0.64	0.83	–0.45		0.07	0.40	0.23					
RANTES	–0.23	–0.12	–0.33	0.16	–	–0.46	–0.16	–0.38	–0.44	–0.29	–0.29	–0.55	–0.51	–0.27	–0.36	–0.40	–0.15	0.06	–0.09	–0.51	0.07		–0.13	–0.24					
TNF-α	0.60	0.63	0.72	0.61	–	0.55	0.35	0.75	0.67	0.38	0.32	0.25	0.61	0.68	0.74	0.30	0.80	0.73	0.55	–0.36	0.40	–0.13							
VEGF	0.83	0.72	0.65	0.19	–	0.56	0.15	0.73	0.68	0.49	0.38	0.35	0.56	0.53	0.71	0.44	0.60	0.63	0.46	0.08	0.23	–0.24	0.69						

After Bonferroni adjusted level:
* $p < 0.00042$ in serum.
** $p < 0.00029$ in hippocampus.
*** $p < 0.00020$ in cortex.

may rejuvenate cognition and neurogenesis in aging rats by modulating immune and neuroimmune signaling pathways.

Although the exercise protocol employed may rejuvenate cognition and neurogenesis in aging rats by modulating immune and neuroimmune signaling pathways, the observed benefits may also be due to a caloric restriction associated with the exercise protocol. Exercise and caloric restriction beneficially affect a number of biological processes in aged rats that include the modulation of inflammatory signaling pathways (see Chung et al., 2009). However, in the current study any caloric restriction was voluntary and very mild producing body weight changes of less than 10% (compare masses in Section 2.1; Bondolfi et al., 2004; Lee et al., 2000; Van der Borght et al., 2007), which would also be consistent with an exercise-induced increase in fitness. Exercise is known to stimulate neurogenesis in young and aged animals (Albeck et al., 2006; Brown et al., 2003; Kobila et al., 2011; Parachikova et al., 2008; van Praag et al., 2005) while the effects of caloric restriction on neurogenesis may be limited to younger animals (Lee et al., 2000; Van der Borght, 2007; Bondolfi, 2004). Regardless, the inflammation associated with obesity and a sedentary lifestyle is thought to contribute to diseases of aging and an understanding how exercise with and without caloric restriction influences inflammatory signaling cascades will be important for the development of treatments.

Consistent with previous studies conducted using socially isolated animals, wheel running improved cognition (Albeck et al., 2006; Parachikova et al., 2008; van Praag et al., 2005) and amplified basal levels of hippocampal neurogenesis without altering the percentage of new cells that acquired neuronal or glial fates (Brown et al., 2003; Farmer et al., 2004; Kannangara et al., 2011, 2009; Kronenberg et al., 2006; Mustroph et al., 2012; Snyder et al., 2009). Although our multiple injection paradigm and ~16–21 d-long survival period cannot dissociate the between the effects of exercise on NPC proliferation versus the survivability of new cells, these data are consistent with those of several other studies showing that physical exercise increases NPC proliferation (Kempermann et al., 2010; van Praag et al., 1999). New neuron number tended to correlate positively with immediate and delayed water maze probe trial discrimination index scores but not inhibitory avoidance retention latency scores. Importantly, the 900 s latency ceiling employed in the inhibitory avoidance task may have masked the relationship between the memory of contextual information and new neuron number. We recently reported that new neuron number strongly correlated with discrimination index scores in environmentally and socially enriched aged rats and their controls (Speisman et al., 2012). While physical activity is typically considered to induce neurogenesis, environmental enrichment is typically considered to promote the survival and potentially the integration of new neurons into active hippocampal networks (Deisseroth et al., 2004; Deng et al., 2010; Gould et al., 1999a; Leuner et al., 2006; Stephens et al., 2012 but see Kobilo et al., 2011), which may more profoundly impact hippocampal integrity (Kempermann et al., 2010).

Fig. 8 illustrates a potential link between serum inflammatory markers and hippocampal cytokines associated with cognition and neurogenesis. Exercise-modulated circulating leptin level (Chennaoui et al., 2008; Novelli et al., 2004) correlated negatively with maze discrimination index scores, new neuron number and the hippocampal expression of IL-18 but positively with hippocampal IL-1β expression. Although leptin's pleiotropic effects are typically associated with energy regulation, recent work suggests that leptin can directly stimulate the proliferation of neural progenitor cells both *in vitro* and *in vivo* (Garza et al., 2008; Perez-Gonzalez et al., 2011). These data would suggest that serum leptin influences hippocampal neurogenesis in the aging rat through an intermediary signaling molecule or simply that the serum leptin

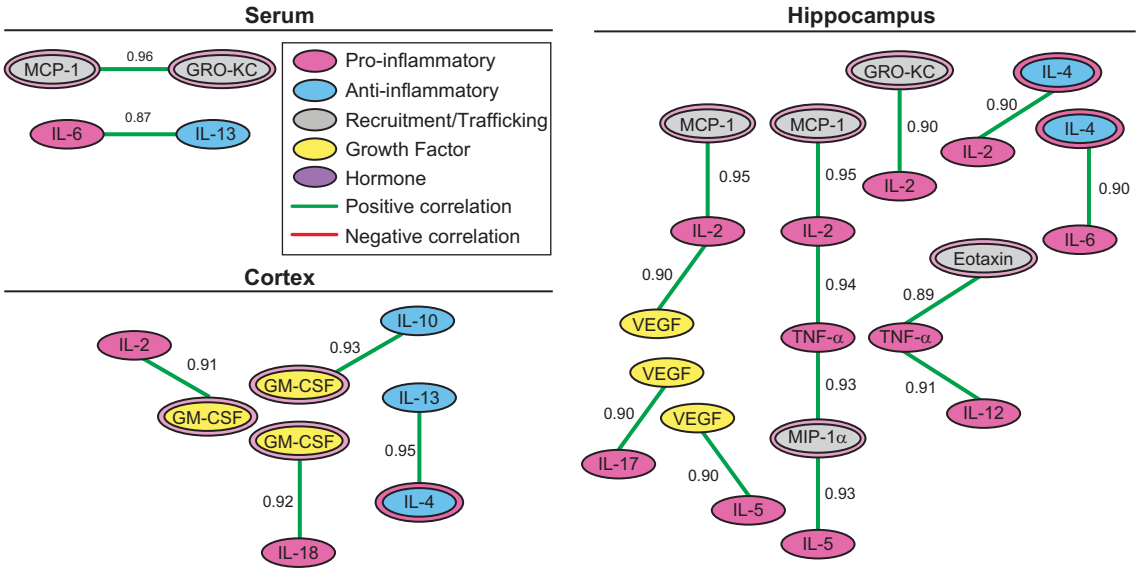

Fig. 7. Cytokine clusters detected in the serum, hippocampal and cortical samples obtained from aging rats. To confirm and expand upon known cytokine pathways, we examined cytokines with concentrations that changed in a coordinated fashion. Cytokine pairs were plotted in descending order based upon Spearman *r* values deemed statistically significant after Bonferroni corrections. If one cytokine in a correlated pair about to be plotted was already part of a plotted cluster, and the unplotted cytokine was correlated with all cytokines in the plotted cluster, then the new pair was added to the cluster. If the unplotted cytokine of the pair about to be plotted was not significantly correlated with all cytokines in the existing cluster, the pair was plotted as a new cluster. Proteins are color coded by their known primary function and green and red lines represent positive and negative correlations, respectively. We detected two serum cytokine clusters, eight hippocampus cytokine clusters and four cortical clusters in aging rats. Note that no between-compartment clusters indicative of immune-to-brain signaling pathways modulated by running in aged rats were detected. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Measures of several variables significantly modulated by daily exercise in aging rats correlate. Spearman rank correlation coefficients (r_s) were calculated test the strength of the relationships between concentrations of serum (S), hippocampal (H) and cortical (C) cytokines, and measures of spatial and hippoocampal neurogenesis that were significantly modulated by conditioned running.

	Serum			Hippocampus			Cortex	Neurogenesis and behavior			
	MCP-1	leptin	GRO-KC	IL-1β	IL-18	GRO-KC	VEGF	New Neuron #	Immed. DI	24hr DI	24hr IA
MCP-1 (S)		0.65†	0.96***	0.54	−0.36	−0.14	0.13	−0.53	−0.81**	−0.39	−0.30
Leptin (S)	0.65†		0.56†	0.76*	−0.61†	−0.58†	0.52	−0.77**	−0.73*	−0.78**	−0.46
GRO-KC (S)	0.96***	0.56†		0.42	−0.29	−0.20	0.07	−0.41	−0.75**	−0.48	−0.18
IL-1β (H)	0.54	0.76*	0.42		−0.60*	−0.25	0.57†	−0.58†	−0.58†	−0.68*	−0.63*
IL-18 (H)	−0.36	−0.61†	−0.29	−0.60*		0.85***	−0.51	0.94***	0.51	0.54	0.16
GRO-KC (H)	−0.14	−0.58†	−0.20	−0.25	0.85***		−0.37	0.79**	0.38	0.41	−0.11
VEGF (C)	0.13	0.52	0.07	0.57†	−0.51	−0.37		−0.43	−0.64*	−0.51	−0.60*
New Neuron #	−0.53	−0.77**	−0.41	−0.58†	0.94***	0.79**	−0.43		0.59†	0.55†	0.18
Immed. DI	−0.81**	−0.73*	−0.75**	−0.58†	0.51	0.38	−0.64*	0.59†		0.47	0.45
24-h DI	−0.39	−0.78**	−0.48	−0.68*	0.54	0.41	−0.51	0.55†	0.47		0.62*
24-h IA	−0.30	−0.46	−0.18	−0.63*	0.16	−0.11	−0.60†	0.18	0.45	0.62*	

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
† $0.05 < p < 0.01$.

levels detected in aged rats exceed those found in healthy young animals to the point that they become detrimental. Consistent with the latter notion, exercise decreased leptin levels in our aging rats. Indeed, leptin is emerging as a potential immune-to-brain signaling mediator (Hosoi et al., 2002) because leptin levels are elevated by peripheral inflammatory stimuli (Mastronardi et al., 2005; Sarraf et al., 1997) that incidentally decrease neurogenesis (Monje et al., 2003) and leptin treatment increases brain levels of hippocampal IL-1β (Hosoi et al., 2002). Alternatively, serum leptin concentration in the current study may simply be a marker for an immune signaling cascade containing the molecule that affects neurogenesis.

Indeed, serum GRO-KC and MCP-1 levels were also decreased by exercise, strongly correlated with one another, and independently (along with leptin) correlated with the acquisition of a spatial search strategy in the water maze (Tables 1 and 3 and Fig. 8). Previous work has shown that MCP-1 levels that are elevated by high fat diet-induced obesity in young mice, can be reduced by daily exercise (Kizaki et al., 2011). Recently, Villeda and colleagues found that circulating CCL2, along with eotaxin (CCL11), MCP-5 (CCL12), MIP-3β (CCL19), haptoglobin and β_2 microglobulin levels were related to age-impaired neurogenesis and performance in a working/reference memory radial water maze task. They then showed that circulating eotaxin levels alone could impair neurogenesis, synaptic plasticity, working/reference memory and contextual fear conditioning (Villeda et al., 2011). We neither detected an effect of exercise on circulating eotaxin levels, nor relationships between circulating eotaxin levels and measures of neurogenesis or water maze performance in aging rats. However, age-related changes in circulating eotaxin may be species-dependent

Please cite this article in press as: Speisman, R.B., et al. Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. Brain Behav. Immun. (2012), http://dx.doi.org/10.1016/j.bbi.2012.09.013

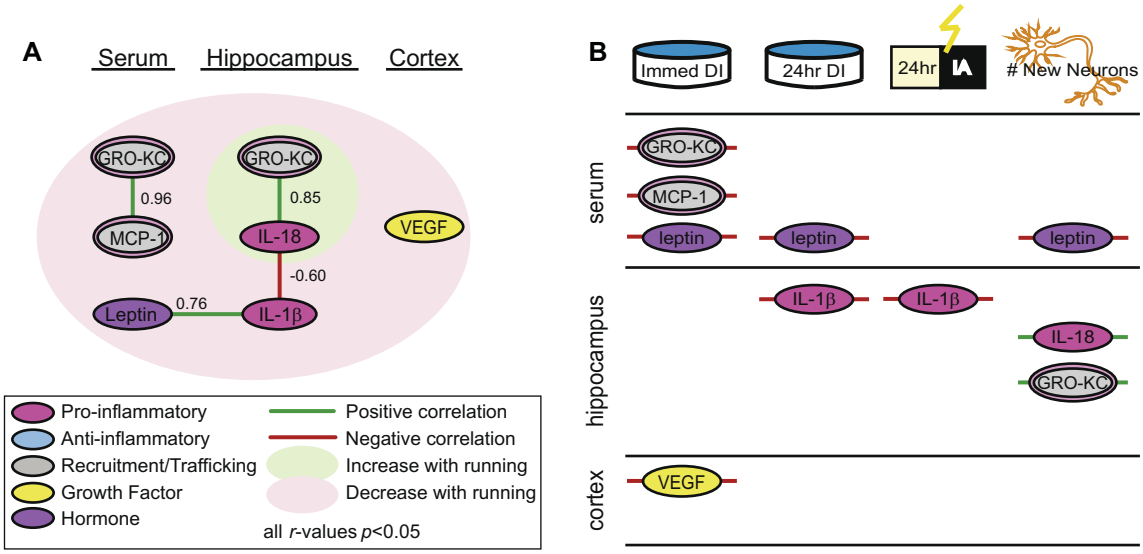

Fig. 8. Some cytokines are modulated in a coordinated fashion by conditioned running in aging rats and relate to measures of hippocampus-dependent behavior and hippocampal neurogenesis. Spearman rank correlations were run on immune and neuroimmune cytokines with concentrations that were modulated by running (see Table 1), water maze DI scores, inhibitory avoidance retention latencies and total new neuron number. Of the cytokines altered by daily exercise, several were modulated in a coordinated fashion. Cytokines are color-coded to denote their primary, typically systemic, known function. Concentrations increased by running are plotted in the green circle while those that decrease are plotted in the red circle. Negatively correlated cytokines are linked with red lines while positively correlated cytokines are linked with green lines. (B) Depicts relationships between cytokines, behavioral measures and measures of neurogenesis that were modulated by running. Water maze discrimination index scores, inhibitory avoidance 24 h retention latencies and new neuron number were significantly affected by conditioned running. Note that only statistically significant correlations ($p < 0.05$) are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

or relate to cognition and neurogenesis by interacting with another variable that differed between studies. Nonetheless, exploring the molecular mechanisms by which circulating factors, such as leptin, GRO-KC, MCP-1 and perhaps eotaxin relate to measures of cognition and plasticity are important future research avenues.

Another important finding of the current study is the compartmental specificity of exercise-associated changes in cytokine levels. After several months of daily exercise, leptin levels decreased only in serum and IL-1β was reduced and IL-18 increased only in the hippocampus (Table 1). A previous report also found that daily treadmill exercise decreases hippocampal, but not circulating, cortical, cerebellar or pituitary levels of IL-1β in young rats (Chennaoui et al., 2008). Our data extends these findings to aging rats, by showing that hippocampal, but not cortical or circulating IL-1β levels are reduced by exercise. Furthermore, hippocampal IL-1β expression was correlated positively with serum leptin and negatively correlated with both water maze and inhibitory avoidance memory scores (Table 3 and Fig. 8). Leptin is actively transported across the blood–brain-barrier (Morrison, 2009) and previous work has demonstrated that leptin treatment increases IL-1β in the hippocampus (Hosoi et al., 2002), providing one possible mechanism for the observed relationship. Moreover, the inverse relationship between IL-1β and cognition is consistent with a growing body of work indicating that elevated hippocampal IL-1β levels impair memory and synaptic plasticity in young and aged rats (Barrientos et al., 2009, 2006, 2003; Chennaoui et al., 2008; Hein et al., 2010; O'Callaghan et al., 2009). Together these data suggest that hippocampal IL-1β concentration may be a reliable biomarker of mnemonic decline and, along with circulating leptin, a target for nootropic drug development.

In contrast to IL-1β, IL-18 and GRO-KC levels were increased in the hippocampus of the exercise group. Hippocampal IL-18 and GRO-KC concentrations correlated positively with one another and independently with new neuron number, while hippocampal IL-18 concentration correlated negatively with hippocampal IL-1β concentration (Fig. 8). Daily exercise has been shown previously to potentiate hippocampal GROα (murine CXCL1) mRNA levels in aging Tg2576 mice that express human amyloid protein (Parachikova et al., 2008). Interestingly, these mice also exhibited improved water radial arm maze performance and decreased hippocampal IL-1β levels (Nichol et al., 2008; Parachikova et al., 2008). GRO-KC stimulates adult rat spinal cord oligodendrocyte (Robinson et al., 1998) and fetal ventral midbrain precursor (Edman et al., 2008) proliferation and IL-18 may attenuate neuronal differentiation in cultured fetal rat-derived neural progenitor cells (Liu et al., 2005), but this is the first report of a relationship between either factor and adult NPC behavior, to our knowledge. Conflicting reports suggest that IL-18 promotes neuroprotection and spatial ability (Ryu et al., 2010; Yaguchi et al., 2010.) but also age-related cognitive decline (Blalock et al., 2003; Mawhinney et al., 2011). Our data showing that exercise-increased hippocampal IL-18 levels correlate positively with new neuron number (which correlate positively with spatial ability in aging rats; Table 2 and Speisman et al., 2012) are consistent with the former notion. The question remains as to how IL-18 could be linked to improved hippocampal function. One possibility is that exercise may improve hippocampal health and stimulate neurogenesis in the aging brain by improving vascular health (Palmer et al., 2000). GRO-KC and IL-18 exhibit pro-angiogenic properties and are linked through ras-raf-ERK-MAPK signaling (Park et al., 2001; Zhong et al., 2008). Although we did not observe an exercise-induced shift in hippocampal VEGF levels, previous research indicates that running potentiates hippocampal neurogenesis through VEGF activity in young mice (Fabel et al., 2003; Tang et al., 2010) and increases hippocampal VEGF levels in middle-aged mice (Latimer et al., 2011). However, VEGF levels are known to decline in the aged brain (Shetty et al., 2005) and may require rejuvenation before exercise can potentiate neurogenesis beyond a basal level (Fig. 6 versus Speisman et al., 2012). Note that although every analyte measured was detected in the hippocampus and/or cortex (from which similar amounts of protein were harvested), the volume of protein obtained from ½ of the hippocampus could have been too small to

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quantify changes in the concentration of low-level analytes, such as VEGF.

Our cluster analyses (see Fig. 7) further suggest that cytokine signaling in aging rat runners and their controls is compartmentalized. In serum, we found strong positive correlations between GRO-KC and MCP-1 and between IL-6 and IL-13 concentrations. Coordinated monocyte/macrophage-derived serum MCP-1 and GRO-KC concentrations have been reported in models of wound repair-induced angiogenesis and in the serum of LPS-treated mice (Barcelos et al., 2004; Erickson and Banks, 2011) and coordinated circulating IL-13 and IL-6 concentrations may be consistent with the heightened T_h2 response hypothesized to occur with age (Grolleau-Julius et al., 2010). Although IL-13 was detected in cortical clusters and GRO-KC and MCP-1 were detected hippocampal clusters we did not detect between-compartments correlations that would indicate direct diffusion or transport across the blood–brain-barrier to either location.

In the brain, we detected correlated concentrations of cytokines that were distinct in the cortex and hippocampus. In the cortex, concentrations of structurally and functionally homologous IL-13 and IL-4, which are expressed by microglia and typically associated with anti-inflammatory and neuroprotective effects in the brain were correlated (Opal and DePalo, 2000; Ponomarev et al., 2007; Shin et al., 2004). Cortical GM-CSF was detected in separate clusters with IL-2, IL-10 and IL-18. Astrocytic GM-CSF acts on its receptors expressed by microglia and oligodendrocytes (Kimura et al., 2000). IL-2 and its receptor protein is thought to be expressed by neurons, glia and microglia while IL-18 mRNA is expressed by microglia with its receptors being expressed by neurons, astrocytes and microglia throughout the brain (Hanisch and Quirion, 1995; Tambuyzer et al., 2009). While the relationship between brain IL-2 and GM-CSF is unclear, IL-10 decreases but IL-18 increases GM-CSF production by peripheral immune cells and IL-10 may suppress microglial inflammatory responses (Lee et al., 2010).

In the hippocampus, VEGF correlated independently with IL-2 and MCP-1, IL-5, and finally IL-17. Endothelial VEGF production can be stimulated by IL-2, and microglial, endothelial cell or smooth muscle cell MCP-1 expression in response to vascular injury (Parenti et al., 2004). IL-5 and IL-17, often associated with allergic reactions, can also stimulate VEGF production. Interestingly, the injury-induced expression of MIP-1α, MCP-1, GM-CSF, and TNF-α and in some cases IL-1β appears to recruit macrophages and induce phagocytosis (Ousman and David, 2001). The coordinated concentration of IL-2 with this cluster is interesting because IL-2 is often associated with self-recognition (Kolls and Linden, 2004). IL-2 and IL-4 are co-regulated by exhaustive acute exercise in muscle and in serum presumably to stimulate repair processes (Rosa Neto et al., 2011). Relationships between IL-2 and IL-4, IL-2 and GRO-KC, and between IL4 and IL-6 that we detected in the hippocampus have not yet been reported in the brain, to our knowledge. Future work that confirms and expands these regionally distinct neuroimmune signaling pathways and tests their effects in the aging brain will be critical for understanding their impact on cognition and plasticity. Certainly, regional changes in other signaling systems are under exploration in the aging brain (for example, see McQuail et al., 2012).

5. Implications

Daily exercise improved spatial/contextual ability, perhaps by stimulating hippocampal plasticity in the form of neurogenesis and by modulating immune and neuroimmune signaling. Daily exercise was associated with the decreased expression of factors that correlated negatively with learning, memory and neurogenesis measures but the increased expression of factors that correlated positively with our neurogenesis measure. The picture of how immune and neuroimmune signaling impacts cognition and plasticity is growing. We add to this picture by showing that exercise modulates factors distinctly in serum and in the brain, suggesting that immune factors do not appreciably diffuse or are transported into the brains of aging rats that exercise and their controls. We also found that exercise modulated neuroimmune factors distinctly in the cortex and hippocampus, which supports the notion that in the brain, neuroimmune signaling is region-specific. Serum leptin emerged as a biomarker for both brain and cognitive aging. Along with serum leptin, serum MCP-1 and GRO-KC levels may predict spatial ability. We confirmed that hippocampal IL-1β levels may be a marker of mnemonic ability and we discovered that hippocampal IL-18 and GRO-KC levels correlated positively with neurogenesis. In summary, our work suggests that engaging in physical activity may reverse some aspects of age-related cognitive decline, perhaps by stimulating neurogenesis and by modulating beneficial and detrimental aspects of immune and neuroimmune signaling. Our correlational data begin to provide a framework for systematically manipulating these immune and neuroimmune signaling molecules to test their effects on cognition and neurogenesis across lifespan in future experiments.

Acknowledgments

The authors thank Dr. Gerry Shaw for his gift of anti-chicken GFAP. The study was supported by grants from the National Institutes of Health (AG014979, AG037984) to T.C.F., the Evelyn F. McKnight Brain Research Foundation and NIH Grant AG038600 to T.C.F. and B.K.O., and the Broad Foundation for Biomedical Research to B.K.O. R.B.S. was the recipient of a National Science Foundation Graduate Research Fellowship (DGE-0802270).

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Please cite this article in press as: Speisman, R.B., et al. Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. Brain Behav. Immun. (2012), http://dx.doi.org/10.1016/j.bbi.2012.09.013

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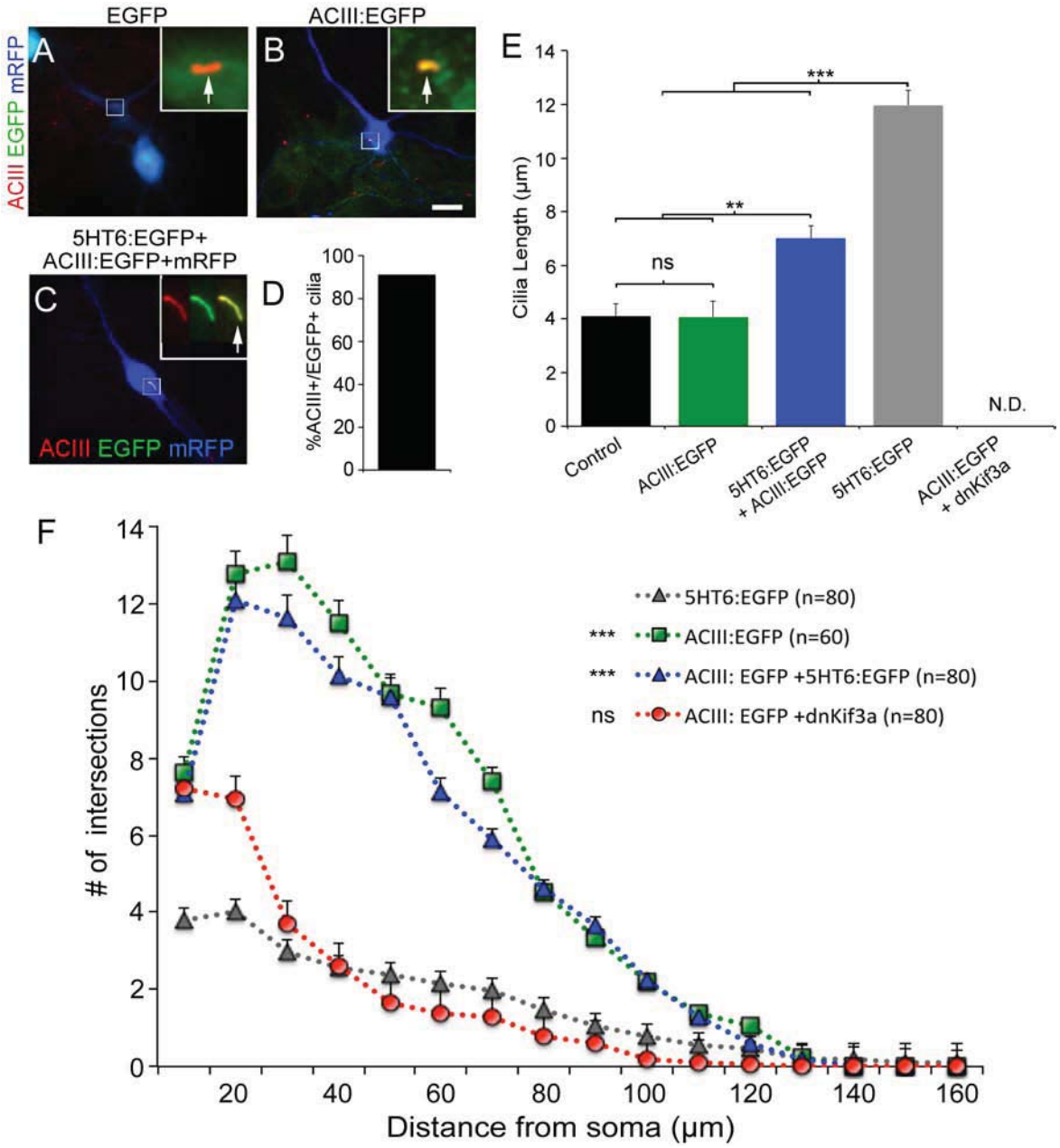
EGFP
ACIII:EGFP
A
B
ACIII EGFP mRFP
5HT6:EGFP+
ACIII:EGFP+mRFP
C
ACIII EGFP mRFP
D
%ACIII+/EGFP+ cilia
100
80
60
40
20
0
E
Cilia Length (µm)
14
12
10
8
6
4
2
0
***
**
ns
N.D.
Control
ACIII:EGFP
5HT6:EGFP
+ ACIII:EGFP
5HT6:EGFP
ACIII:EGFP
+ dnKif3a
F
# of intersections
Distance from soma (µm)
20
40
60
80
100
120
140
160
5HT6:EGFP (n=80)
*** ACIII:EGFP (n=60)
*** ACIII: EGFP +5HT6:EGFP (n=80)
ns ACIII: EGFP +dnKif3a (n=80)

The Journal of Neuroscience

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Arborization of Dendrites by Developing Neocortical Neurons is Dependent on Primary Cilia and Type 3 Adenylyl Cyclase

JN-RM-2906-12R2

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Commercial Interest: No

Arborization of Dendrites by Developing Neocortical Neurons is Dependent on Primary Cilia and Type 3 Adenylyl Cyclase

Abbreviated title: Cilia and Dendrite Formation in Developing Cortex

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Number of figures: 7
Number of tables: 1
Number of words in: Abstract (246), Introduction (622), Discussion (1,638)

Keywords: cilium, pyramidal neuron, neocortex, neurites, intraflagellar transport, axoneme, cerebral cortex, GPCR

Acknowledgements
We would thank J. Eggenschwiler for the TULP3 antibody and S. Ge for the dnKif3a cDNA construct. We also thank D. Siebzehnrubl, and D. Smith in the Cell and Tissue Analysis Core for technical assistance. This work was supported by funds from the Bryan Robinson Endowment (to S.M.G.), the McKnight Brain Research Foundation and University of Florida College of Medicine (to M.R.S.).

ABSTRACT

The formation of primary cilia is a highly choreographed process that can be disrupted in developing neurons by overexpressing neuromodulatory G protein-coupled receptors (GPCRs) or by blocking intraflagellar transport (IFT). Here, we examined the effects of overexpressing the ciliary GPCRs, 5HT6 and SSTR3, on cilia structure and the differentiation of neocortical neurons. Neuronal overexpression of 5HT6 and SSTR3 was achieved by electroporating mouse embryo cortex *in utero* with vectors encoding these receptors. We found that overexpression of ciliary GPCRs in cortical neurons, especially 5HT6, induced the formation of long (>30μm) and oftentimes forked cilia. These changes were associated with increased levels of IFT proteins and accelerated ciliogenesis in neonatal neocortex, the induction of which required Kif3a, an anterograde motor critical for cilia protein trafficking and growth. GPCR overexpression also altered the complement of signaling molecules within the cilia. We found that SSTR3 and type III adenylyl cyclase (ACIII), proteins normally enriched in neuronal cilia, were rarely detected in 5HT6-elongated cilia. Intriguingly, the changes in cilia structure were accompanied by changes in neuronal morphology. Specifically, disruption of normal ciliogenesis in developing neocortical neurons, either by overexpressing cilia GPCRs or a dominant negative form of Kif3a (dnKif3a), significantly impaired dendrite outgrowth. Remarkably, co-expression of ACIII with 5HT6 restored ACIII to cilia, normalized cilia structure, and restored dendrite outgrowth, effects that were not observed in neurons co-expressing ACIII and dnKif3a. Collectively, our data suggest the formation of neuronal dendrites in developing neocortex requires structurally normal cilia enriched with ACIII.

INTRODUCTION

Interest in the biological functions of the primary cilia of cortical neurons and their influence on neuronal maturation and cortical development has dramatically increased in recent years (Louvi and Grove, 2011), in part because several developmental and neurological disorders have now been linked to defects in ciliogenesis (Green and Mykytyn, 2010; Bennouna-Greene et al., 2011; Lee and Gleeson, 2011; Novarino et al., 2011; Sattar and Gleeson, 2011). As a first step toward understanding the impact that primary cilia have on neuron function and maturation, we characterized the time course of neuronal ciliogenesis in developing mouse neocortex (Arellano et al., 2012). Neuronal ciliogenesis progresses through several stages in post-migratory neurons, the first of which is distinguished by the appearance of the "procilium" that is formed as a result of outgrowth of the early stage ciliary plasma membrane (Arellano et al., 2012). Shortly after birth, the elongation stage begins during which time microtubules within the procilium assemble and organize to form the axoneme that continues to elongate over the first 8-12 weeks of life. During the procilium and elongation stages of ciliogenesis, the cilia become enriched with signaling enzymes (e.g. type 3 adenylyl cyclase (ACIII)(Berbari et al., 2007; Bishop et al., 2007; Anastas et al., 2011; Arellano et al., 2012)), nerve growth factor receptors (e.g. $p75^{NTR}$ (Chakravarthy et al., 2010)), and specific GPCRs (e.g. 5HT6, SSTR3, MCHR1, and D1(Handel et al., 1999; Brailov et al., 2000; Miyoshi et al., 2006; Berbari et al., 2008a; Berbari et al., 2008b; Stanic et al., 2009; Marley and von Zastrow, 2010)) that enable the cilia to respond to ligands in the extracellular environment. The process of intraflagellar transport (IFT) not only promotes neuronal ciliogenesis but also supports the bidirectional trafficking of these ciliary signaling molecules within the cilium.

There is growing evidence that disruption of the formation or function of neural cilia adversely affects neuronal differentiation. For example, in neurons born in the adult hippocampus, blocking the function of Kif3a, an anterograde motor protein that is required for CNS ciliogenesis (Chizhikov et al., 2007; Davenport et al., 2007; Han et al., 2008; Spassky et al., 2008), has been reported to disrupt dendritic arborization of these neurons and their synaptic integration into the hippocampus (Kumamoto et al., 2012). In addition, overexpression of the doublecortin domain-containing protein 2 (DCDC2) in cultured rat hippocampal neurons, a protein that interacts with Kif3a, has been reported to induce cilia elongation and alter the dendritic branching of these neurons (Massinen et al., 2011). These findings raise the question as to whether the growth and differentiation of other cortical neuron subtypes are altered by disruption of their cilia. For example, while targeted ablation of cilia hindered dendritic outgrowth in adult-born hippocampal granule neurons (Kumamoto et al., 2012), ablating cilia in post-migratory cortical interneurons did not significantly alter differentiation (Higginbotham et al., 2012). Given the range of these observations, we wondered whether cilia regulate the differentiation and maturation of projection neurons in developing neocortex.

The purpose of this study was to investigate the relationship between ciliogenesis and the differentiation of neocortical neurons in developing cortex. We hypothesized that disruption of ciliogenesis in developing neocortical neurons would induce abnormal dendritic outgrowth. In this study, we overexpressed neuronal cilia GPCRs in developing neocortical neurons to disrupt ciliogenesis; a strategy that we found induces significant lengthening of primary cilia of immortalized cells. We found that neurons overexpressing cilia GPCRs developed exceedingly long, malformed cilia, and that dendrite outgrowth from

these neurons was severely stunted in a manner similar to that observed for neurons lacking cilia. Moreover, our findings suggest that changes in the complement of signaling proteins present in the cilia that were induced by overexpression of the GPCRs contributed to the abnormal dendritic phenotype exhibited by these neurons.

METHODS

Mice

All animal protocols were approved by and carried out in accordance with the Institutional Animal Care and Use Committee at the University of Florida. CD1 mouse brains were collected on embryonic (E) day 14.5 (n=9), E16.5 (n = 92), postnatal (P) day 1 (n=5), P10 (n=10), and P14 (n= 37). Postnatal mice of either sex were intracardially perfused with saline followed by 4% paraformaldehyde in 0.1M phosphate buffer solution (4% PFA). All brain tissues were post-fixed overnight in 4% PFA at 4°C. Following fixation, the brains were rinsed, cryoprotected in sucrose, frozen over liquid N_2, and sectioned (40-50μm coronal) using a cryostat.

In Utero Electroporation

Vectors were delivered to the developing cortices of E15.5 mice using in utero electroporation (IUE) as previously described (Sarkisian et al., 2006). Briefly, at 15.5 days into gestation, female mice were anesthesized by intraperitoneal injections of ketamine (100mg/kg) and xylazine (10mg/kg) diluted in sterile saline. Mice received Meloxicam (1mg/kg) as an analgesic. The uterine horns were exposed and approximately 1μl of DNA

([0.5-2μg/μl] mixed with 0.025% Fast-Green) was microinjected through the uterine wall into the cerebral lateral ventricles of the mouse embryos using pulled glass capillaries. Electroporation was achieved by discharging 50V across the cortex in 5-pulse series spaced 50msec apart (pulse duration =950msec) using a BTX ECM 830 Square Wave Electroporator. Following injections, the dams were sutured and allowed to recover on heating pads.

Vectors

Table 1 describes the vectors used in this study and their promoter/protein tag information if applicable. pEGFP-N3 vectors were constructed that encoded either mouse SSTR3 or mouse 5HT6 that were fused to EGFP on the C-termini. The expression of SSTR3:EGFP and 5HT6:EGFP in these vectors was controlled by a human CMV immediate early promoter. Lentiviral vectors were generated that encoded mCherry tagged with AU1 upstream of dnKif3a, or either SSTR3 or 5HT6 that were fused to EGFP on the C-termini. mCherry(AU1) and dnKif3a, SSTR3:EGFP or 5HT6:EGFP in these vectors were fused to each other using the pTV1 2A cleavage peptide. The expression of all lentiviral transgenes was controlled by an elongation factor 1 alpha (EF1α) promoter (Verrier et al., 2011). Additional 5HT6 vectors used included pcDNA3.1-HA:5HT6 encoding 5HT6 with a HA tag fused to the N-terminus, and two signaling defective 5HT6 receptor constructs 5HT6 (D72A):EGFP and 5HT6 (K265A):EGFP, which have been previously described (Kang et al., 2005; Zhang et al., 2006). The K265A point mutation completely abolishes the signaling capabilities of the 5HT6 receptor and reduces cAMP levels to 5% of those found in wild type cells. The D72A point mutation reduces the binding affinity of the 5HT6 receptor for

5HT and reduces downstream activation of adenylyl cyclase III by 60%. The PKHD-1C1-68 vector encodes 68 amino acids immediately downstream of the transmembrane domain of fibrocystin, the human autosomal recessive polycystic kidney disease protein. These 68 amino acids, which include the 18-residues near the N-terminus that contains the cilia targeting sequence, were fused to the N-terminus of EGFP (Follit et al., 2010). The PC2-TRFN vector encodes the cilia targeting sequence of polycystin-2 (PC2) (Geng et al., 2006) fused to the first 61 amino acids of the N-terminus of the human transferrin receptor (hTFR), all of which were fused to the N-terminus of EGFP. The transferrin receptor (hTFR) imports iron into cells and is not normally trafficked to the cilium (Geng et al., 2006; Avasthi et al., 2012). The vector encoding full-length mouse type 3 adenylyl cyclase (ACIII) was fused to the C-terminus (ACIII:EGFP) and under control of the ubiquitin-C (UbiC)promoter. All control experiments were performed using pCAGGS-EGFP, pCAGGS-mRFP, or EF1α-EGFP-2A-mCherry expressing vectors.

Cell Culture

NIH3T3 cells were seeded onto glass coverslips in DMEM supplemented with 10% fetal bovine serum (FBS) and 1X antibiotic-antimycotic solution (ABX, Life Technologies) (cDMEM). Cells were seeded into 24-well plates at a density of 3.6 x 10^6 cells/well and were transfected 24 hours later. The cells were transfected with the indicated cDNAs (0.8µg/well) using Lipofectamine™2000 (Life Technologies) in serum free DMEM (Mediatech Cellgro # 10-013-CV). After 4-6 hours, the transfection media was replaced with cDMEM and the cells were allowed to grow for 48 hrs at 37°C in 5% CO_2. After 48 hrs

of growth, the cells were fixed in 4% PFA for 15 min at room temperature (RT) and washed 3X with PBS.

Electroporated mRFP or EGFP+ neurons located in the dorsal telencephalon were dissected from the brains of E16.5 mice (6-8 fetal cortices/group) and were placed into ice-cold Hanks Balanced Salt Solution (HBSS) containing 25mM HEPES buffer and 0.5% (w/v) glucose. The tissues were transferred into pre-warmed Trypsin LE™ solution (Life Technologies) supplemented with 10mM HEPES and were then dissociated by trituration with a fire-polished glass pipette. The dissociated cells were re-suspended in Neurobasal medium (Life Technologies) supplemented with 2μM sodium pyruvate, 4μM L-glutamine, 1X antibiotic-anti-mycotic liquid (Life Technologies), 5% FBS, and 2% (v/v) B27 (Life Technologies) and were seeded at a density of 1.5 x 10^5 cells/well in 24-well plates containing sterile glass coverslips that had been coated with poly-ornithine (0.001%) and laminin (5μg/ml). To promote neuron differentiation, 50% of the culture media was replaced with media lacking serum 24 hr after seeding followed by fifty percent of the media being replaced every other day. Throughout the experiment the cultures were maintained at 37°C in 5% CO_2. Neurons were cultured for up to 12 days in vitro (DIV) and were then fixed in 4% PFA for 15 min at RT and washed 3X with PBS.

Immunostaining

Cultured cells or brain cryosections were incubated overnight at 4°C with the following primary antibodies: mouse anti-acetylated α-tubulin (1:2000; Sigma), rabbit anti-adenylyl cyclase (ACIII) (1:10,000; EnCor BioTechnology, 1:1000; Santa Cruz), mouse anti-pericentrin (1:500; BD Biosciences), rabbit anti-IFT88 (1:500; Covance), chicken anti-GFP

(1:5,000; Abcam), and goat anti-SSTR3 (1:200; Santa Cruz). Appropriate, species-specific, secondary antibodies conjugated to fluorescent tags were used to visualize the primary antibodies (1:200; JacksonImmunoResearch). Immunostained sections were cover slipped using ProLong Gold Antifade media containing 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) (Life Technologies).

Analyses and Quantification of Cilia

Immunostained cell cultures and brain sections were examined using an Olympus IX81-DSU spinning disc confocal microscope. Z-stack images (0.5-0.75 μm per step) of ACIII, acetylated α-tubulin or EGFP-positive cilia were collected and then collapsed to create maximum projection images that were saved as tiff files and subsequently analyzed using Image J64 (http://rsbweb.nih.gov/ij/). We analyzed the brains of P1 and P14 electroporated mice (6-8 collapsed z-stack images/brain). Cilia lengths were measured in pixels by tracing ACIII+, α-tubulin+ or EGFP+ structures. The pixel values were converted to microns and were plotted as the mean ± SEM for each group. A One-Way ANOVA (with Fisher's PLSD post-hoc analysis) was used to compare groups. A p-value <0.05 was considered significant.

Western Blots

Protein lysates were prepared from mouse cortex by homogenizing tissue in 1X RIPA buffer (Cell Signaling Technology). Lysate samples (10 μg total protein/lane) were separated on 4-12% NuPAGE gels (Life Technologies) and transferred onto PVDF membranes using an iBlot (Life Technologies). Blots were blocked in Tris-buffered saline containing 0.1% Tween (TBST) and 5.0% BSA (w/v) for 1h at RT and were then incubated

overnight at 4°C with one of the following primary antibodies diluted in TBST containing 2.5% BSA: rabbit anti-Kif3a (1: 1000; Protein Tech); rabbit anti-TULP3 (1: 500; gift from J Eggenschwiler); rabbit anti-cytoplasmic dynein (74.1) (1:5000; Covance); rabbit anti-IFT88 (1:1500, Covance); mouse β-actin (1:10,000; Sigma). The next day, the blots were washed with TBST, incubated for 1hr at RT with appropriate HRP-conjugated secondary antibodies (1;10,000; BioRad), and were developed using an ECL-Plus chemiluminescence kit according to the manufacturer's instructions (GE HealthCare). Images of the blots were captured and analyzed using an Alpha Innotech FluorChemQ Imaging System (ProteinSimple). The signal intensities of the protein bands of interest were quantified and normalized to the intensity of the β-actin on the same blot which served as a loading control. Each protein sample was analyzed a minimum of three times.

Sholl Analysis

To visualize cell morphology, cells were electroporated in utero with vectors encoding either EGFP or mRFP alone [0.5μg/μl], or one of these vectors was paired with a vector encoding either ACIII:EGFP, 5HT6:EGFP, SSTR3:EGFP, EGFP:dnKif3a, or mCher(AU1)-2A-dnKif3a [2μg/μl]. Sholl analyses were carried out as previously described (Sarkisian and Siebzehnrubl, 2012). Briefly, from several experiments, fixed EGFP+ or mRFP+ cultured neurons selected from 4-8 coverslips (~10-15 neurons/coverslip) were photographed using an Olympus IX81 spinning disc confocal microscope fitted with a 40x dry objective. All raw images of EGFP+ or mRFP+ cells were resampled to obtain smaller, higher resolution grayscale images (e.g. 18.667"x14.222" at 72 pixels/in to 3"x2.286" at 300 pixels/in). The grayscale images were thresholded to create high-contrast, binary images

using Adobe Photoshop (Version 11.01) and were saved as tiff files. Images were then opened in Image J64 (http://rsbweb.nih.gov/ij/) and analyzed using the Sholl Analysis plugin. The intersections of each cell's processes with concentric rings placed every 10 μm up to 200 μm from a point positioned in the center of each soma was counted and the means of the various treatment groups were compared using Two-Way ANOVA (with Fisher's PLSD posthoc analysis), with $p < 0.05$ considered significant.

RESULTS

Overexpression of neuronal cilia GPCRs in developing mouse cortical neurons dramatically increases cilia length and disrupts cilia morphology

In this study we targeted neocortical neurons and their cilia by delivering ciliary genes to developing E15.5 neural progenitors using in utero electroporation. The effects of expression of these genes by the pyramidal neurons were examined in culture or in situ at different postnatal stages of neocortical development (Fig. 1A). We found that expression of control plasmids encoding cytoplasmic EGFP by electroporated neurons did not induce any gross morphological changes in the cilia of these cells or alter the normal position of the cilia on the soma, which emerges near the base of the apical dendrite (Fig. 1B). In addition, the staining patterns for ACIII and pericentrin in EGFP-positive, control neurons and neighboring non-electroporated pyramidal neurons in layers 2/3 were similar; ACIII was enriched in the axoneme and pericentrin was localized to the basal body (Anastas et al., 2011; Arellano et al., 2012) (Fig. 1B). We also observed that cytoplasmic EGFP was not detected in ACIII-positive cilia (Fig. 1B).

5HT6 and SSTR3 receptors are normally expressed in the neocortex of early postnatal brain (Breunig et al., 2008; Riccio et al., 2009; Stanic et al., 2009; Anastas et al., 2011) and localize to the plasma membranes of neuronal primary cilia (Hamon et al., 1999; Handel et al., 1999; Brailov et al., 2000). We previously noted that overexpression of GPCRs in immortalized cell lines induced abnormal growth of the cilia of these cells. In this experiment, we used in utero electroporation to deliver bicistronic 2A vectors encoding mCherry and either SSTR3:EGFP or 5HT6:EGFP to the brains of E15.5 mice to determine if similar changes in the cilia of developing neurons would occur if we overexpressed GPCRs in these cells. Examination of electroporated neurons in layers 2/3 of P14 neocortex revealed that both 5HT6:EGFP and SSTR3:EGFP were trafficked into the cilia (Fig. 1 C, D); however, unlike 5HT6:EGFP, SSTR3:EGFP also appeared to be diffusely distributed throughout the somas and neurites of the electroporated cells (Fig. 1D). Notably, the cilia elaborated by neurons expressing 5HT6:EGFP were not only significantly longer than those of neurons expressing SSTR3:EGFP, but were also abnormally branched (Fig. 1 E, F). The robust primary and secondary order branching of 5HT6:EGFP+ cilia occurred at varicosities that were distributed along the lengths of the cilia (Fig. 1C, G). Branching of the cilia of neurons expressing SSTR3:EGFP, while not common, was occasionally observed (Fig. 1H). We confirmed that the cilia-like processes elaborated by the electroporated cells were in fact cilia by staining the cells for pericentrin. Examination of the stained cells showed that pericentrin was localized at the base of all hyper-elongated EGFP+ organelles (Fig. 1I). In sum, our data show that overexpression of ciliary GPCRs in developing neocortical neurons dramatically alters ciliogenesis, inducing growth of cilia that are abnormally long and branched.

Aberrant lengthening of neuronal cilia by GPCR overexpression is linked to enhanced IFT function.

While conducting our cortical experiments, we found that overexpression of 5HT6:EGFP and SSTR3:EGFP in NIH3T3 cells induced changes in the structures and lengths of their cilia that were similar to those observed in neurons (Fig. 2A, B, G). We took advantage of this observation and used these cells as a model system to examine possible factors that could explain the effects of overexpression of these receptor proteins on ciliogenesis. To determine whether the function of the overexpressed receptor was critical to obtaining the cilia phenotype induced by overexpression of 5HT6, we examined the effects of overexpression of two signaling defective 5HT6 receptors, K265A and D72A, on ciliogenesis. We also determined if fusion of 5HT6 to protein tags contributed to the cilia phenotype. Finally, we examined the specificity of this effect by overexpressing non-ciliary fusion proteins targeted to the cilia by ciliary targeting sequences; the two proteins examined were EGFP fused to the fibrocystin ciliary targeting sequence (PKD-1C1-68:EGFP), a protein that is normally excluded from neuronal cilia (e.g. Fig. 1B), and the transferrin receptor fused to EGFP, a single transmembrane-spanning protein not normally found in cilia (PC2-TRFR:EGFP). The results of these experiments suggest that the abnormal cilia growth that accompanies overexpression of 5HT6:EGFP is not dependent on the activity of this receptor (Fig. 2C, D, G) and is only modestly influenced by the size or the position of the EGFP or HA protein tags fused to the receptor (Fig. 2G). More importantly, we found that abnormal cilia growth could not be induced in cells overexpressing non-

ciliary transmembrane (PC2-TRFR:EGFP) or soluble proteins (PKD-1C1-68:EGFP) targeted to the cilia (Fig. 2E, F, G).

Trafficking of GPCRs within the cilium is believed to depend on IFT, a process that is mediated by proteins that are also critical for promoting ciliogenesis (Nachury et al., 2007; Berbari et al., 2008b; Mukhopadhyay et al., 2010; Garcia-Gonzalo and Reiter, 2012). We next asked if overexpression of GPCRs in neurons alters IFT in a manner that would support the increased growth of the primary cilia of these cells. Western blot analyses of proteins extracted from electroporated cortex revealed that the levels of Kif3a, an anterograde motor subunit required for neuronal ciliogenesis (Chizhikov et al., 2007; Davenport et al., 2007; Han et al., 2008; Kumamoto et al., 2012), were significantly elevated within sub-dissected regions of P14 cortex overexpressing either SSTR3:EGFP or 5HT6:EGFP (n=3-4 pooled hemispheres/group) compared to extracts from electroporated and non-electroporated control tissues (Fig. 3A-D). We also found that the levels of other IFT-associated proteins were elevated in these cortical regions: the retrograde transport protein cytoplasmic dynein, D1 IC74 (Pazour et al., 1998; Tai et al., 1999; Grissom et al., 2002; Makokha et al., 2002); the IFT complex B protein, IFT88(Kozminski et al., 1993; Willaredt et al., 2008; Goetz and Anderson, 2010; Satir et al., 2010; Taschner et al., 2012); and the GPCR ciliary trafficking protein, Tubby-like protein 3 (TULP3) (Mukhopadhyay et al., 2010). Examination of neurons overexpressing 5HT6:EGFP that were stained with IFT88 antibodies showed that this transport protein was localized to the cilium and was distributed along its entire length (Fig. 3E, F). Together, our Western and immunohistochemical data show that overexpression of 5HT6:EGFP induces an increased levels of several proteins associated with IFT and suggest that these proteins are

distributed to the cilia and would support increased IFT and cilia growth. Interestingly, the levels of TULP3, which were barely detectable in control cortex and cortex overexpressing 5HT6:EGFP, were increased in cortices overexpressing SSTR3:EGFP (Fig. 3D).

We next asked whether overexpressing GPCRs in neurons not yet elaborating cilia could trigger IFT and premature ciliogenesis. In normal developing neocortex, neurons begin to elaborate 'procilia' at P1 that resemble puncta when visualized by ACIII immunostaining (Arellano et al., 2012). To address this question, we electroporated E15.5 neocortex with a vector encoding mCherry and 5HT6:EGFP (mCherry(AU1)-2A-5HT6:EGFP) and examined the cilia of the electroporated neurons at P1. We found that many of the 5HT6:EGFP-positive cilia that were elaborated by mCherry-positive P1 neurons were significantly longer than the ACIII-positive procilia associated with neighboring, non-electroporated neurons (Fig. 3G, H), a result which showed overexpression of 5HT6 was able to induce premature ciliogenesis. To determine if IFT was essential to support the early onset of ciliogenesis in developing layer 2/3 neurons, we electroporated neurons at E15.5 with either a plasmid encoding 5HT6:EGFP (mCherry(AU1)-2A-5HT6:EGFP) to visualize cilia or a mixture of this plasmid and one encoding dominant negative Kif3a (mCherry(AU1)-2A-dnKif3a). Dominant negative Kif3a (dnKif3a) is a truncated, non-functional form of Kif3a that does not support IFT and has recently been reported to block ciliogenesis in adult-born granule neurons (Kumamoto et al., 2012). As expected, examination of neurons in P1 brains expressing 5HT6:EGFP alone revealed numerous cells bearing long, EGFP-positive cilia (Fig. 3I). In contrast, neurons co-expressing 5HT6:EGFP and dnKif3a, failed to localize 5HT6:EGFP to the developing cilia; in

these cells 5HT6:EGFP was largely restricted to the neurons' cell bodies (Fig. 3J). This result indicates that 5HT6-induced ciliogenesis in P1 neocortical neurons requires IFT.

Collectively, these data show that the premature cilia growth in developing neocortical neurons that is induced by overexpression of ciliary GPCRs is accompanied by increased levels of IFT proteins, which may reflect a demand for increased transport of the GPCRs into or within the cilia.

5HT6 overexpression is associated with a marked decrease in ciliary SSTR3 and ACIII localization

Next, we asked whether the abnormal length and branching of 5HT6:EGFP+ cilia could compromise trafficking of other cilia-targeted molecules to the cilia that are required for normal cilia function. In normal neocortex, SSTR3 is trafficked into the majority (>90%) of ACIII+ neuronal cilia (Fig. 4A, C) (Stanic et al., 2009; Einstein et al., 2010). Thus, in our first experiment, we examined the cilia of neurons overexpressing 5HT6:EGFP for the presence of SSTR3. Strikingly, we found that the cilia elaborated by ~95% of the neurons overexpressing 5HT6:EGFP failed to stain for SSTR3 (Fig. 4B, C), an observation that suggests that overexpression of 5HT6:EGFP disrupts trafficking of SSTR3 into the cilium.

In view of this result, we next asked if overexpression of 5HT6:EGFP alters the levels of other cilia-targeted signaling molecules in the cilia. One key signaling molecule associated with primary cilia is ACIII. ACIII, which is localized to the primary cilia of most neurons, is believed to participate in the signal transduction cascades triggered by many receptors in the ciliary membrane (Berbari et al., 2007; Bishop et al., 2007; Ou et al., 2009; Arellano et al., 2012). Given its central role in cilia function, we asked whether levels of

ACIII are altered in the cilia of cortical neurons overexpressing either 5HT6:EGFP or SSTR3:EGFP. Intriguingly, ACIII was not detected in the cilia of layer 2/3 neurons overexpressing 5HT6:EGFP (Fig. 5A), but was present in the cilia of neurons overexpressing SSTR3:EGFP (Fig. 5B). Quantification of ACIII staining revealed that >90% of the cilia produced by neurons overexpressing SSTR3:EGFP were ACIII+ whereas <10% of the cilia elaborated by neurons overexpressing 5HT6:EGFP were ACIII+ (Fig. 5C). Collectively, these data suggest that the growth and structural changes observed in the primary cilia of neurons overexpressing 5HT6:EGFP are accompanied by a dramatic reduction in ciliary ACIII levels that we predict would compromise the signaling capabilities of these organelles.

Neurons with long, malformed cilia and those with blocked cilia formation exhibit abnormal dendritic outgrowth.

Recent studies suggest that abnormal neuronal ciliogenesis is associated with dendrite outgrowth defects (Massinen et al., 2011; Kumamoto et al., 2012). To determine whether the abnormal ciliogenesis induced by overexpression of GPCRs in neocortical neurons is accompanied by changes in dendrite outgrowth, we compared the dendritic arbors of cortical neurons that had been electroporated at E15.5 with vectors encoding either EGFP (control) (Fig. 6A), SSTR3:EGFP (Fig. 6B), or 5HT6:EGFP (Fig. 6C) and then cultured for 12 days. Visual comparisons of the confocal images of these neurons revealed that overexpression of 5HT6:EGFP dramatically reduced dendrite outgrowth while overexpression of SSTR3:EGFP produced moderate reductions in dendrite outgrowth compared to controls. Using Sholl analyses to quantify these observations, we found that

dendritic outgrowth from 5HT6:EGFP and SSTR3:EGFP neurons was significantly less than from control neurons ($p<0.001$; two-way ANOVA with repeated measures) (Fig. 6E). The complexity of the dendritic arbors elaborated by neurons overexpressing SSTR3:EGFP resembled that of control neurons within a radius of ~20 μm of the soma center, but then abruptly decreased between ~30 to 150 μm of the soma center. Within this later region, the complexity of the dendritic arbors was greater than that observed for 5HT6:EGFP neurons but less than that of control neurons. Within 100 μm of the soma, the complexity of the dendritic arbors elaborated by SSTR3:EGFP+ neurons was significantly greater than that exhibited by 5HT6:EGFP+ neurons. We also found that overexpression of 5HT6:EGFP (n= 80 cells) compared to EGFP alone (n= 80 cells) hindered formation of dendritic arbors by neurons electroporated at E13.5 that were destined to form the deeper layers of neocortex (data not shown). This observation shows that the effects of overexpression of 5HT6:EGFP on dendrite formation are not limited to neurons that populate layers 2/3 but are also observed in other neocortical neuron subtypes.

To determine if the absence of a cilium would induce similar effects on the dendrite arbor formation, we electroporated developing cortical neurons with a vector encoding dnKif3a fused to EGFP (EGFP:dnKif3a). We found that overexpression of EGFP:dnKif3a in NIH3T3 cells and cultured neurons blocks ciliogenesis as evidenced by the absence of acetylated alpha tubulin and ACIII staining, respectively (data not shown). The dendritic arbors of neurons expressing EGFP:dnKif3a were significantly less complex than those of neurons overexpressing SSTR3:EGFP and control neurons (Fig. 6D, E). It is noteworthy that the effects of overexpression of 5HT6: EGFP on dendritic complexity, while the most severe, closely resembled those induced by overexpression of EGFP:dnKif3a. Collectively, our data

suggest that developing neurons that either elongate malformed cilia or lack cilia fail to elaborate normal dendritic arbors.

Co-expression of 5HT6:EGFP with ACIII but not dnKif3a reverses dendrite arbor defects

We observed that the inability of neurons to arborize dendrites was most pronounced when their cilia were either both very long and branched or were absent. In either case, ACIII could not be detected in the cilia of these cells (Figs. 5 and 6). In contrast, the cilia of neurons overexpressing SSTR3:EGFP, although longer than control cilia, were ACIII+ and their dendritic arbors were less severely attenuated than those of neurons expressing either 5HT6:EGFP or dnKif3a. Thus, we asked whether overexpression of ACIII in neurons overexpressing 5HT6:EGFP could reverse the dendritic arbor defects exhibited by these neurons. First, we generated an EGFP-tagged ACIII construct which, unlike EGFP (Fig. 7A), was able to localize to cilia in cultured electroporated neurons (Fig 7B). We then electroporated E15.5 cortex with a mixture of vectors encoding ACIII:EGFP, 5HT6:EGFP and mRFP, harvested the electroporated neurons at E16.5, and examined them after 12 days in culture. Examination of mRFP+ neurons revealed that >90% of EGFP+ cilia also stained positively for ACIII (Fig. 7C, D). Compared to neurons overexpressing ACIII:EGFP alone, whose cilia lengths were similar to control, co-expression of 5HT6:EGFP and ACIII:EGFP in neurons significantly reduced the lengths of the cilia elaborated by these cells compared to neurons expressing 5HT6:EGFP alone (Fig. 7E). As expected, in neurons co-expressing ACIII:EGFP and dnKif3a, we were unable to detect ACIII+ cilia (Fig 7E). These results indicate that co-expression of ACIII:EGFP with 5HT6:EGFP in neurons reduced the

ciliary defects induced by overexpression of 5HT6:EGFP alone and increased the levels of ACIII in the cilium.

We next asked whether there were any differences in the dendritic arbors of neurons expressing either ACIII:EGFP alone, 5HT6:EGFP alone, or ACIII:EGFP and 5HT6:EGFP. As shown above (Fig 6C,E), we found that the dendritic arbors of neurons overexpressing 5HT6:EGFP alone were the most poorly arborized (Fig. 7F). In contrast, the dendritic arbors of neurons overexpressing ACIII:EGFP were significantly more elaborate than neurons expressing 5HT6:EGFP alone (Fig. 7F). Remarkably, the dendritic arbors of neurons co-expressing ACIII:EGFP and 5HT6:EGFP were significantly more arborized than those of neurons expressing 5HT6:EGFP alone, resembling those of neurons expressing ACIII:EGFP alone (Fig. 7F). To determine whether the effects of overexpression of ACIII on dendritic arborization were dependent on localization of the ACIII to the cilium, we examined the dendritic arbors of cultured neurons that had been co-electroporated with vectors encoding dnKif3a and ACIII:EGFP. Strikingly, we found that the dendritic processes elaborated by these neurons were not significantly different from those elaborated by neurons overexpressing 5HT6:EGFP alone (Fig. 7F). Collectively, these results strongly suggest that the formation of normal dendritic arbors by developing cortical neurons requires ACIII to be localized to their cilia. Whether the regulation of arborization is mediated directly by ACIII or whether ACIII works in concert with other ciliary molecules to regulate dendrite arborization requires further investigation.

DISCUSSION

The results of our study show that disruption of ciliogenesis in developing cortical neurons by either overexpressing cilia-associated GPCRs or by blocking IFT inhibits the ability of these neurons to form normal dendritic arbors. Overexpression of ciliary GPCRs in these neurons induces an upregulation of IFT-associated molecules and premature elongation of primary cilia by developing neurons. The most striking changes in cilia morphology were induced by overexpression of 5HT6:EGFP, which were accompanied by a lack of SSTR3 and ACIII in the cilia. Co-expression of ACIII:EGFP and 5HT6:EGFP reversed the abnormal dendrite phenotype associated with overexpression of 5HT6:EGFP alone. However, overexpression of ACIII:EGFP in neurons lacking cilia due to co-expression with dnKif3a was unable to reverse this dendritic phenotype. Collectively, our data suggest that the process of neuronal dendritogenesis is dependent on the ability of neurons to generate ACIII-enriched primary cilia. Mutations that disrupt ciliogenesis or the ability of signaling proteins to localize to the cilium are thus likely to alter dendritogenesis and therefore the ability of neurons to form normal network connections and brain circuitry.

GPCR-induced changes in neuronal cilia length

The primary cilia of cortical neurons in the postnatal brain elongate over a period of many weeks (Arellano et al., 2012; Kumamoto et al., 2012). While many factors are believed to affect cilia length (Ou et al., 2009; Miyoshi et al., 2011; Sharma et al., 2011; Avasthi and Marshall, 2012), our data show that cilia length homeostasis is dramatically disrupted by overexpression of GPCRs. Our observation is consistent with and extends recent results showing that class A GPCRs (e.g. dopamine 1) can induce elongation of NIH3T3 primary cilia (Avasthi et al., 2012) and that the primary cilia of neurons in the amygdalae of *BBS4*$^{-/-}$

mice become elongated due to increased ciliary accumulation of dopamine 1 receptors (Domire et al., 2011)(K. Mykytyn unpublished observation). In contrast, the absence of neuronal cilia GPCRs does not appear to have an appreciable effect on cilia length homoeostasis. For example, the cilia of hippocampal or nucleus accumbens neurons in mice lacking either BBS2 or BBS4 appear normal even though they do not contain detectable levels of SSTR3 or MCHR1 (Berbari et al., 2008b). Similarly, the primary cilia of neurons in SSTR3 knockout mice also appear to be structurally normal (Einstein et al., 2010).

We found that overexpression of either SSTR3:EGFP or 5HT6:EGFP in neurons not only induced premature growth of their primary cilia but also increased expression levels of Kif3A, IFT88, and cytoplasmic dynein in these cells, all of which support IFT. Interestingly, we found increased levels of TULP3 in neocortical regions overexpressing SSTR3:EGFP, but not in those overexpressing 5HT6:EGFP. TULP3 binds to the IFT-A complex and promotes ciliary trafficking of SSTR3 and MCHR1 into neural cilia but not Smoothened (Smo) (Mukhopadhyay et al., 2010). The increase in TULP3 in neurons overexpressing SSTR3 could reflect differences between 5HT6 and SSTR3 transport within and/or to neuronal cilia, and suggests that the neuronal response induced by overexpression of specific GPCRs may be receptor specific.

Over the course of our experiments we noticed that the cilia of neurons overexpressing 5HT6:EGFP were typically longer and more irregular than neurons overexpressing SSTR3:EGFP. The underlying cause(s) for this difference is not clear. It is possible that 5HT6 is trafficked into the cilia more efficiently or that neurons regulate the levels of expression of these GPCRs differently. An additional possibility is suggested by recent reports that active $G\alpha_s$ can bind tubulin and mobilize microtubule plus-ends (Dave

et al., 2009; Yu et al., 2009; Dave et al., 2012). The 5HT6 receptor is coupled to Gs, whereas the SSTR3 receptor is coupled to Gi (Law et al., 1994). Thus, it is tempting to speculate that the dramatic effects of overexpression of 5HT6 on cilia formation is, at least in part, due to an increase in the amount and activity of Gs within the cilium that leads to increased axoneme growth and demand for IFT proteins.

GPCR overexpression may compromise neuronal cilia signaling

It is likely that increased trafficking of ciliary GPCRs (especially 5HT6) into developing cilia alters the signaling properties of the cilia. In addition to ciliary branching and varicosities, we found that native SSTR3 was rarely detected in the cilia of neurons overexpressing 5HT6:EGFP. The reduced levels of SSTR3 in these cilia could reflect impaired trafficking of SSTR3 into the cilium in the presence of the more abundant 5HT6:EGFP receptor which would be expected to alter ciliary signaling. A recent study suggests that the various GPCRs present in cilia physically interact with each other (Green et al., 2012). Since heteromerization of ciliary GPCRs has been proposed to increase the complexity of ciliary signaling, overexpression of specific receptors would cause an imbalance in the ratios of these receptors and may compromise ciliary signaling. Moreover, the forced exclusion of receptors such as SSTR3 from the cilia of neurons overexpressing 5HT6 could contribute to learning and memory deficits as observed in SSTR3 knockout mice (Einstein et al., 2010).

Normally, ACIII is trafficked into cilia during the earliest stages of neuronal ciliogenesis and persists throughout adulthood (Bishop et al., 2007; Arellano et al., 2012). We were unable to detect ACIII in the cilia of neurons overexpressing 5HT6:EGFP and did not detect an accumulation of ACIII around the bases of the elongated cilia or in the soma

from these cells. Surprisingly, overexpression of SSTR3:EGFP, which lengthened neuronal cilia, was not accompanied by a loss of ciliary ACIII staining. In addition, the ciliary phenotype associated with overexpression of SSTR3:EGFP was not as severe as that observed in cells overexpressing 5HT6:EGFP. Together, these results suggest that the unique transport demands created by overexpression of 5HT6:EGFP reduced the trafficking of native ACIII, SSTR3, and perhaps other important signaling molecules into the cilium. The reduced levels of these molecules in these cilia would likely impair the ability of the cilia to detect and respond to cues in the local extracellular milieu.

Cilia malformation, dendrite abnormalities, and ACIII

We found that neocortical neurons overexpressing cilia GPCRs grow excessively long/malformed cilia and defective dendritic arbors. Intriguingly, the severity of the dendritic defect was positively correlated with the severity of the ciliary structural defect. The dendritic defects that we observed in our study were more severe than those reported in a recent study of the adult dentate gyrus. In that study, blocking the formation of cilia by expressing dnKif3a in neurons born in the adult dentate gyrus shortened the dendrites elaborated by these cells and delayed their arborization, but did not alter their complexity as assessed by Sholl analysis (Kumamoto et al., 2012). Although we did not measure the lengths of the dendrites elaborated by our cultured neocortical neurons expressing dnKif3a, we did find that overexpression of either dnKif3a or ciliary GPCRs in these cells reduced the complexity of the perisomal dendrites elaborated by these cells. The differences observed in the results of these two studies could indicate that different populations of neurons, in this case adult born dentate granule versus developing neocortical pyramidal

neurons, respond differently to disruption of Kif3a function. It is noteworthy that overexpression of DCDC2, a molecule that interacts with KIF3A, has recently been reported to induce abnormal cilia growth in hippocampal neuron cultures and in ciliated *C. elegans* neurons that was associated with abnormal dendritic outgrowth (Massinen et al., 2011).

To what extent are ciliogenesis and dendrite formation in neocortical neurons linked? Our data suggest these two processes are interdependent. We observed that overexpression of 5HT6:EGFP induced abnormal growth of the cilia, significantly reduced ciliary ACIII levels, and disrupted dendrite growth. We also found that overexpression of ACIII in these cells reversed these effects. Importantly, overexpression of ACIII and dnKif3a did not alter the dendritic defects exhibited by these cells. Together, these results suggest that ciliary ACIII is required for normal dendritogenesis. Whether ACIII itself directly regulates dendrite formation, or is functions in concert with other signaling molecules requires further study. Interestingly, a recent study of ACIII deficient mice shows that these mice exhibit deficits in learning and memory (Wang et al., 2011). Our results suggest these deficits may, in part, reflect defects in growth of neuronal dendrites. At this time, it is unclear whether the reduction in the lengths of the cilia produced by neurons co-expressing 5HT6:EGFP and ACIII:EGFP was due to a reduction in the amount of 5HT6:EGFP trafficked into the cilia or restoration of ACIII signaling from the cilia.

In sum, our data and those of recent studies support a link between cilia and dendrite growth. We observed that disruption of ciliogenesis in layer 2/3 cortical neurons, as well as in cells destined for the deeper layers of neocortex, reduced dendritogenesis, suggesting that the effects of cilia on cytoarchitecture are not limited to one neuronal cell type. It is possible that this effect does not generalize to all neocortical neuron subtypes.

For example, a recent study of developing neocortical inhibitory interneurons shows that targeted ablation of the cilia of these cells induced aberrant migration of these cells and their final position in neocortex, but did not dramatically alter their post-migratory differentiation (Higginbotham et al., 2012). Interestingly, these authors also found that targeted ablation of Arl13b in projection neurons did not alter their migration, but did induce axonal outgrowth and connectivity defects. Taken together, these results suggest that the impact that ciliogenesis has on neuronal development and maturation may depend on the developmental age and subtype of the neuron. Unraveling these phenotypes may represent a key to understanding the various neurological symptoms exhibited by patients diagnosed with ciliopathies, including cognitive deficits, autism spectrum disorders, seizures, schizophrenia, and developmental dyslexia (Marley and von Zastrow, 2010; Lee and Gleeson, 2011; Louvi and Grove, 2011; Massinen et al., 2011). The specific changes induced in the neural network or in signaling pathways by cilia defects may depend on the nature of the defect and the type of neuron affected.

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Figure Legends

Figure 1. **Overexpression of 5HT6 and SSTR3 in mouse neocortical neurons induces abnormal growth of their primary cilia.**

(**A**) Flow diagram of electroporation and experimental procedures used to induce and analyze the effects of overexpression of ciliary GPCRs on ciliogenesis in cultured and in situ neocortical neurons. Neurons analyzed in culture were dissected from electroporated cortex at E16.5, dissociated, and cultured up to 12 days (12DIV). Neurons in situ were analyzed in sections of brains between postnatal day (P) 1 and P40 electroporated mice. (**B**) Examples of layer 2/3 neurons electroporated at E15.5 with a vector encoding EGFP and labeled at P14. EGFP+ pyramidal neurons show normal positioning and morphology of ACIII+ cilia (red, arrows) with pericentrin+ basal bodies (blue, arrowheads) near the base of the apical dendrite, and are comparable to neighboring non-electroporated neurons. EGFP does not colocalize with ACIII in the EGFP+ neurons. (**C, D**) E15.5 cortices were electroporated with vectors encoding either 5HT6:EGFP (**C**) or SSTR3:EGFP (**D**) under control of the EF1α promoter and were analyzed in situ at P14. (**C**) Example of layer 2/3 neurons overexpressing 5HT6:EGFP which is enriched in cilia (arrows). (**D**) Example of layer 2/3 neurons overexpressing SSTR3:EGFP which is detected in cilia (arrows) and throughout the cell bodies (asterisk). (**E**) Tracings of EGFP+ cilia elongated by neurons expressing either 5HT6:EGFP and SSTR3:EGFP. Bar = 10μm. (**F**) Comparison of cilia lengths from control (ACIII+), SSTR3:EGFP+ and 5HT6:EGFP+ neurons in layers 2/3 of P14 neocortex. **p<0.01, ***p<0.001 (ANOVA). (**G, H**) Higher magnification confocal z-stacks of branching cilia with varicosities (arrows) synthesized by mCherry+ pyramidal neurons expressing either 5HT6:EGFP (**E**) or SSTR3:EGFP (**F**). Bar= 10μm. (**I**) Sections of P14

neocortex containing mCherry+ neurons (red) with abnormally long and malformed 5HT6:EGFP+ cilia (green) were immunostained for pericentrin(blue). Five EGFP+ cilia with basal bodies were numbered and their magnified images are displayed as separate channels (3 images) grouped vertically by cilium on the right. Pericentrin+ basal bodies are present at the base of each EGFP+ cilium (**I**, arrows). Bar = 10μm.

Figure 2. **Cilia growth induced by GPCR overexpression is not significantly affected by loss of GPCR function or the presence of protein tags.**

(**A-F**) Representative confocal images of NIH3T3 cells expressing the proteins indicated above each panel whose expression was driven by the CMV promoter. All cells were immunostained with antibodies against the axoneme-enriched protein, acetylated alpha tubulin (AAtub) (red) and GFP (green). Nuclei were labeled with DAPI (blue). (**A**) Cilium of a cell transfected with a vector encoding SSTR3:EGFP (arrow). (**B**) Cilium of cell overexpressing 5HT6:EGFP (arrow) adjacent to a cilium of a non-transfected control cell (arrowhead). The insets show single channel EGFP and AAtub staining of the cilium of the transfected cell. (**C** and **D**) Cilia elaborated by cells overexpressing the signaling defective 5HT6 receptors, 5HT6(K265A):EGFP (**C**) or 5HT6(D72A):EGFP (**D**) (arrows). (**E** and **F**) Overexpression of EGFP fused to fibrocystin (PKHD-1C1-68:EGFP) (**E**) or human transferrin receptor (PC2-TRFR:EGFP) (**F**), two non-cilia proteins fused to a cilia localization signal. (**G**) Mean axoneme lengths of cilia produced by cells expressing the experimental vectors shown in panels A-F and HA:5HT6. From left to right, n = 50, 34, 44, 56, 38, 22, 26, and 30 cilia analyzed/group, respectively. Each bar represents the mean ± SEM. *** = $p<0.001$, ns = not significant (One-way ANOVA).

Figure 3. GPCR overexpression induces upregulation of IFT proteins and premature cilia lengthening.

(**A-D**) Comparisons were made between protein expression in non-electroporated control cortex (**A**), fetal cortex that was electroporated at E15.5 with either a vector encoding EGFP and mCherry:AU1 (IUE control) (**B**), or mCherry:AU1 and either SSTR3:EGFP or 5HT6:EGFP (**C**). Expression of all transgenes was under the control of the EF1α promoter. Dashed lines indicate cortical regions of P14 brains that were used to prepare the protein lysates analyzed by Western blot. (**D**) Western blots (10 μg total protein/group) were probed for proteins associated with either anterograde (Kif3a) or retrograde (cytoplasmic dynein, D1 IC74) IFT complex B protein (IFT88), or GPCR trafficking into cilia (TULP3). β-actin was used as a loading control. (**E**) Cultured, non-electroporated control cortical neuron immunostained for pericentrin (basal body, red), IFT88 (green), and the neuronal marker, MAP2 (blue). The arrow in the middle panel points to an IFT88+ cilium extending from a pericentrin+ basal body (arrow left panel). (**F**) Example of an abnormally long, branched 5HT6:EGFP+ cilium synthesized by a cultured neuron expressing 5HT6:EGFP (green) under the control of the CMV promoter and mRFP (pseudocolored blue). Ift88 (red) and EGFP were co-localized along the length and branches of the cilium (white arrows). Bar = 5μm. (**G**) E15.5 brains were electroporated with a vector encoding mCherry(AU1)-2a-5HT6:EGFP. At P1, electroporated brains were sectioned and stained with an antibody against ACIII. Examination of the upper layers of the cortical plate revealed mCherry+ neurons (red) that possessed longer 5HT6:EGFP+ cilia (arrowheads) than their neighboring non-electroporated cells whose ACIII stained cilia appear punctate

(blue, arrows). Bar=10μm. (**H**) Comparison of the lengths of the cilia of neurons overexpressing 5HT6:EGFP and control neurons. (***Student's t-test) (**I**) Section of brain electroporated and processed as described for (**G**) but not including the red channel used to visualize mCherry. Numerous, often long cilia (arrows) were present in the upper layers of the cortical plate. (**J**) P1 neurons in the upper cortical plate that were co-electroporated at E15.5 with vectors encoding mCherry and 5HT6:EGFP (mCherry(AU1)-2a-5HT6:EGFP) and mCherry and dnKif3a (mCherry(AU1)-2a-dnKif3a). The elongated 5HT6:EGFP+ cilia of neurons expressing 5HT6:EGFP alone (**I**) are not observed in cells co-expressing 5HT6:EGFP and dnKif3a.

Figure 4. Cilia of neurons overexpressing 5HT6:EGFP do not contain detectable levels of SSTR3.

(**A**) Control P14 neocortex stained with antibodies against ACIII (green) and SSTR3 (red). The majority of ACIII+ cilia are also SSTR3+ (arrows). Bar = 10μm. (**B**) Cilia synthesized by neurons expressing 5HT6:EGFP (arrows) are not correspondingly SSTR3+. Bar = 10μm. (**C**) The percentage of ACIII+ control (n= 1056) or 5HT6:EGFP+ (n=688) cilia that are also SSTR3+.

Figure 5. Cilia of neurons overexpressing 5HT6:EGFP do not contain detectable levels of ACIII.

The brains of E15.5 embryos were electroporated with vectors encoding either 5HT6:EGFP or SSTR3:EGFP under control of the EF1α promoter and were immunostained for ACIII at P14. (**A**) Pyramidal neurons in layers 2/3 of neocortex expressing mCherry:AU1 (blue) and

5HT6:EGFP (green). Sections were immunostained for ACIII (red), which normally is enriched in cilia of virtually all neocortical neurons. White arrows point to 5HT6:EGFP+ cilia projecting from mCherry:AU1+ neurons that lack detectable ACIII staining. Bar = 10μm. (**B**) Pyramidal neurons in layers 2/3 of neocortex expressing mCherry:AU1 (blue) and SSTR3:EGFP (green). SSTR3:EGFP+ cilia also stain for ACIII (yellow arrowheads). (**C**) The percentage of SSTR3:EGFP+ (n=123) or 5HT6:EGFP+ (n=89) cilia that are also ACIII+.

Figure 6. **5HT6, SSTR3 and dnKif3a overexpression reduce dendrite outgrowth of cultured cortical neurons**.

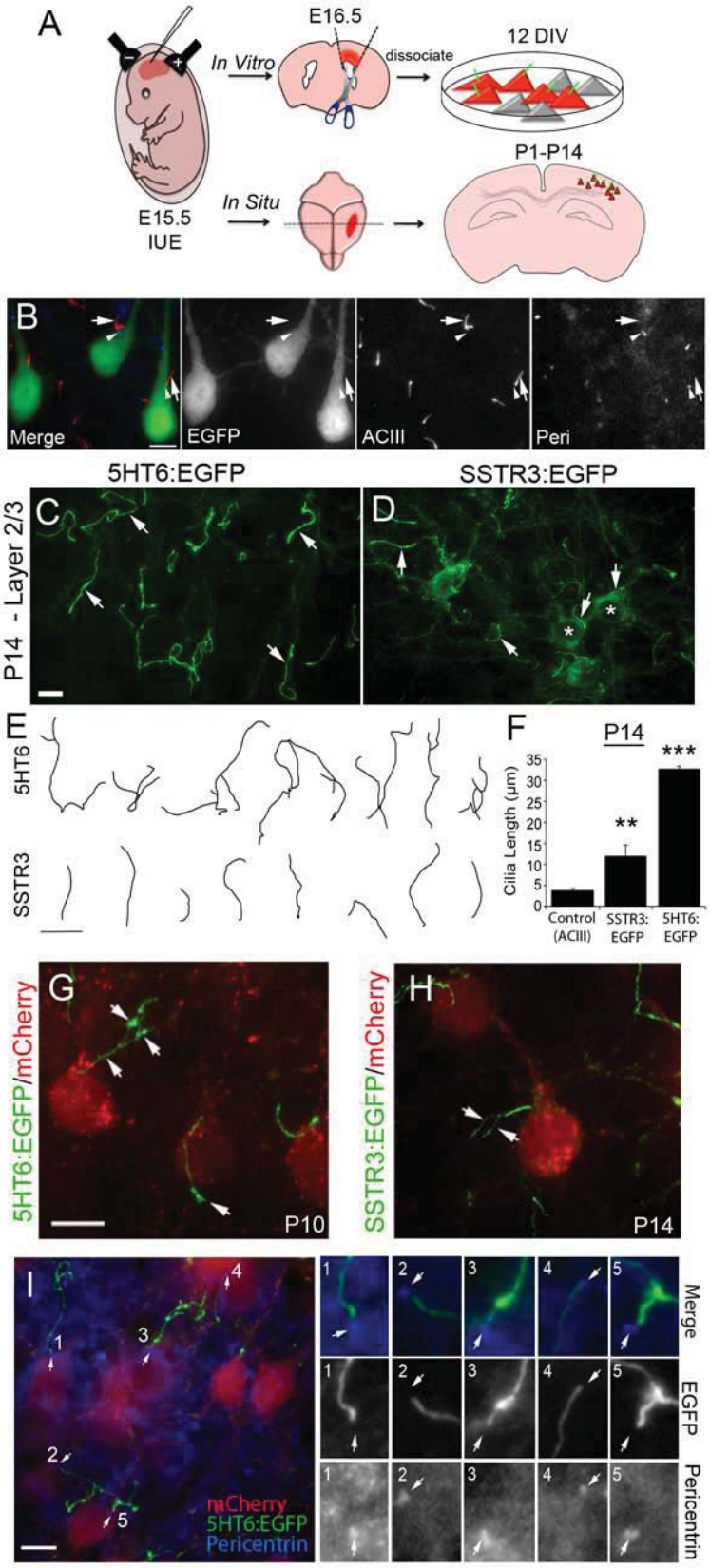
Neurons were electroporated at E15.5 with a vector encoding EGFP (Control) or the vector encoding EGFP plus vectors encoding either SSTR3:EGFP, 5HT6:EGFP or EGFP:dnKif3a under the CMV promoter. Typical cilia phenotypes associated with each group is indicated. At E16.5, cells were harvested, dissociated and fixed after 12DIV. Confocal images of EGFP+ cells were converted to black and white images. (**A-C**) Examples of neurons at 12DIV expressing (**A**) EGFP alone, (**B**) SSTR3:EGFP, (**C**) 5HT6:EGFP, and (D) EGFP:dnKif3a. (**D**) Sholl analyses reveal the extent of arborization of EGFP control (green line), SSTR3:EGFP (blue), dnKif3a (red) and 5HT6:EGFP (grey) neurons. N= total number of cells analyzed. Statistical comparisons were against EGFP using Two-way ANOVA with repeated measures (***$p < 0.001$, **$p<0.01$, *$p<0.05$).

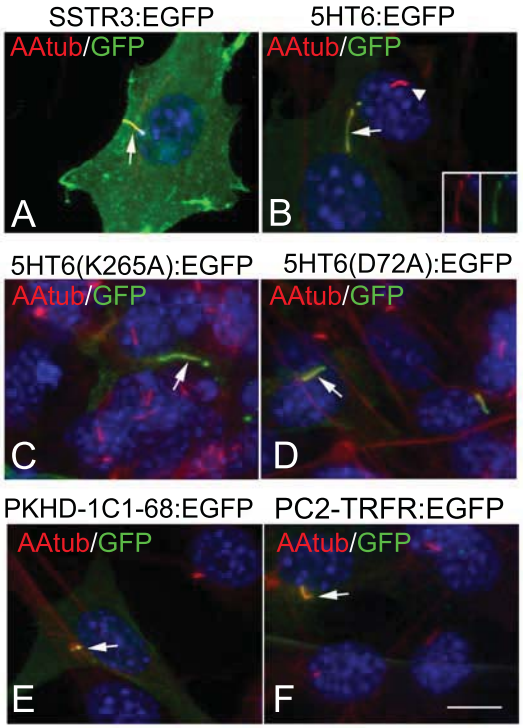
Figure 7. **Co-expression of ACIII:EGFP with 5HT6:EGFP, but not dnKif3a, restores ciliary ACIII, cilia structure and dendrite outgrowth**.

(**A, B**) Neurons electroporated at E15.5 with vectors encoding either (**A**) mRFP and EGFP or (**B**) mRFP and ACIII:EGFP. Electroporated neurons were cultured for 6DIV, fixed, and immunostained for ACIII (red). Analyses of mRFP+ neurons (blue) showed that EGFP did not traffic into the cilia as evidenced by an absence of co-localization with ACIII (A; arrow in inset). When fused to ACIII, the cilia were positive for both ACIII staining and EGFP fluorescence (B; arrow in inset). (**C**) Example of a cultured neuron co-electroporated with 5HT6:EGFP and ACIII:EGFP possessing a cilium that is positive for both ACIII staining and EGFP (inset shows zoom of cilia in individual channels and merge). (**D**) Quantification of the number of cells co-electroporated with 5HT6:EGFP and ACIII:EGFP whose cilia were both ACIII and EGFP positive (N= 139/153). (**E**) Comparisons of the lengths of the cilia elaborated by neighboring non-electroporated (control) neurons (n=142) or neurons transfected with vectors encoding mRFP plus either ACIII:EGFP (n=60), ACIII:EGFP and 5HT6:EGFP (n=120), 5HT6:EGFP (n=120) or dnKif3a (n=118) and cultured for 12DIV (**p<0.01, ***p<0.001, ns=not significant, N.D.= not determinable). (**F**) Sholl analyses of the complexity of the dendritic arbors elaborated by neurons transfected with mRFP plus either 5HT6:EGFP (grey), ACIII:EGFP (green), EGFP:dnKif3a + ACIII:EGFP (red), or ACIII:EGFP + 5HT6:EGFP (blue) and maintained in culture for 12 days (n= number of cells analyzed). The complexity of the arbors of neurons expressing 5HT6:EGFP were statistically compared to those of the other groups using two-way ANOVA (***p < 0.001, ns=not significant).

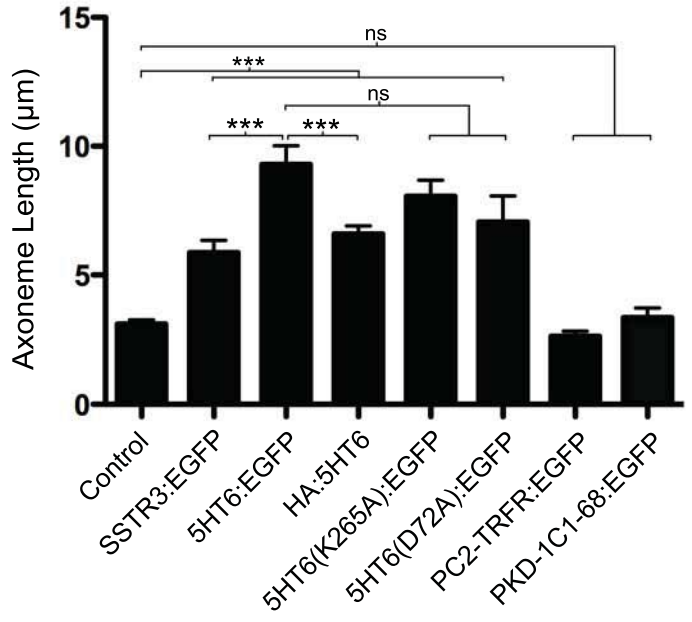
Table 1. Plasmid cDNA vectors used in this study.

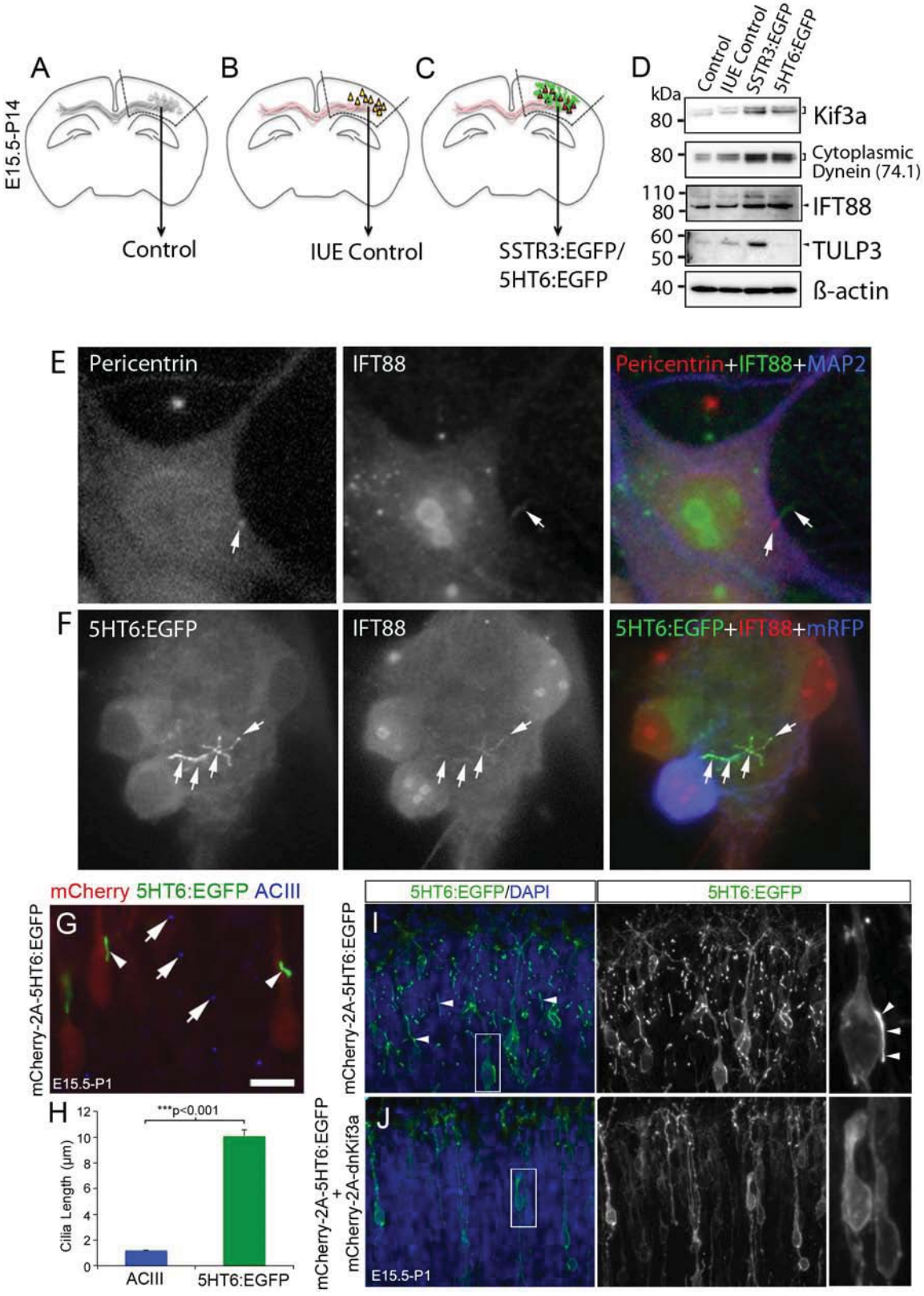
Plasmid	Vector backbone	Promoter	Source Species (if applicable)	Fusion Tag Location	Obtained From
5HT6:EGFP	pEGFPN3	CMV	mouse	CT	K Mykytyn
SSTR3:EGFP	pEGFPN3	CMV	mouse	CT	K Mykytyn
mCherry(AU1)-2A-5HT6:EGFP	pFIN	EF1α	mouse	CT	S Rowland
mCherry(AU1)-2A-SSTR3:EGFP	pFIN	EF1α	mouse	CT	S Rowland
EGFP-2a-mCherry(AU1)	pFIN	EF1α	n/a	CT	S Rowland
PC2-TRFR:EGFP	pEGFP-N	CMV	human (TRFR)	CT	K Mykytyn
PKHD-1C1-68:EGFP	pEGFP-N	CMV	mouse	CT	K Mykytyn
5HT6 (D72A):EGFP	pEGFPN3	CMV	mouse	CT	K Mykytyn
5HT6 (K265A):EGFP	pEGFPN3	CMV	mouse	CT	K Mykytyn
HA:5HT6	pcDNA3.1	CMV	mouse	NT	K Mykytyn
mRFP	pCAGGS	CAG	n/a	none	J LoTurco
EGFP	pCAGGS	CAG	n/a	none	P Rakic
EGFP:dnKif3a	pEGFPC1	CMV	bp1252-2255 of mouse Kif3a	NT	S Ge
mCherry(AU1)-2A-dnKif3a	pFIN	EF1α	bp1252-2255 of mouse Kif3a	none	S Rowland
ACIII:EGFP	pUB	UbiC	mouse	CT	J Breunig

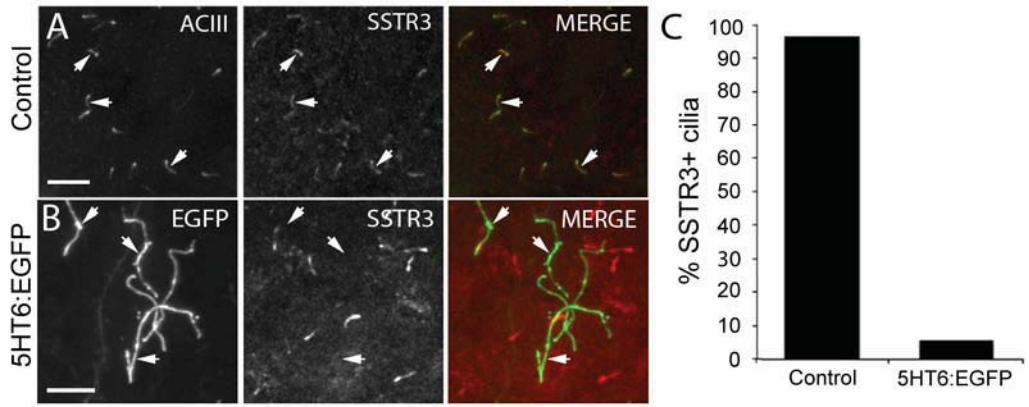

A
E16.5
In Vitro
dissociate
12 DIV
P1-P14
In Situ
E15.5
IUE
B
Merge
EGFP
ACIII
Peri
5HT6:EGFP
SSTR3:EGFP
C
D
P14 - Layer 2/3
E
5HT6
SSTR3
F
P14
Cilia Length (µm)
Control (ACIII)
SSTR3: EGFP
5HT6: EGFP
G
5HT6:EGFP/mCherry
P10
H
SSTR3:EGFP/mCherry
P14
I
mCherry
5HT6:EGFP
Pericentrin
Merge
EGFP
Pericentrin

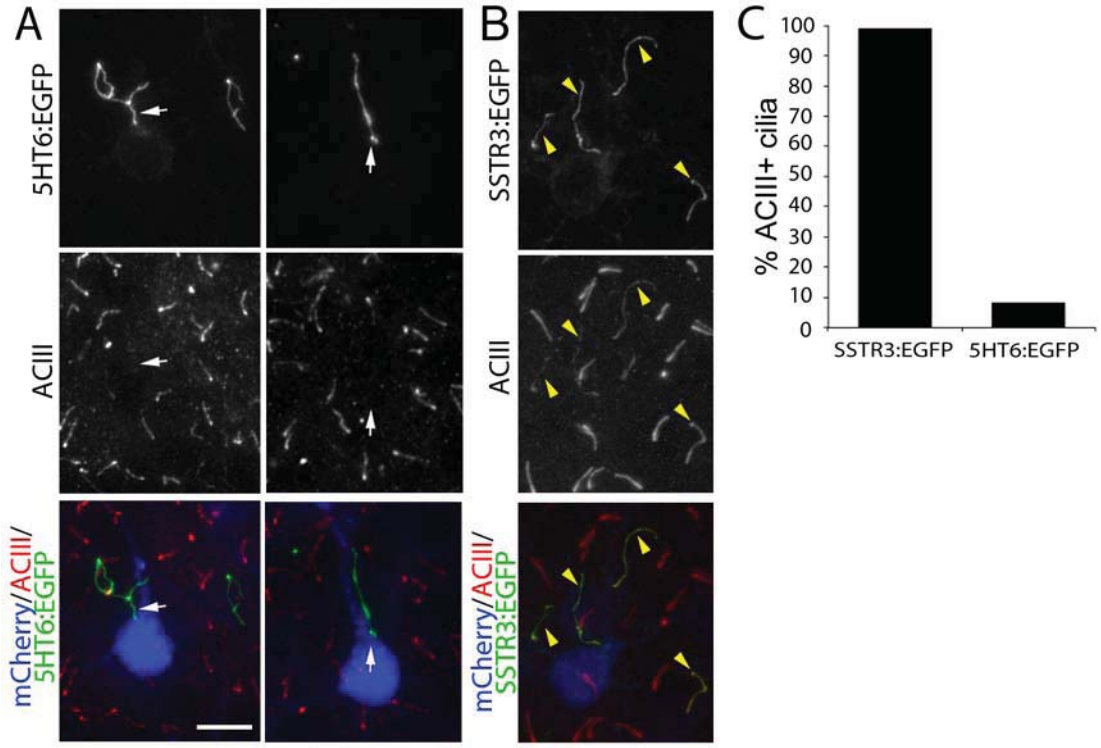

SSTR3:EGFP
5HT6:EGFP
AAtub/GFP
AAtub/GFP
A
B
5HT6(K265A):EGFP
5HT6(D72A):EGFP
AAtub/GFP
AAtub/GFP
C
D
PKHD-1C1-68:EGFP
PC2-TRFR:EGFP
AAtub/GFP
AAtub/GFP
E
F

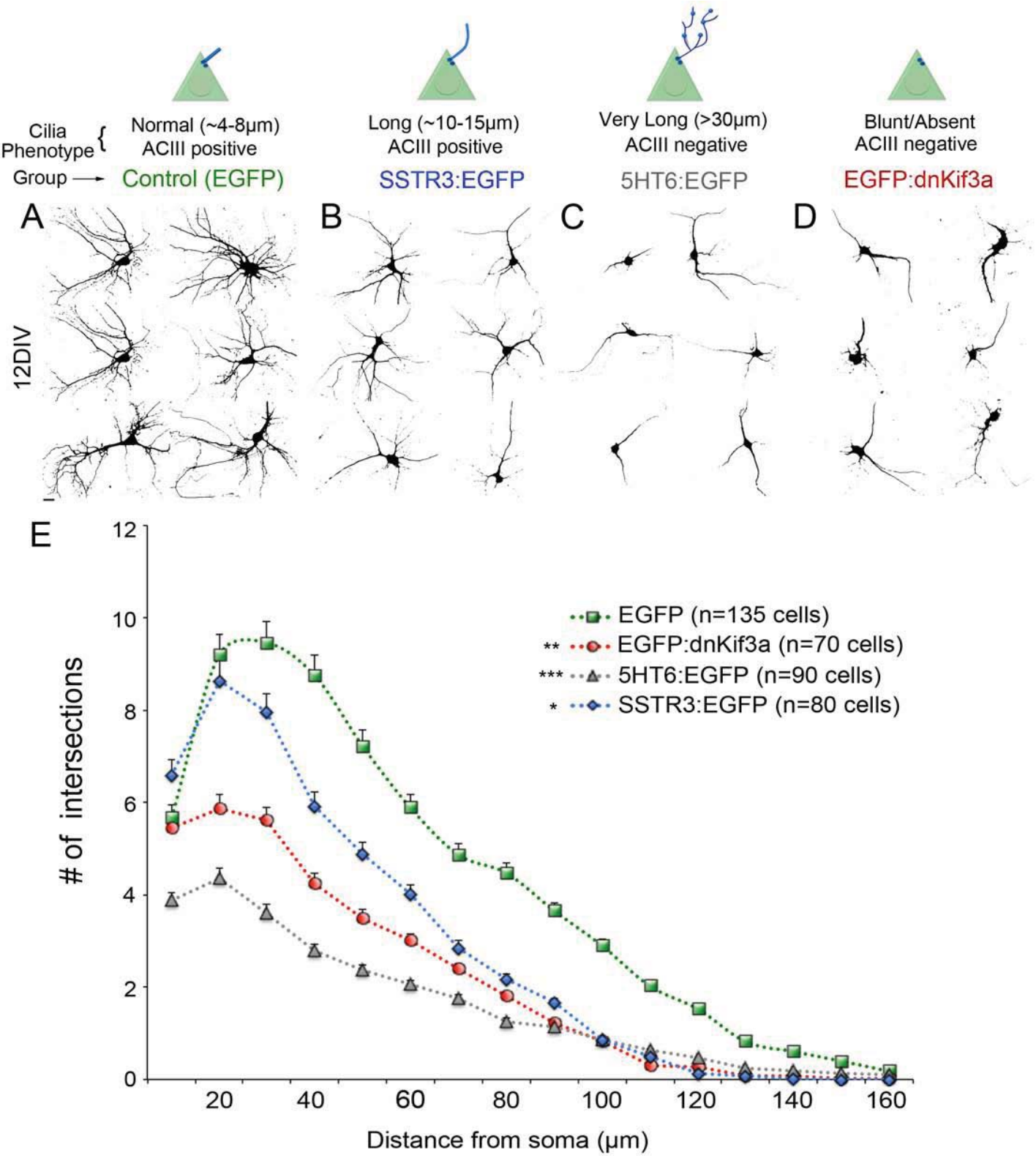
G

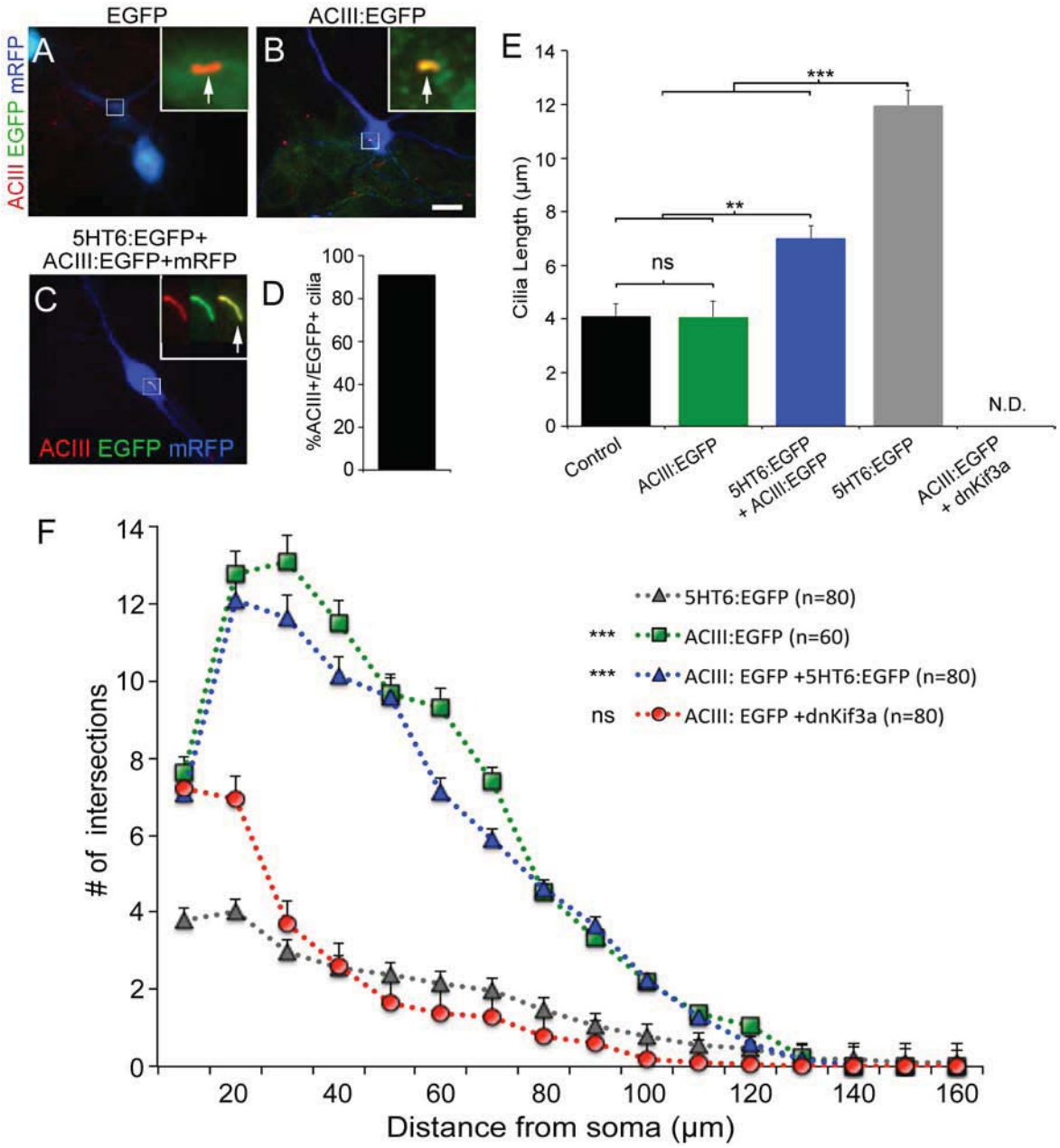

Axoneme Length (μm)
15
10
5
0
ns
***
ns
***
***
Control
SSTR3:EGFP
5HT6:EGFP
HA:5HT6
5HT6(K265A):EGFP
5HT6(D72A):EGFP
PC2-TRFR:EGFP
PKD-1C1-68:EGFP


A
B
C
D
E15.5-P14
Control
IUE Control
SSTR3:EGFP/
5HT6:EGFP
kDa
Control
IUE Control
SSTR3:EGFP
5HT6:EGFP
80
Kif3a
80
Cytoplasmic
Dynein (74.1)
110
80
IFT88
60
50
TULP3
40
ß-actin
E
Pericentrin
IFT88
Pericentrin+IFT88+MAP2
F
5HT6:EGFP
IFT88
5HT6:EGFP+IFT88+mRFP
mCherry 5HT6:EGFP ACIII
G
mCherry-2A-5HT6:EGFP
E15.5-P1
H
***p<0.001
Cilia Length (µm)
12
10
8
6
4
2
0
ACIII
5HT6:EGFP
5HT6:EGFP/DAPI
5HT6:EGFP
I
mCherry-2A-5HT6:EGFP
J
mCherry-2A-5HT6:EGFP
+
mCherry-2A-dnKif3a
E15.5-P1


A
ACIII
SSTR3
MERGE
Control
B
EGFP
SSTR3
MERGE
5HT6:EGFP
C
% SSTR3+ cilia
100
90
80
70
60
50
40
30
20
10
0
Control
5HT6:EGFP


A
5HT6:EGFP
ACIII
mCherry/ACIII/
5HT6:EGFP
B
SSTR3:EGFP
ACIII
mCherry/ACIII/
SSTR3:EGFP
C
% ACIII+ cilia
100
90
80
70
60
50
40
30
20
10
0
SSTR3:EGFP
5HT6:EGFP


Cilia Phenotype
Normal (~4-8μm) ACIII positive
Long (~10-15μm) ACIII positive
Very Long (>30μm) ACIII negative
Blunt/Absent ACIII negative
Group
Control (EGFP)
SSTR3:EGFP
5HT6:EGFP
EGFP:dnKif3a
A
B
C
D
12DIV
E
# of intersections
Distance from soma (μm)
EGFP (n=135 cells)
** EGFP:dnKif3a (n=70 cells)
*** 5HT6:EGFP (n=90 cells)
* SSTR3:EGFP (n=80 cells)
0
2
4
6
8
10
12
20
40
60
80
100
120
140
160


EGFP
ACIII:EGFP
A
B
ACIII EGFP mRFP
5HT6:EGFP+
ACIII:EGFP+mRFP
C
ACIII EGFP mRFP
D
%ACIII+/EGFP+ cilia
100
80
60
40
20
0
E
Cilia Length (µm)
14
12
10
8
6
4
2
0
***
**
ns
N.D.
Control
ACIII:EGFP
5HT6:EGFP
+ ACIII:EGFP
5HT6:EGFP
ACIII:EGFP
+ dnKif3a
F
# of intersections
Distance from soma (µm)
20
40
60
80
100
120
140
160
5HT6:EGFP (n=80)
*** ACIII:EGFP (n=60)
*** ACIII: EGFP +5HT6:EGFP (n=80)
ns ACIII: EGFP +dnKif3a (n=80)

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