Stony Brook University

Comparison of Sustained Cognitive Training vs. Cardiovascular Exercise on Cognitive Decline and Pathology in a Vascular Model of Alzheimer's Disease

A Dissertation Presented

by

Maria E. Anderson

to

The Graduate School

in Partial Fulfillment of the

Requirements

for the Degree of

Doctor of Philosophy

in

Integrative Neuroscience

Stony Brook University

August 2015

Stony Brook University

The Graduate School

Maria E. Anderson

We, the dissertation committee for the above candidate for the

Doctor of Philosophy degree, hereby recommend

acceptance of this dissertation.

Dr. John K. Robinson
Professor of Integrative Neuroscience, Department of Psychology
(Advisor)

Dr. Brenda J. Anderson
Professor of Integrative Neuroscience, Department of Psychology
(Defense Chairperson)

Dr. Richard Gerrig
Professor of Cognitive Science, Department of Psychology

Dr. William E. VanNostrand
Professor, Department of Neurosurgery

This dissertation is accepted by the Graduate School

Charles Taber
Dean of the Graduate School

Abstract of the Dissertation

Comparison of Sustained Cognitive Training vs. Cardiovascular Exercise on Cognitive Decline and Pathology in a Vascular Model of Alzheimer's Disease

by

Maria E. Anderson

Doctor of Philosophy

in

Integrative Neuroscience

Stony Brook University

2015

Despite numerous advances in our understanding of the mechanisms that underlie Alzheimer's disease (AD), there is still no cure, and the disease continues to pose a severe public health problem. It has become clear that many factors, including certain lifestyle practices, may contribute to the development of the disease; however, much remains to be learned about how lifestyle factors may prevent or delay cognitive decline. Unlike human epidemiological studies, murine models can provide clearer inferences about causality. The present studies focus on two lifestyle factors: cognitive enrichment and cardio-vascular exercise. Specifically, the effects of lifestyle interventions on behavioral performance and pathology were studied in a transgenic murine model of vascular amyloid pathology, the Tg-SwDI. The vascular component of this model's pathology is an important feature of AD pathology that shows particular promise for being responsive to these interventions. The first two studies examined the effects of a novel, progressive cognitive training intervention on pathology and behavior in three-month-old Tg-SwDI mice, as well as in a model of parenchymal amyloid (Tg-5xFAD mice). Only marginal

improvements were observed in cognitive measures, without corresponding changes in gross or regional levels of amyloid pathology or in levels of regional neuroinflammation. These data do not support the value of isolated cognitive training regimens, though the feasibility of this approach in murine models is demonstrated. The final study examined the impact of four months of 1, 3, or 12-hours of voluntary cardiovascular exercise on cognitive-behavioral measures in three-month-old Tg-SwDI and healthy wild-type mice. Exercise effects were evident across multiple behavioral measures, but did not provide reliable improvement of performance across tests. Taken together, these results highlight the potential for differential susceptibility of particular features of AD to the effects of lifestyle.

Table of Contents

List of Figures/Tables/Illustrations

List of Abbreviations

Aß - Amyloid ß protein

ACh – Acetylcholine

AChEIs – Acetylcholinesterase inhibitors

ACT – Amyloid Cascade Theory

AD – Alzheimer's Disease

ALT - Alternation

ANOVA – Analysis of Variance

APP – Amyloid Precursor Protein

BBB – Blood Brain Barrier

CAA – Cerebral Amyloid Angiopathy

DNMTP – Delayed Non-Match to Position

EE – Environmental Enrichment

ELISA – Enzyme-linked Immunosorbent Assay

FR – Fixed Ratio

LDD – Light Dark Discrimination

M – Mean

MCI – Mild Cognitive Impairment

NMTP – Non-Matched to Position

RUN – Mice in running condition

SD – Standard Deviation

SED – Mice in sedentary condition

SEM – Standard Error of the Mean

SHUT – Shuttle Task

WT – Wild Type

Acknowledgments

The completion of this dissertation would not have been possible without the support and guidance of multiple people. First, I would like to thank my advisor, Professor John K. Robinson, for his unwavering support. He made my experience at Stony Brook intellectually challenging, productive and fun. I owe so much of my intellectual and personal growth over the last five years to him.

I have also had the opportunity to work with many faculty members in the psychology department, and would like to thank them for their insight and encouragement. In particular, I would like to thank my committee members, Professor Brenda Anderson, Professor Richard Gerrig and Professor William VanNostrand. In addition, I must thank all of the post-docs, graduate and undergraduate students who were of the utmost importance in designing and conducting multiple studies over the last five years. In particular I would like to thank Dr. Jimmy Xu, Dr. Alice Blackshear, Dr. Ilan McNamara, Dr. Ming Ou-Yang, Dr. Feng Xu, Judianne Davis, Lisa Robison, Arjun Kumar, Matthew Mayers, Dominique Popescu, Steven Beigelman, and Pelin Ayyildiz. Thank you all for your instruction, patience and friendship.

Finally, I would like to express my sincere gratitude to my family and friends, who have kept my life outside of my graduate studies full of love and joy. To my mother and father, thank you for your unconditional love and support. I thank my brother, Dave, for always blazing the trail. And to my closest friends: Pete Manza, Leni Jochner, Kayla Birnbaum, Tara Thomas, Jose Cordova, Adrian Soto Cambres, Giulia Suarato, Moriah Jacobson, Melissa Auerbach, Jeremy Konar, Elizabeth Gallea, Kate Lavelle, Sam Pokorny and Julie Zhang.

CHAPTER I: GENERAL INTRODUCTION

Alzheimer's disease: Introduction and Background

Alzheimer's disease (AD) is a debilitating neurodegenerative disease that is the leading cause of irreversible dementia in the United States. Individuals afflicted by AD initially present with impairments in their ability to form and retrieve recent memories; but as the disease progresses, it robs them of all intellectual functions, including reasoning, abstraction, language and emotional control (Alzheimer's Association, 2014; Selkoe, 2012). Two pathological features that were first identified by Alois Alzheimer over a century ago characterize the AD brain: the deposition of amyloid- β (Aβ) peptides in brain tissue, and the accumulation of hyperphosphorylated tau in intracellular neurofibrillary tangles (Alzheimer, 1907). These features spread progressively throughout the brain and are thought to be linked with chronic neuroinflammation and synaptic dysfunction (Mucke & Selkoe, 2011). It has been reported that 90-96% of patients with AD also show signs of vascular dysfunction, including cerebral amyloid angiopathy (CAA), a condition that is characterized by the deposition of Aβ in the walls of cerebral arteries and capillaries (Vinters et al., 1987). Along with (1) quantities of oligomeric Aβ in the brain (Cleary et al., 2005; Lue et al., 1999), and (2) the number of neurofibrillary tangles (Braak et al., 2006), the severity of CAA is thought to be one of the pathologies most strongly associated with clinical dementia (Attems & Jellinger, 2004; Pfeifer et al., 2002; Thal et al., 2003).

Despite steady advances in our understanding of the pathological features and mechanisms that underlie AD, current pharmacological treatments have continued to show minimal efficacy in stopping or slowing the disease (Alzheimer's Association Report, 2014; Milgram, 2006). This has generated a sense of urgency for the identification of effective

therapies for a growing AD population. AD is currently the 6th leading cause of death in the United States, with 5.4 million Americans thought to be currently living with the disease (Alzheimer's Association Report, 2014). Because the average lifespan continues to increase, it is projected that the incidence of AD will double over the next 50 years absent new ways to delay its onset and slow its progression. AD is also a severe financial burden - the United States alone spends $200 billion annually to cover Medicare, Medicaid and other healthcare-related costs for individuals with AD (Alzheimer's Association Report, 2014).

Theories of AD etiology and available treatments

Over the last few decades, several theories have been advanced to explain the cause of AD-associated pathogenesis and loss of cognitive function. The cholinergic theory is the oldest hypothesis of AD etiology, and the one on which most available drug therapies are based (Alzheimer's Association Report, 2014; Bartus, 2000). In addition to the accumulation of senile plaques and neurofibrillary tangles previously mentioned, AD is also characterized by a significant reduction in levels of acetylcholine (ACh), a neurotransmitter that has been shown to play a major role in learning and memory (Bartus et al., 1982; Davies & Maloney, 1976). This discovery led to clinical trials of acetylcholinesterase inhibitors (AChEIs) that reduce the breakdown of acetylcholine in the brain, thus making more of this chemical available to neurons. Currently, there are four AChEIs (donepezil, rivastigmine galantamine and tacrine) available and being prescribed to individuals with AD. Unfortunately, it became apparent soon after they went on the market that while some patients gain short-term relief from these treatments, they are not effective at halting disease progression (Qaseem et al., 2008).

More recently, the amyloid cascade theory (ACT) has played a prominent role in describing AD etiology. The theory was proposed after rare mutations in the gene that encodes for the Amyloid Precursor Protein (APP) were found to invariably lead to a severe, early-onset form of AD. This familial form of AD is quite rare (~ 1%) compared to the more common, sporadic form of the disease (90-95% of individuals with AD) (Alzheimer's Association Report, 2014), but the ACT was nevertheless generalized across types because of the similarities in progression of pathogenesis that exist between the two. Briefly, the ACT proposes that the deposition of the naturally-derived Aß peptide is the initial pathological event in AD that triggers the formation of amyloid plaques, neurofibrillary tangles, gliosis, neuroinflammation and ultimately clinical dementia. Despite its popularity, there have been multiple criticisms of the ACT. For example, post-mortem studies have shown repeatedly that levels of amyloid in the brain are not consistently correlated with levels of dementia (Bennett et al., 2006; Katzman, 1998; Snowdon, 1997; Stern, 2001; Whalley et al., 2004). In addition, drugs developed to target the amyloid pathway have largely yielded inconclusive results. Between the years 2002 and 2012, there were 244 compounds tested in 413 clinical trials. A large proportion (221/244) of the compounds target the production and/or clearance of the Aß protein via different mechanisms, some through inhibition of the enzymes gamma and beta secretase that cleave APP, others through active and passive Aß immunotherapy (Cummings, Morstorf & Zhong, 2014). Out of the 221 Aß-targeting compounds tested, none were advanced to the Food and Drug Administration (FDA) and approved for marketing.

Modifiable risk factors

Due to the lack of effective pharmacotherapy, a fundamental research goal has been to intervene early in the progression from healthy aging to AD. It is becoming clear that, similar to other conditions such as heart disease and type II diabetes, some of the risk for AD can be attributed to modifiable risk factors. A recent study reported that as many as *one in three cases* of Alzheimer's disease worldwide may be attributable to factors such as type II diabetes, smoking, physical inactivity, midlife hypertension and low educational attainment (Barnes & Yaffe, 2011; Norton et al., 2014). This presents a real opportunity for the development of feasible preventative strategies inside and outside of clinical settings.

The current studies focus on two modifiable risk factors in particular: cognitive enrichment and physical exercise, both of which have been examined and discussed in basic research laboratories, in clinical settings and by the media and general public. Despite broad and growing interest in these factors, there remains a large amount of work necessary to thoroughly understand the potential impacts they may have on preventing, delaying or slowing cognitive decline, as well as their influence at the neural level and on pathological features of the disease.

The motivation behind this work derives ultimately from a hope that we will be able to provide clear clinical intervention guidelines that will help prevent and/or curb the progression of this insidious disease. The scientific literature that has emerged from these fields is reviewed in the following chapter.

CHAPTER II: REVIEW OF LITERATURE: COGNITIVE ENRICHMENT, CARDIOVASCULAR EXERCISE AND ALZHEIMER'S DISEASE

Cognitive Enrichment

Multiple epidemiological studies have reported that higher levels of education and engagement in cognitively stimulating activities may be correlated with lower risk of acquiring

AD, as well as the severity of AD symptoms (Review by Katzman, 1993; Stern & Gurland, 1994; Wilson et al., 2002). These results have been replicated in subsequent studies that included in their analyses covariates such as socio-economic status, (Karp et al., 2004), family medical history (Schmand, 1997) physical activity (Wilson, 2002) and ethnicity (Scarmeas, 2006). A seminal longitudinal study that followed 800 nuns, priests and monks found that those individuals who spent more time engaging in cognitively stimulating activities (e.g., reading newspapers, books, completing crossword puzzles) were less likely to develop AD (Snowdon, 2003; Wilson et al., 2002). One theory often proposed to explain the underlying mechanisms of the reduction in risk is that these mentally-stimulating activities result in an increased level of 'cognitive reserve,' a compensatory ability at the neuronal level to withstand great amounts of pathological damage and protect against cognitive decline (Stern, 2001, 2006, & 2009).

Brain training as an intervention

Epidemiological findings have generated a large interest, both clinically and commercially, in the possibility of using cognitive training regimens as non-pharmaceutical forms of treatment for patients with AD (Ballard, 2011). The findings on whether cognitive-specific training has an impact on cognitive decline in AD, however, have been mixed. One large-scale study assessing cognitive function in 11,430 healthy adults found that while participants improved significantly on the specific tasks that they had trained on, this improvement did not generalize to other types of cognitive abilities (Owen et al., 2010). These results were replicated in a sample of older adults (Ball, 2002), again showing that there were no generalizable benefits following cognitive stimulation interventions. Conversely, one study found that AD patients who underwent a combination of reality orientation (RO), a technique

where caregivers repetitively present the patient with information that orients them, such as time, date, place, and a version of cognitive stimulation therapy (CST), reported significant improvements in the mini mental state examination (MMSE), a widely used test of general cognitive function, and in tests assessing overall quality of life (Spector, 2003).

In a pilot study by Rosen et al. (2011), participants with mild cognitive impairment (MCI) were asked to spend 2 months (90 minutes/day, 5 days/week) playing 7 online games that exclusively targeted processing speed and accuracy in auditory processing (e.g., distinguishing between similar sounds or words, determining whether sounds are moving upward or downward). The exercises were adaptive, such that the tasks continuously adjusted to match the participant's level based on performance. Results showed that verbal memory scores improved significantly and that there was an increase in activation of the left hippocampus in the experimental group during the task.

Although these results are encouraging, it remains unclear whether the cognitive training regimens provide any sustained measurable benefit, mainly because it is difficult to control for potential confounds and social interactions in human intervention studies. In a study by Spector et al. (2003), individuals in the intervention group reported a better quality of life and improved cognitive function as measured by the MMSE; however, this intervention also included a social component of meeting and interacting in groups several times a week. It has been reported that social interactions may independently protect against dementia (e.g., Fratiglioni, et al., 2004), making it difficult to tease apart what effects the training in particular had on the endpoints measured. It should also be noted that the study by Rosen et al. (2011) utilized a small sample size (n=12), making it difficult to generalize the findings and warranting careful interpretation. It is also possible that the reported changes are reflective of a shift in strategic abilities that alter the

way individuals approach a particular task, as opposed to sustainable improvements in general cognitive function.

Importantly, there have been multiple studies suggesting *no evidence of cognitive improvement* following isolated cognitive training in experimental studies (Noack et al., 2014; Redick et al., 2013; Shipstead et al., 2012) or popular brain training games like Lumosity or Happy Neuron (Ballard, 2011; Stanford Center on Longevity, 2014). Although certain changes in neural systems may be occurring following brain training (as they generally do, following most types of learning or changes to our environment) we may not yet be able to confidently conclude that the changes following brain training go beyond the specific skills learned during training or promote any sort of general 'brain health.'

Cardiovascular Exercise

Recently, evidence has begun to emerge that the benefits of cardiovascular exercise extend beyond general physical health to aspects of cognition, including learning and memory. It has been well documented that increased cardiovascular exercise is associated with improved cognitive functioning in older adults (Ahlskog et al., 2011; Blomquist & Danner, 1987; Colcombe & Kramer, 2003; Erickson, 2011**;** Lautenschlager et al., 2008; Rolland et al., 2007; Radak et al., 2010; Roig et al., 2013; Scarmeas et al., 2009). Studies on patients at various stages of the disease have reported that exercise interventions generally produce positive benefits in cognitive function, mood, and in Activities of Daily Living scores (e.g., Baker et al., 2010; Geda et al., 2012; Hernandez et al., 2010; Lautenschlager et al., 2008; Liu-Ambrose et al., 2010; Rolland et al., 2007; Williams, 2007). In a well-known study conducted by Erikson et al. (2011), 120 healthy older adults were assigned to participate in either moderate aerobic exercise or

stretching exercises (control group) for 3days/week. They found that aerobic exercise increased the size of both left and right anterior hippocampus by an average of 2%, whereas the stretching control group showed a slight, aging-associated decrease in the size of their hippocampus (~1.4% reduction). In addition, while both groups showed improvements in a spatial memory task administered at three separate time points (baseline, six months and one year), the authors found that larger hippocampal volumes before and after the intervention were associated with better memory performance (Erickson et al., 2011).

In a similar study that examined the effects of aerobic exercise on mild cognitive impairment (MCI), Baker et al. (2010) randomly assigned 33 older adults with MCI to a high-intensity aerobic exercise or stretching control group. The experimental group maintained their heart rate at 75-85% of their heart rate reserve for 45 to 60 min/day, 4 days/week for 6 months. Following the intervention, a battery of performance measures were given that examined verbal fluency, recall ability and participant's task switching ability. Results showed that aerobic exercise improved performance on multiple tests of executive function, which was particularly pronounced in women, and led to a series of physiological changes such as increased BDNF, reduced cortisol and stabilization of insulin (Baker et al., 2010).

Importantly, these studies suggest that physical activity may lead to physiological changes that alter the course of cognitive decline. Although there are still no consistent official clinical guidelines that describe optimal levels and durations of aerobic exercise for individuals at different stages of the disease, these findings provide support for the continued study of exercise interventions in healthy aging populations as well as in individuals who may be already experiencing cognitive decline.

Animal Studies

Although results from human studies have been encouraging, they are limited in their ability to clearly describe the causal relationship between lifestyle factors and the cognitive decline and pathology associated with AD. Animal research has contributed a great deal to our understanding of the mechanisms that drive lifestyle-related reductions in risk and severity of Alzheimer's disease. For the most part, transgenic mouse models of AD, genetically modified to express one or more of the human genes responsible for the heritable forms of the disease, have been very important to the furthering of knowledge in the field. Although no mouse line fully replicates the disease as it is manifested in humans, these models have provided insight into the relationship between neuropathology and behavioral deficits. For example, they have contributed to the understanding of how Aß pathology progresses in the brain (e.g., Domnitz et al., 2005), the involvement of the tau protein in cognitive decline (e.g., Santacruz, 2005), and the influence of carrying an ApoE4 allele (e.g., Nichol, 2009). In addition to expanding our knowledge of the biological bases of AD, they have also been used widely in preclinical testing of potential therapeutics (Elder, 2010).

Using Animal Models to Study Cognitive Enrichment

For the most part, studies seeking to improve our understanding of the impact of cognitive enrichment have employed variations of the classic 'environmental enrichment' (EE) paradigm, designed to simulate a generally enriched and cognitively stimulating atmosphere for rodents (van Praag, 2000). First discussed anecdotally by Donald Hebb in 1947, 'enriched environments' have since been used to understand how our external environments can impact our brain (e.g., Black, Isaacs, Anderson, Alcantara & Greenough, 1990; Bruel-Jungerman et al., 2005; Diamond,

Krech, & Rosenzweig, 1964; Holloway, 1966; Kempermann, Kuhn, & Gage, 1997; Leggio et al., 2005; Rosenzweig, Krech, Bennett, & Diamond, 1962; van Praag et al., 2000; Volkmar & Greenough ,1972).

Researchers that study the effects of lifestyle on Alzheimer's disease have used EE paradigms to understand how a cognitively, socially and physically stimulating environment may offset cognitive decline and slow pathogenesis in transgenic models of AD. While there have been multiple versions of the enriched environment reported in the literature (e.g., Van Dellen et al., 2000) most use a variation of a relatively complex environment (Nithianantharajah & Hannan, 2006; van Praag, 1999, 2000) that exposes mice to exercise wheels, toys of various colors and textures, blocks, passageways and novel habitats. Results from these experiments have been generally positive; many reporting improved cognitive performance on various spatial and working memory tasks following exposure to enriched environments (e.g., Gobbo et al., 2004; Jankowsky et al., 2005; Maesako et al., 2012). The effects on AD pathology, however, have been mixed (e.g., Arendash et al., 2004; Jankowsky et al., 2003, 2005; Lazarov et al., 2005).

Despite the successes of this approach, there are several questions that warrant attention. First, given the multi-dimensional design of the EE paradigm, it is difficult to determine if and how cognitive-specific enrichment is having an impact. Also of interest is the *kind* of cognitive stimulation provided in an enriched environment Exposure to colorful toys and tunnels is relatively static, and may not properly model the progressive nature that is a common characteristic of human learning. The first study discussed in this dissertation introduces a novel, highly controlled paradigm that employs progressive, cognitive-specific training in mice in isolation from other potential confounds. Given the current, popular outlook on the benefits of

cognitive-specific training, the hope is that this highly-controlled study will help begin to clarify whether cognitive stimulation leads to actual, measurable benefits in cognitive function.

Using Animal Models to Study Cardiovascular Exercise

Mouse models of AD have also been used to delineate the underlying mechanisms through which physical activity modulates brain function. These studies have reported a variety of benefits following exercise interventions, including alleviating amyloid burden and neuroinflammation (Adlard et al., 2005; Parachikova et al., 2008; Yuede et al., 2009), increases in certain growth factors such as BDNF (Cotman & Berchtold, 2002; Stranahan et al., 2009), and proteins related to synaptic terminals, such as synaptophysin (Nichol et al., 2009). Studies have also reported improvements in spatial memory and hippocampal function following various durations of exercise (review by Intlekofer & Cotman, 2013; Nichol et al., 2009; Parachikova et al., 2008).

These studies typically employ either voluntary exercise interventions on running wheels or forced exercise on treadmills. While the methods outlined in these studies vary in their exercise-duration parameters, none systematically compare the effects of different doses of exercise on cognitive function and AD-associated pathologies. In fact, exercise is typically presented as a treatment that is compared only with a sedentary control condition, and does not assume that the intervention may have potential dose-response properties. One of the studies described below addresses this issue by examining the potential benefits of different doses of voluntary aerobic exercise in both transgenic mouse models and healthy mice.

Different transgenic mouse lines used in research

Over the last several decades, multiple transgenic mouse lines have been developed to study Alzheimer's disease in a controlled setting. Depending on the human gene inserted into the genome and the specific neural-promoter used, different transgenic lines emphasize certain distinct features of AD pathology and symptomology. For example, many popular transgenic mouse lines, such as the Tg-2576 (Hsiao et al., 1996) and Tg-5xFAD (Oakley et al., 2006), develop parenchymal Aβ plaques that progress at varying rates. Other transgenic lines have been developed to express tau pathologies (e.g., Yoshiyama et al., 2007) and both Aβ and tau simultaneously (e.g., Oddo et al. 2003), to name a few.

The current studies primarily use the Tg-SwDI mouse line (Davis et al., 2004), a model unique in its expression of fibrillar amyloid that accumulates in the cerebral vasculature, a condition known as cerebral amyloid angiopathy (CAA). Unlike other commonly used models of amyloid pathology, the Tg-SwDI allows for an examination of the role that the vasculature plays in AD onset and progression. This literature will be briefly reviewed below.

CAA and Alzheimer's disease

Brain function is reliant upon an elaborate, tightly packed vascular network that provides glucose, oxygen and life-sustaining amino acids to brain cells. The brain's vascular network expands 400 miles, and its structural and functional integrity must remain perfectly intact in order to maintain healthy function (Zlokovic, 2005). Because accumulation of amyloid in and around vessels in the brain is a common feature found in the brains of patients with AD (Nicoll, 2004), there have been several theories proposed that link cerebrovascular dysfunction with AD pathogenesis and related cognitive decline.

In addition to accumulating in brain parenchyma, amyloid deposition is also observed in medium-sized and small cerebral vessels, a condition known as Cerebral Amyloid Angiopathy (CAA) (Attems, 2005; Jellinger, 2002; Levy et al., 1990; Rensink et al., 2003; Vinters, 1987). Although CAA is at times found in aged brains independent of AD, it is widely believed that it plays a direct role in the pathogenesis of the disease and associated cognitive decline (Nicoll, 2004). One study found that among a sample of 82 patients with AD, 87% presented with CAA, compared with only 35% of the 119 non-AD patients (Yamada, 2002). Several studies have suggested, in fact, that CAA may correlate *more strongly* with cognitive decline than the other pathological features of AD (Neuropathology Group, 2001), and that it may be a more sensitive predictor of early dementia (Xu et al., 2014). In light of this information, some researchers have proposed that CAA should be included as a criterion for AD diagnosis, along with amyloid plaques and neurofibrillary tangles (Nicoll, 2004).

It has been suggested that the buildup of amyloid pathology along cerebral blood vessels may be a result of blood brain barrier (BBB) breakdown (Zlokovic, 2005). The BBB is made up of vascular endothelial cells that form a tight junction and control the flow of molecules that pass from the blood into the extracellular brain fluid. Oxygen, glucose and certain amino acids, for example, are allowed to pass or are transported through the barrier to the brain, while other molecules, like blood cells that would be toxic for the brain, are not permitted to pass. The BBB also promotes the clearance of toxic products, such as Aß, from the brain back into the blood where they are drained by perivascular clearance pathways (Weller et al., 2008). One theory suggests that as the BBB breaks down, Aß is no longer efficiently cleared from the brain, and begins to deposit within and around vessel walls, leading to features of CAA (Zlokovic, 2005).

In addition to the sporadic, non-heritable form of CAA, there are several familial forms that result from mutations of the Aß sequence on the APP gene (Grabowski et al., 2001; Levy et al., 1990; Van Broeckhoven et al., 1990). The Dutch mutation involves a substitution at position 22 (E22Q) and leads to fibrillar Aß deposition in the cerebral blood vessels, along with progressive cognitive impairment (Rozemuller et al., 1993; van Duinen et al., 1987; Wattendorff et al., 1995). Patients with the Dutch mutation develop fibrillar amyloid that is confined within the walls of larger vessels. They do not have any parenchymal fibrillar plaques and do not show signs of neural inflammation, but have recurrent vessel hemorrhages and associated cognitive impairment. The Iowa mutation is one amino acid downstream of the Dutch mutation, at position 23 of the Aß sequence (D23N), and leads to amyloid deposition specifically in the microvasculature. Since the amyloid is not contained within the vessel wall and engages the surrounding parenchyma, individuals with the Iowa mutation have severe neuroinflammation along with progressive cognitive impairment (Shin et al., 2002).

These genes were used to develop the aforementioned Tg-SwDI, the first transgenic mouse model of cerebral amyloid angiopathy. The Tg-SwDI expresses low level s of familial Dutch/Iowa CAA mutant human APP in brain and develops early-onset and progressive accumulation of Microvascular CAA (Davis et al., 2004). Notably, Tg-SwDI do not develop parenchymal fibrillar plaque found in Dutch and Iowa-type human patients, however they do produce diffuse Aß in brain parenchyma and also show robust neuroinflammation The choice to use this particular model for the current studies was based on the assumption that, given the important role that the vasculature plays in the onset and progression of AD, it is crucial to examine how non-pharmaceutical interventions may impact this particular aspect of AD pathology.

Contributions of current studies

The studies described in this dissertation are designed to make the following contributions: First, although the results of many human-based population studies have been encouraging, they are limited in their ability to describe causal relationships between lifestyle interventions and their influence on cognitive function. To that end, our studies used transgenic murine models that develop specific aspects of AD pathology and symptomology, making it possible to design studies with highly controlled experimental conditions.

Second, studies using animal models have not yet thoroughly examined the impact that specific lifestyle factors might be having on the disease. As was explained in previous chapters, the commonly used enriched environment paradigm, central to our current understanding of how environments shape the brain, does not provide a clear picture of how lifestyle factors may be independently impacting AD symptomology. Our studies extract from the enriched environment specific varieties of stimulation and look at them in isolation. The hope is that understanding the influence of single factors will ultimately help inform the guidelines established in clinical settings.

Lastly, the transgenic mouse lines most commonly used in reported studies on lifestyle factors and AD tend to express a limited variety of AD neuropathologies. These mice deposit amyloid primarily in brain parenchyma; a feature of AD that supporters of the amyloid cascade theory credit as the initial step in a cascade of pathological events that ultimately result in cognitive decline. Our studies broaden this examination by investigating cerebral amyloid angiopathy; another common and interrelated feature of AD that is thought to play a direct role in cognitive dysfunction and until now has not been examined in this context.

CHAPTER III: EFFECTS OF A PROGRESSIVE COGNITIVE STIMULATION INTERVENTION ON COGNITIVE FUNCTION AND PATHOLOGY IN A TRANSGENIC MURINE MODEL OF CAA

INTRODUCTION

Previous studies have reported enhancements in spatial memory and learning, as well as alterations of AD pathology, following varying periods of exposure to enriched environments that include various forms of cognitive, social and motor stimulation (e.g., Arendash, 2004; Jankowsky, 2005). However, the multi-dimensional feature of the classic rodent enriched environment does not allow for a detailed examination of whether cognitive stimulation in isolation may produce benefits. This is of particular interest given the advertised benefits of commercialized 'brain training' regimens in humans that are grounded in the theory that specific, isolated cognitive training may lead to generalized improvements in every-day cognitive function (Ballard, 2011). In the current study, we implement a highly controlled, isolated cognitive training intervention where mice progress through a series of increasingly challenging operant tasks. The intervention shares many features with several commercialized cognitive training regimens, as well as regimens being tested in clinical research settings (e.g., Rosen et al., 2011), such that the mice advance through these tasks at their own speed, are constantly working at their limits, and are introduced to new cognitive challenges. Aspects of spatial memory, exploratory behaviors and potential modifications to levels of pathology were examined following the 4-month intervention.

Taking into account the previously reported benefits of exposure to a cognitively stimulating environmental enrichment paradigm, the putative benefits of cognitive-specific training in humans, and the features of the transgenic mouse model used in the current studies, we expect that the findings from the current study will support the following predictions:

1. Transgenic mice will show a measurable degree of difficulty in mastering the operant tasks over the 4 months, considering the increasing difficulty of the tasks over time in addition to age-related accumulation of pathology.
2. Transgenic mice trained in the operant tasks will show improvements in cognitive function when they are presented with new cognitive challenges provided by the Barnes Maze and Radial Arm Maze tasks.
3. Transgenic mice will show modifications in levels of pathology following the PCS intervention, most likely in hippocampal regions.

MATERIALS AND METHODS

Subjects

The Tg-SwDI, a transgenic mouse line developed by Davis et al. (2004) at Stony Brook University, is the first transgenic mouse model that expresses aspects of cerebral amyloid angiopathy (CAA). The Tg-SwDI expresses low levels of familial Dutch/Iowa CAA mutant human APP on a C57B1/6 background with a Thy 1.2 promoter (Davis et al., 2004; Miao et al., 2005). Histologically, the Tg-SwDI produces fibrillar amyloid that is localized in the cerebral vasculature surrounding the thalamus and subiculum beginning as early as 3 months of age. This is accompanied by neuroinflammation, microgliosis and astrogliosis around these areas (Fan et al., 2007; Miao et al., 2005). Accumulation of diffuse Aß is also seen in the brain parenchyma of these mice, appearing first in the subiculum, hippocampus and cortex and then spreading to the olfactory bulbs, thalamus and forebrain by 12 months of age. The Tg-SwDI mouse has also been reported to exhibit deficits in tasks of spatial memory (Xu et al., 2007; Xu et al., 2014), making it

an ideal model for the investigation of cerebral vascular amyloid and its contributions to AD pathology and symptomology

Twenty female Tg-SwDI mice (nine PCS, five Fixed-Ratio (FR) six Sedentary) were an average of three months of age at the start of the intervention period and were matched with seventeen female C57BL/6 (wild type) controls (five PCS, five FR, seven sedentary). Mice weighed 21-30 grams at the beginning of the intervention period. Mice were deprived of water for twenty-three hours/day, five days/week. They received water as a reinforcer for thirty minutes during their time in the operant chambers, and had thirty minutes free-access to water once their session was complete. Mice in the FR group were placed in the operant chambers but remained on the FR task for the entirety of the intervention. This protocol was adapted from Blackshear et al. (2011). All mice had free access to water on Saturdays and Sundays, and body weights were taken weekly. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the SBU Institutional Animal Care and Use Committee.

PCS Intervention

Progressive Cognitive Stimulation is a novel, individualized operant cognitive training program for mice that spans approximately four months. The operant chambers (MED Associates, St. Albans, VT) were located inside a sound-attenuating chamber, with an exhaust fan providing white noise. The chambers were 19 x 22 cm at the floor with two front nose-poke ports 4.5 cm directly to the right and left of the water dipper, and one nose-poke port in the rear, directly opposite of the dipper. Nose poke ports were 1.5 cm in diameter and contained a small

light and a single photo-beam that registered nose-poke responses. The chamber had a metal rung floor and was lit by a small house light.

Over a 4-month period, the mice progressed through the increasingly difficult series of operant tasks, where the ability to perform one task was contingent upon the mastery of rules from the previous task. Because the intervention was designed as an individualized training program, mice were moved up to the next task once they had successfully reached the previous task's criterion. Progression of programs was as follows:

1. Magazine (DIPPER) training: In this first task, mice were simply exposed to the functionality of the water dipper. The dipper was activated automatically every thirty seconds, and remained raised and available for mice to drink from for ten seconds. Additionally, both nose-ports remained illuminated, and a nose-poke into either produced an additional reinforcer. All mice were exposed to this procedure for two days.
2. Fixed Ratio: During this task, both left and right nose ports were illuminated simultaneously, and mice were required to nose poke into either port in order to receive a reinforcer. This program continued until mice made a total of fifteen or more responses in one session.
3. Alternation: Here, the goal was to have mice become comfortable using both left and right nose ports. In this task, only *one* of the front nose ports was illuminated at a time, and the location of the lit port alternated from left to right following a response. Mice had to poke their nose only into the lit port in order to receive a reinforcer. The program continued until mice made thirty or more responses total (fifteen responses to each side) in a single session.

4. Light Dark Discrimination: The goal of this task was to teach mice to nose poke only into illuminated ports, while ignoring unlit ports. Similar to Alternation, only one port was illuminated at a time, but this time the illumination of nose ports was randomized, and any nose-poke into an unlit port was recorded as an error. The program continued until mice reached 90% correct responses (correct responses/total responses) in one session.
5. Shuttle: This task added another level of complexity, such that mice learned to follow a specific pattern of light cues. Here, the trial began with one front nose port well illuminated (left or right) that required a nose poke. Mice then turn around to nose-poke a rear well that became illuminated after the initial response. Following the rear-port response, the front well *not* initially illuminated was lit, and required a nose-poke that lead to a reinforcer. Errors were measured as a nose-poke into any unlit port. This program continued until mice reached 90% correct responses in one session.
6. Non-Match to Position: This task was similar to the Shuttle task, except for the addition of a short-term memory component. That is, instead of the last response being cued, *both* nose ports were illuminated and the mice had to remember to nose-poke the port opposite of the first light in order to receive a reinforcer. Errors were measured as a final nose poke into an incorrect port, and mice remained on this task until they reached 75% correct responses in one session.
7. Delayed Non-match to Position: This procedure was identical to Non-match to position, except for an added delay between the first (front) and second (back) response. This delay required mice to hold the initial position of the illuminated front port in their short-term memory as they progressed through the sequence of responses. All mice began with a 3 second delay. Performance was measured by an error (defined by incorrect

responses/total responses). If the errors were fewer than 25% in a single session, the delay would increase to a 5 second delay in the subsequent session. If they were higher than 25%, it would decrease to a 1 second delay. This task continued for 28 days.

Battery of Behavioral Assessment Tasks

Following their assigned intervention, all mice completed a battery of behavioral tests that allowed for an examination of spatial working memory, exploratory behaviors and general activity levels. All mice were an average of seven months of age at the beginning of behavioral testing, which lasted approximately two weeks. The behavioral battery included the Barnes Maze task that was completed during the first five consecutive days of testing, and the Radial Arm Maze and DigiScan that were completed during the second week.

Barnes Circular Maze

The circular platform is an adaptation of the Barnes Maze apparatus originally developed for rats (Barnes, 1979). It consisted of a wooden circular platform with 8 escape holes along the edge, spaced equally apart. Shelves were built under the holes such that an escape box could sit securely underneath each escape hole. The platform measured 91cm in diameter and the escape hole was 5cm in diameter. Holes were spaced 24.5cm apart. The escape box measured 10cm x 8.5 cm x 4cm. The circular platform was placed at the center of the behavioral testing room and elevated 75cm off the ground. Each mouse was given 2 trials per day separated by a 15 minute intertrial interval. Testing lasted a total of 5 consecutive days. Visible distal cues were placed around the room and remained constant throughout testing. At the start of a trial, each mouse was gently placed onto the center of the maze. The mouse was allowed a maximum of 5 minutes to

find its assigned hole and escape box. When the mouse entered the box, the experimenter stopped the timer and the mouse was left in the escape box for 1 minute before being transferred back to the home cage. If the mouse did not find the escape hole within 5-minutes, they were placed into the hole and left there for 1 minute. During the trial, the following was recorded: the time it took the mice to find the escape box (latency to find) the time it took them to enter into the escape box (latency to enter) and the number of hole visits that were made before finding the escape hole (hole visits or errors). A hole visit was defined as a nose poke into a hole that did not have an escape box beneath it prior to finding the escape position.

Unreinforced 8 Radial Arm Maze (URAM)

The unreinforced radial arm maze used in these studies is an adaptation of the original designed by Olton and Samuelson in 1976, initially developed to measure spatial learning and memory in rats. In the current version, the complex environment was used to examine potential changes in exploratory behavior of mice in a novel environment, following the interventions. Eight equally spaced arms radiated from a circular center area. Each mouse was placed in the center of the maze and allowed 5 minutes to explore freely. During the 5-minute trial, number of entries and number of entries into previously entered arms (% re-entries) were recorded.

DigiScan

The Digiscan container is a clear, plexiglass container measureing 43cm x 21cm x 21cm. Photobeams record ambulation, defined as movement parallel to the floor, as well as horizontal activity, defined as all movement (including grooming and rearing activity). This task was used to measure general activity levels of mice. Mice were placed in the Digiscan for 5 minutes. In

addition to recording of ambulatory and horizontal activity, the number of assisted rearings (defined as a mouse rearing while using the sides of the container for support) and unassisted rearings (mouse rearing up on its hind legs without using the container for support) were recorded.

Biochemical and Histological Measures

Tissue preparation

This procedure was adapted from Vasilevko et al. (2007) and Xu et al., (2007). Mice were overdosed with 2.5% avertin and intracardially perfused with PBS. Brains were then removed and bisected along the midsagittal plane. One hemisphere was snap frozen and used for ELISA, the other hemisphere was placed in 70% ethanol, then treated with xylene and embedded in paraffin for immunohistochemical and immunoflourescence evaluation.

Enzyme Linked Immunosorbent Assay (ELISA) For Aβ species

Brains of Tg-SwDI (as well as Tg-5XFAD in study #2) groups were analyzed for soluble (TBS), membrane (TBS/TX100) and insoluble (guanidine) forms of Aβ40 and Aβ42 using ELISA. The brains of wild type animals do not display any amyloid pathology as assessed by ELISA, and were therefore not analyzed.

Hemispheres that were flash frozen were pulverized into three aliquots. To obtain a soluble fraction, brain aliquots were homogenized with TBS (10ul/mg) using a bullet blender (Next Advance, Inc.) and .5mm glass beads (Next Advance, Inc.). Aliquots were then spun in a centrifuge for 20 minutes at 16,000 x g at 4ºC. The supernatant is the soluble fraction, and was removed. The next extraction used TBS/1% Triton X-100 following the same procedure to

obtain a membrane-associated fraction. The remaining pellet was suspended in 5M guanidine–HCL (pH 8.0) and rotated at room temperature for 3 hours. Following centrifugation, the supernatant was removed as the insoluble fraction. For each fraction a sandwich ELISA was performed. Ninety-six well plates (Costar 3590) were coated with 1µg/well of antibody: either Aß42-specific antibody m21F12 (Eli Lily) or Aß40-specific antibody M2G3 (Eli Lily). Plates were blocked and then incubated with samples on a shaker overnight at 4ºC. Aß was detected using biotinylated-m3DG (Eli Lily), followed by streptavidin-HRP (Amdex RPN4401V). The plates were then developed using SureBlue (KPL) and reactions were stopped by using 1M HCL. Plates were read with Spectramax (Molecular device).

Immunohistochemical Analysis

Sagittal brain sections were stained for fibrillar amyloid in the microvasculature, total amyloid burden and activated microglia. Specifically, the thalamus, cortex, hippocampus and subiculum were inspected. These regions were selected because of their propensity to accumulate pathology in this particular mouse model (Davis et al., 2004) and their vulnerability to degeneration in Alzheimer's disease.

Immunohistochemistry was performed following similar procedures used in Xu et al., (2007). For each hemisphere, a total of 100 sections were cut starting at the most distal region of the thalamus. Paraffin sections were cut in the sagittal plane at 10µm thickness using a sled microtome. These were then floated on a 43°C water bath and transferred onto glass slides. Each slide contained 3 sections that were separated by 10 cuts (i.e. slide #1 had section #1, #11 and #21; slide #2 had section #2, #22, #32, etc). Slides were deparaffined through immersion into xylenes and rehydrated with decreasing concentrations of EtOH (100% (2x), 95%, 90%, 75%,

50%). Antigen retrieval was conducted via 5-minute incubation with protease K. Sections were blocked with 0.3% triton X-100 buffer and incubated with primary antibody in 1:10 Superblock (Life Technologies) with 0.1% triton X-100 at 4ºC overnight. Primary antibodies used are as follows: Collagen Type IV (1:200) for blood vessels, 66.1 (1:250) for Aß species, Keratan sulfate (5D4) (1:20) for activated microglia. These incubated overnight at 4ºC. The next day, secondary antibodies were added and left to incubate for 2 hours. The secondary antibodies used are as follows: Biotinylated anti-mouse IgG (1:1000) and anti-Rabbit-AP (1:1000). Tissue sections were then coated with vectastain ABC (Vector labs) for DAB staining. FAST RED staining was used primarily to develop collagen staining.

Percentage Immunoreactivity

Images of stains (Collagen type IV, Keratan sulfate, Thioflavin-S and 66.1) were collected using an Olympus BX60 microscope with an attached Olympus Dp72 camera. Images from the hippocampus (CA1, CA2, CA3 and dentate gyrus) subiculum, cortex and thalamus were collected from each section. Every tenth section was sampled in order to cover the region in a comprehensive fashion, which yielded 8-10 images for each brain region (thalamus, cortex, subiculum, and hippocampus) for each mouse (n=4-7). The pictures of cortex, thalamus and hippocampus were taken under 4X magnification, while the subicular regions were analyzed under 10X magnification. Using NIH ImageJ software, an appropriate threshold was set for each stain and the percent area occupied with positive stain was quantified.

Statistical Analyses

All data were analyzed using StatView statistical software (SAS) or Microsoft Excel, and figures were generated in GraphPad Prism 6. One and two-way ANOVAs were used to analyze operant data, as well as data from the Unreinforced Radial Arm Maze and the DigiScan tasks. Two and three-way repeated measures ANOVA were used to analyze data from the Barnes Maze task. Student t-tests were used to assess differences in positive immunostaining as well as differences in total Aβ levels determined through ELISA. A series of Pearson correlations were used to examine preliminary relationships between behavioral data and performance on various intervention measures, behavioral data and specific measures of pathology, and intervention measures and measures of pathology. Significance level was set at $p = .05$.

The current study investigated whether an isolated, progressive cognitive training intervention would impact cognitive function and neuropathology in a transgenic murine model of CAA.

RESULTS

Operant Responding

The Fixed Ratio (FR) task required that mice poke their nose into a lit nose port. The goal of this task was for mice to begin making the association between nose-pokes into lit ports and the delivery of a water reinforcer. The number of sessions required to reach criterion (a total of 15 or more responses in a single session) was measured. Two, one-way ANOVAs were performed to compare each group (PCS or FR) with their age-matched wild type control group. In the PCS group, the wild type mice required a higher number of sessions to reach criterion (M=6.8, SD=1.3) than the Tg-SwDI mice (M=5.0, SD= 2.179), but there was no statistically

significant difference between the two [$F(1,12)=2.790$, $p>.05$]. In the FR group, wild type mice again required a slightly higher number of sessions ($M=6.6$, $SD= 2.8$) when compared to the Tg-SwDI mice ($M=5.6$, $SD=1.36$), but again there was no statistically significant difference between the two groups [$F(1,9)=.503$, $p>.05$] (Figure 1A)

Because the FR group remained on the FR schedule for the entirety of the 4-month intervention, only data from the PCS group will be analyzed and reported from here on. The Alternation task required that mice generalize nose-pokes into both nose ports (on both the left and right side of the main chamber wall). Number of sessions required to reach criterion were again measured and analyzed using a one-way ANOVA. There was no statistically significant different found between the two groups (WT: $M=7.2$, $SD=1.3$; Tg-SwDI: $M=6.7$, $SD=.83$), [$F(1,12)=.55$, $p>.05$]. (Figure 1B)

In the Light-Dark Discrimination task, mice were also required to nose-poke into a lit port, but the ports alternated at random. The mice, therefore, could not rely on a clear pattern (left, right, left, right) the way they could with the Alternation task, and had to solely follow the randomly placed lit port. As such, an error was measured as a nose poke into an unlit nose port. Number of sessions required to reach criterion, (a 90% correct response in one trial) was again measured and analyzed using a one-way ANOVA. Results showed that Tg-SwDI mice required a higher number of sessions ($M=8$, $SD=1.4$) compared to their wild type controls ($M=5$, $SD=.894$). This difference was statistically significant [$F(1,11)=5.038$, $p=.046$], suggesting that the Tg-SwDI required more time to learn the rule of this particular task. (Figure 1C)

The Shuttle task adds another layer of complexity by requiring that mice follow a specific sequence of events to completion that includes a response requirement in the back of the chamber. There were no differences in the number of sessions required to reach the criterion of

90% correct response in a single trial (Tg-SwDI: M=7.87, SD=2.2; wild type: M=8.6, SD=1.67; $F(1,11)$=.386,p>.05). (Figure 1D).

The Non-Match to Position task has the same response sequence as the Shuttle schedule, except for the addition of a short-term memory component that went as follows: after nose-poking the rear nose port, both front ports were illuminated and the mouse was required to first recall the port they poked previously, and then poke the *opposite* nose port in order to receive a reinforcer. In this case, all mice spent an entire 40 days on this task because none reached criterion in that time. We took this opportunity to examine the differences in response accuracy over the 40 days. When looking at the entire 40 days of the task, we see a trending significant difference between groups [$F(1,558)$=3.74, p=.06]. (Figure 1E). In order to examine potential trends in response accuracy over the 40 days period, the data were broken up into 10-day spans and analyzed separately. If we look at the first, second and third 10-day blocks, we see no differences between groups (all p>.05). However, during the last 10 days, Tg-SwDI mice had a significantly higher percentage of correct responses compared to wild types [Tg-SwDI: M=66.5%, SD=20.3%; wild type: M=56.4%, SD=19.5%; F(1,138)=8.256, p=.0047], indicating that the Tg-SwDI, while comparable in their abilities toward the beginning of the task, eventually perform even better than their wild type counterparts. These data support results from a previous study in our laboratory (Blackshear, 2009)

The average delays across sessions were calculated and potential differences between groups analyzed. Interestingly, the Tg-SwDI mice maintained a significantly higher average delay when compared to the wild type group [wild type: M=2.37, SD=16; Tg-SwDI: M=3.14, SD=2.7; F(1,390)=9.34, p=.002]. (Figure 1F).

Behavior

Barnes Maze

A three-way repeated measures ANOVA, with experimental condition (PCS, FR and sedentary), genotype (wild-type or Tg-SwDI) and day set as factors, was first used to determine potential differences in spatial working memory performance and exploratory behavior in the Barnes Maze. A significant main effect of genotype [$F(1,30) = 18.125$, $p = .0002$] showed that latency to find measures were significantly lower in the wild-type groups compared to the transgenic groups, and a main effect of time [$F(4,30)=20.7$, $p<.0001$] revealed that performance improved over the five-day testing period. However, the analyses revealed no significant main effect of experimental condition [$F(2,30) = .902$, $p>.05$], a significant genotype x condition interaction [$F(2,30) = 1.370$, $p>.05$], a time x genotype interaction or a time x condition interaction, suggesting that the PCS intervention did not modify performance on the Barnes Maze. (Figure 2A).

The number of hole-visits mice made prior to finding their assigned escape box was also analyzed. Again, there was a significant main effect of genotype [$F(1,30) = 18.372$ $p= .0002$], with the wild-type mice making fewer hole-visits, but no significant main effect of condition [$F(1,30) = .746$, $p>.05$] or a genotype x condition interaction [$F(1,30) =.080$, $p>.05$] (Figure 2B). How we examine these results depends on how we choose to interpret the hole-visit measure. One possibility is that the results suggest that the PCS intervention did not modify exploratory characteristics of the mice during their trials on the maze; another is that the intervention did not impact the number of 'errors' mice made into holes that did not contain their goal box.

Because there is a possible dissociation between performance on the first and second trial of the Barnes Maze, latency to find on the two separate trials of each day of Barnes maze testing were analyzed separately using repeated measures ANOVAs. Examining potential 1st trial differences may give us some insight into effects on 'reference memory,' where the inter-trial interval was substantial (24 hours), while examining potential 2nd trial differences may give us insight into effects on 'working memory,' where the inter-trial interval was minimal (15 minutes). The very first trial on the first day of testing was excluded from the analyses because the mice had not at this point been exposed to the escape position and their memories were not yet being tested. There was no main effect of condition for the 1st [$F(1,30) = .182$, $p>.05$] or 2nd trials [$F(1,30)=.343$, $p>.05$], suggesting that the PCS intervention did not have a specific impact on either reference or working memory in the Barnes Maze task.

Unreinforced Radial Arm Maze

Next, an unreinforced version of the 8-radial arm maze was used to investigate potential exploratory and behavioral differences in a novel environment following the PCS intervention. When measuring the total number of entries made, a two-way ANOVA revealed a significant main effect of genotype [$F(1,30) = 12.402$, $p=.0014$], but no significant main effect of condition [$F(1,30) = 1.015$, $p>.05$] or a genotype x condition interaction [$F(1,30) = .3.541$ $p>.05$] (Figure 2D) suggesting that the PCS intervention had no impact on the number of arms entered during the 5-minute trial period.

When assessing the number of re-entries mice made into previously entered arms, a main effect of genotype was once again found [$F(1,30) = 12.402$, $p=.0014$], but there was no main effect of condition [$F(1,30) = 1.015$, $p>.05$] (Figure 2E) further confirming the lack of

differences in exploratory behavior between PCS and sedentary Tg-SwDI groups. However, the analyses revealed a statistically significant genotype x condition interaction, [$F(1,30)$, 3.541, p=.0416], suggesting that the effect of genotype on exploration depended on the condition the mice were in.

DigiScan

In order to investigate potential differences in general activity levels and limb strength, a series of two-way ANOVAs were used to examine measures from the DigiScan test (Columbus Instruments, Columbus OH). There was no main effect of genotype [$F(1,30) = .958$, $p>.05$], condition [$F(1,30) = .465$, $p>.05$] or a genotype x condition interaction [$F(1,30) = .873$, $p>.05$] for the horizontal activity level measure. However, there was a significant main effect of condition on assisted rearings [$F(1,30) = 10.708$, p=.0003]. Fisher's post-hoc analysis determined that this difference remains when only Tg-SwDI groups were examined [PCS M=30.88, SD= 7.753; FR M= 22.167, SD= 10.226; SED M=19.33, SD=5.61; $F(1,17) = 4.9$, p=.02, suggesting that the PCS intervention may have played a role in increasing the number of assisted rearings made by Tg-SwDI in the DigiScan test. (Figure 2C).

Pearson Correlations

A thorough series of correlations examining relationships between intervention measures, behavioral measures and pathological measures can be found in Figure 8. Although most of the correlations were not significant, there was one that may be of interest: A Pearson correlation revealed a negative relationship ($r(11)$= -0.34, $p>.05$) between DNMTP task performance and individual performance in the Barnes Maze, such that those mice that maintained a higher delay

in the DNMTP task also took less time to find their escape box in the Barnes Maze task. When only the Tg-SwDI group was examined, this negative association became more pronounced ($r(6) = -0.56$, $p=.15$) (Figure 2F).

Enzyme-Linked Immunosorbent Assay (ELISA)

In order to examine whether PCS had an impact on levels of Aβ pathology, an ELISA was used to assess potential differences in whole-brain levels of Aβ between PCS, FR and SED groups. Aβ 42-soluble, Aβ 42-membrane and Aβ 42-insoluble fractions were examined with a student t-test. Wild type mice were not included in the analyses because they do not develop measurable levels of Aβ protein in the brain. As can be seen in Figure 3, there were no statistically significant differences in Aβ levels between any of the groups (all t-tests, $p>.05$).

Given individual differences in both operant and Barnes Maze task performance, the following relationships were explored: First, average delay in DNMTP vs. levels of soluble and insoluble Aβ pathology, in order to examine whether a relationship exists between varying levels of pathology and high performance on the DNMTP task. This may give us an indication of whether mice that were more successful at the short-term memory operant task (and therefore had higher average delays) also had lower overall levels of Aβ pathology. There was no strong association between average delay in the DNMTP task and levels of soluble or insoluble Aβ ($r(7) = -0.2$, $p>.05$; $r(7) = -0.08$, $p>.05$, respectively). Second Barnes Maze performance was examined as measured by the latency to find measure vs. soluble and insoluble levels of Aβ. This allowed me to determine whether varying levels of pathology were related to Barnes Maze task performance. This was particularly interesting given that a previously reported correlation suggested that mice with higher average DNMTP delays also tended to have lower latency to

find measures. Performance on the Barnes Maze and levels of soluble ($r(7) = -0.3$, $p>.05$) and insoluble Aβ levels ($r(7) = -0.14$, $p>.05$) were not strongly correlated.

Immunohistochemistry and Immunoflourescence

ELISAs were performed to assess whole-brain soluble and insoluble Aβ. In order to establish whether *regional differences* in quantity of amyloid plaque or activated microglia varied between PCS, FR and Sedentary groups, image analyses were used to examine hippocampal, subiculum, thalamic and cortical regions. These regions were of interest as they have been the focus of previous similar studies (Adlard et al., 2005; Lazarov et al., 2005; Jankowsky et al. 2003; Xu et al., 2014), and are among the most severely affected by AD (Alzheimer's Association, 2014).

Thioflavin-S Stain of fibrillar amyloid

Independent-sample t-tests were conducted to compare potential differences in percent area occupied with Thioflavin fluorescent stain of fibrillar amyloid in PCS, FR and Sedentary conditions. Four regions of interest (thalamus, cortex, hippocampus and subiculum) were selected because of their propensity to accumulate pathology in this particular mouse model and their vulnerability to degeneration in Alzheimer's disease. There were no statistically significant differences in percent positive stain between the PCS and FR groups in thalamus ($t(9)= 1.513$, $p>.05$), cortex ($t(9)=0.73$, $p>.05$), subiculum ($t(9)=1.344$, $p>.05$) or hippocampus ($t(9)=0.175$, $p>.05$). Similarly, no differences were found between PCS and SED groups in thalamus ($t(11) = 0.107$, $p>.05$), cortex ($t(11)=.944$, $p>.05$), subiculum ($t(11)=.2506$, $p>.05$) or hippocampus ($t(11)=1.009$, $p>.05$). (Figure 7A). These results suggest that the PCS intervention not seem to directly modify the level of amyloid pathology in these regions.

Total Aβ stain (66.1)

Similar to the Thioflavin stain, independent-sample t-tests were conducted to compare potential differences in percent area occupied with a total Aβ stain in PCS, FR and SED conditions. There was no statistically significant difference in percent positive stain between the PCS and FR groups in thalamus ($t(9) = 0.203$, $p>.05$), cortex ($t(9)=0.339$), $p>.05$), subiculum ($t(9)=1.426$, $p>.05$) or hippocampus (t (9)=0.99, $p>.05$). Similarly, no differences were found between PCS and SED groups in thalamus ($t(11)=0.322$, $p>.05$), cortex ($t(11)=2.0$, $p>.05$), subiculum ($t(11)=1.43$, $p>.05$), or hippocampus ($t(11)=0.368$, $p>.05$). (Figure 7B). These results suggest that the PCS intervention did not directly modify the levels of both diffuse and fibrillar Aβ, lending further support to the lack of intervention effectiveness.

Activated Microglia Stain

In order to examine whether there was an impact on activated microglia in these regions following the PCS intervention, independent-sample t-tests were conducted to compare percent area occupied with positive stain in PCS, FR and Sedentary conditions. Similar to the previous results from regional analyses, there were no statistically significant differences in positive staining of activated microglia between the PCS and FR groups in the thalamic region ($t(9)=0.40$, $p>.05$), the cortex ($t(9)=0.51$, $p>.05$), subiculum ($t(9)=0.61$, $p>.05$) or hippocampus ($t(9)= 1.36$, $p>.05$). Similarly, there were no differences found between PCS and Sedentary groups when thalamic regions were analyzed ($t(11)=1.476$, $p>.05$, cortex ($t(11)=0.312$,$p>.05$), subiculum ($t(11)=0.018$, $p>.05$), or hippocampus ($t(11)= 0.39$, $p>.05$). (Figure 7C).

DISCUSSION

The current study focuses on 'cognitive training;' the currently popular phenomenon grounded in the theory that specific, isolated 'cognitive-training' may allow for a generalized improvement in cognitive function and overall improvement in brain health. We test this theory by implementing a highly controlled, isolated cognitive training intervention where mice progress through a series of increasingly challenging operant tasks. The mice advance through these tasks at their own rate, are constantly working at their limits, and are introduced to new cognitive challenges; features that are present in most commercialized cognitive training regimens (i.e. Lumosity) as well as regimens being tested in research and clinical settings (e.g., Rosen et al., 2011).

Operant Results

Importantly, the proof of concept for this intervention was successful: all mice completed each task of the intervention and were able to progress through the tasks at their own pace by consistently working at their limits.

Contrary to our initial prediction, the Tg-SwDI mice showed an interesting variety of both strengths and weaknesses in each task. They were comparable to wild types in their ability to learn basic stimulus-response rules (FR and ALT). Interestingly, while they required significantly more time to master the first complex-rule-associated operant task (Light Dark Discrimination; Figure 1C), they also had a relatively high number of correct responses in the more difficult Non Match to Position tasks, and were comparable if not superior to their wild type controls during the 28-day delayed Non-Match to Position tasks (Figure 1E). These results partially support previous findings from a dissertation from our research group (Blackshear,

2009) that were the first to demonstrate the difficulty Tg-SwDI had in task-switching to the LDD task, and the improvement in NMTP performance in the latter portion of the 40-day task. Importantly, the fact that Tg-SwDI and wild types were more or less matched in their operant task performance was beneficial because it established a baseline in training levels going into the evaluation of performance in subsequent behavioral testing.

One potential explanation for their high performance on these relatively complex short-term memory tasks may, paradoxically, be a result of pathology accumulating in areas of the brain that these tasks are presumably dependent on (hippocampal and subicular regions). The build-up of toxic proteins that pose a threat to neuronal connections may have led to an over-compensation of blood flow and neuronal activity in these regions, thus leading to improved performance. Therefore, while wild type mice were presenting with 'normal' aging deficits, and therefore declining temporally in their performance in each task, the Tg-SwDI may have held a transient advantage on these particular tasks. In fact, there has been some evidence of increased activation in fMRI studies of human AD patients, which was interpreted as evidence of a potential compensatory mechanism that the brain undergoes in response to damage (Deutsch et al., 1993; Grady et al., 1993).

One potential limitation of the PCS manipulation is that water deprivation may have dampened the effects of PCS by increasing the health challenges the Tg-SwDI mice had to endure. However, lack of group differences in pathology would suggest that deprivation did not exacerbate CAA-related pathology and was therefore a relatively benign aspect of the manipulation design.

Behavioral Results

Following the PCS intervention, the Barnes Maze was used to examine aspects of spatial memory, an unreinforced version of the 8 Radial Arm Maze was used to look at exploratory behavior in a novel and relatively complex environment, and the DigiScan allowed for a general measure of overall activity levels.

Rationale behind using Barnes Maze as measure of general cognitive abilities

At this point, it is important to present the reasoning behind choosing the Barnes Maze as our test of 'general' cognitive abilities, and its distinctive characteristics when compared to the cognitive challenges found in PCS.

One very common feature of AD is a general difficulty orienting oneself in extrapersonal space (Alzheimer's Association Report, 2014). Individuals with AD will often become disoriented and lost, even in familiar places, typically because they are lacking a spatial context. So, while they may recognize visual cues and register them as being familiar, they may be unable to understand where those cues are in relation to a specific location. This inability to properly orient oneself in space is undoubtedly a handicap that makes daily functioning problematic, and has been listed as one of the top 10 warning signs of early AD by the Alzheimer's Association (2014).

The Barnes Maze, unlike PCS, examines these hippocampal-dependent spatial abilities. In the Barnes Maze, mice are trained to learn a particular location, and are provided with multiple visual cues that they become familiar with over the five-day session. While a healthy wild type mouse will properly use these visual cues and their internal spatial map to locate their escape box, transgenic mice have much more difficulty with this task, and typically require more time to find their escape box in each trial. While it is possible that the transgenic mice recognize

the visual cues that they have been exposed to over the weeklong period, they have difficulty understanding where their escape box is in relation to those cues. This is illustrated by the high rate of nose poking into holes that lack an escape box prior to finding their own.

It is, of course, difficult to definitively tease apart the brain circuitry required for PCS tasks and compare it to what is most important for the Barnes Maze task. Most likely, all of the cognitive challenges presented to the mice in both the PCS and Barnes Maze task are requiring different as well as overlapping neural networks. However, because the Barnes Maze requires spatial and orientation abilities that the PCS task does not, we thought it appropriate to use this Barnes Maze task *not as a simple continuation of cognitive challenges following the PCS intervention,* but instead as a way to measure more general sets of spatial cognitive abilities that are necessary for daily functioning.

Results

Overall, and contrary again to our initial hypothesis, mastery of the PCS operant tasks did not seem to generalize to consistently improved performance in any of the chosen behavioral tasks. There were no statistically significant differences in Barnes Maze performance between PCS Tg-SwDI and Sedentary Tg-SwDI mice. We do see improved performance in the PCS group on the first day of testing, although their relatively flat learning curve over the 5-day period indicates that their learning over time was limited. In addition, PCS did not lead to a rescuing of a healthy behavioral phenotype in the unreinforced Radial Arm Maze task (Figure 2D&E). There are several ways to interpret these findings. First, it has been demonstrated in the human literature that similarly-designed 'brain training' games, while improving performance on the *specific task* that the individual has trained on, have not been shown to generalize to other

types of tasks (Noack et al., 2014). This holds true for the current study. The lack of differences between PCS and Sedentary groups demonstrates that although the PCS mice became adept at specific complex rule learning, this did not generalize to improved cognitive function in other situations. The function of the Barnes Maze, for example, might be analogous to an individual attempting to find one's car in a parking lot. What this body of research would predict, all else being equal, is that an individual who has been working on brain-training games will have the same amount of difficulty finding their car in the grocery store parking lot as will the individual who has not been engaging in these games. The difference between them would be limited to their performance abilities on the brain-training games themselves.

It is possible, however, that the types of behavioral tasks chosen were not sensitive enough to measure changes in cognitive function following this particular intervention. The Barnes Maze, for example, assumes a rodent's inherent aversion to bright light and open spaces in order to create sufficient motivation for escape (Barnes, 1979). It is possible that the Morris Water Maze, that uses water as the primary aversive property (Morris, 1984), would lead to an even higher level of motivation for escape in rodents and thus be more sensitive to potential changes in cognitive function. It should be noted, however, that water-based tasks risk being *too* aversive; introducing potential confounds that may unintentionally alter results. Also, the Barnes Maze has shown in the past to be measurably sensitive to hippocampal deficits in various transgenic mouse lines (e.g., Xu et al., 2014).

Notably, there was a marginally significant negative correlation found between latency to find and average delay in the DNMTP task (Figure 2F), such that Tg-SwDI mice that had higher average delays (and therefore had higher performance levels) also took less time on average to find their escape box. Given the small sample size, it is possible that this negative relationship

would become more pronounced with a larger number of subjects. This, of course, poses a classic 'chicken or egg' dilemma: while it is possible that spending more time working at a higher level on the DNMTP task led to enhanced spatial memory abilities, via various currently unidentified mechanisms, it also possible that, like humans, mice may have varying degrees of abilities at baseline, which would then lead to corresponding performance on all tasks. The negative relationship was not found when only wild type mice were included in the analysis, however, which suggested that if there were differences in Tg-SwDI mice, they might become evident in histological analyses. While there were no relationships found between pathology levels and performance on the DNMTP or Barnes Maze (Figure 8), it is possible that there may have been changes at the neural level that were not examined.

ELISA and Histological Results

Quantified levels of pathology in all groups, both gross and regional, were similar to those reported from this model in previous studies (Fan et al., 2007; Xu et al., 2014), confirming the validity of the model used in the current study.

There were no differences in overall levels of Aβ pathology, as measured by ELISA, or regional differences in density of fibrillar Aβ, total Aβ or activated microglia in the thalamic, hippocampal, subicular or cortical regions (Figures 3, 4, 6 & 7). There were also no clear relationships found between individual levels of Aβ and performance on the DNMTP task or behavioral tasks.

Levels of Aβ determined by ELISA were similar to levels reported in previous studies of Tg-SwDI mice (Xu et al., 2014).

Conclusions

Progressive cognitive stimulation is a novel intervention that successfully isolates cognitive stimulation, and shares many of the same features that exist in human 'brain-training' games, such that both transgenic and wild type mice were able to progress through each task at their own pace while consistently working at their limits, and were introduced to novel challenges that train attention and require behavioral flexibility. The intervention also included tasks that were dependent on short-term memory, which were of particular interest given their reliance on certain memory-critical brain areas that are also vulnerable in the Tg-SwDI mouse model. However, it became clear after subsequent testing of more general cognitive abilities that the PCS intervention did not have a measurable effect on the tasks chosen for this study (i.e. latency to find the goal box on the Barnes Maze and general activity on the unreinforced Radial Arm Maze). Again, these results highlight the scant evidence available in the human literature in support of cognitive-specific interventions improving general cognitive aptitude in humans (Noack et al., 2014; Redick et al., 2013). The current study further validates the appeals being made by a growing number of scientists against overstating advantages of brain-training products by advertisers.

It should also be noted that the PCS intervention did not lead to obvious benefits, unlike the classic enriched environment paradigm so commonly used to study the effects of an enriched lifestyle (including cognitive stimulation), on cognitive function and pathology (e.g., Arendash et al., 2004; Jankowsky et al., 2005). The current results lend support to the idea that benefits from enriched environment studies may be a product of other particular forms of enrichment found within (e.g., exercise and/or social enrichment), or rather that general improvements in function are driven by interactions between multiple forms of enrichment.

FIGURES: STUDY #1

FIGURE 1

Graphs showing average number of sessions required to reach criterion for individual training programs: A,B&D) All groups performed comparably on the FR, Alternation and Shuttle tasks, respectively. C) PCS wild type mice required fewer number of sessions to reach the criterion in LDD task, p <.05, E) Average percent correct responses in Non-Matched to Position schedule over 40 day period; Tg-SwDI mice had a marginally higher average number of correct responses, p = .06. F) Average number of delays achieved in DNMTP task over 28 day period, Tg-SwDI maintained higher delay, p<.05. Error bars represent SEM.

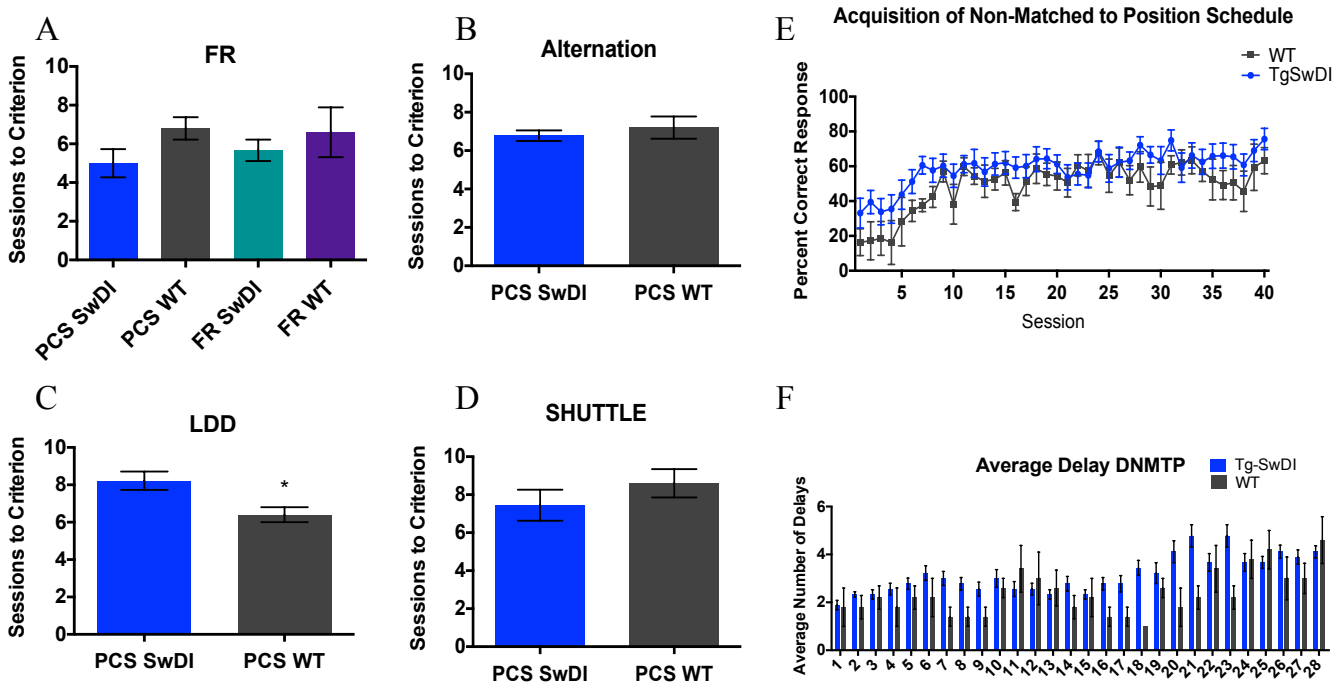


FIGURE 2

Graphs showing performance on a series of behavioral measures, A) Latency to find escape box in Barnes Maze task, there was a significant main effect of genotype (Tg-SwDI vs. wild type, p<.05), but no main effect of condition (PCS vs. Sedentary, p>.05), B) Number of hole visits in Barnes Maze task, again there was a main effect of genotype (p<.05), but no main effect of condition, C) Average number of beam breaks in a DigiScan test of general activity, no differences between groups (p>.05), D&E) Number of entries and percent re-entries in an unreinforced version of the Radial Arm Maze task, significant main effect of genotype (p<.05), no main effect of condition, F) Relationship between average number of delays in DNMTP task vs. Latency to find in Tg-SwDI PCS in the Barnes Maze task, there was a negative, non-significant relationship between the two variables (r=-0.52, p=.15). Error bars represent SEM.

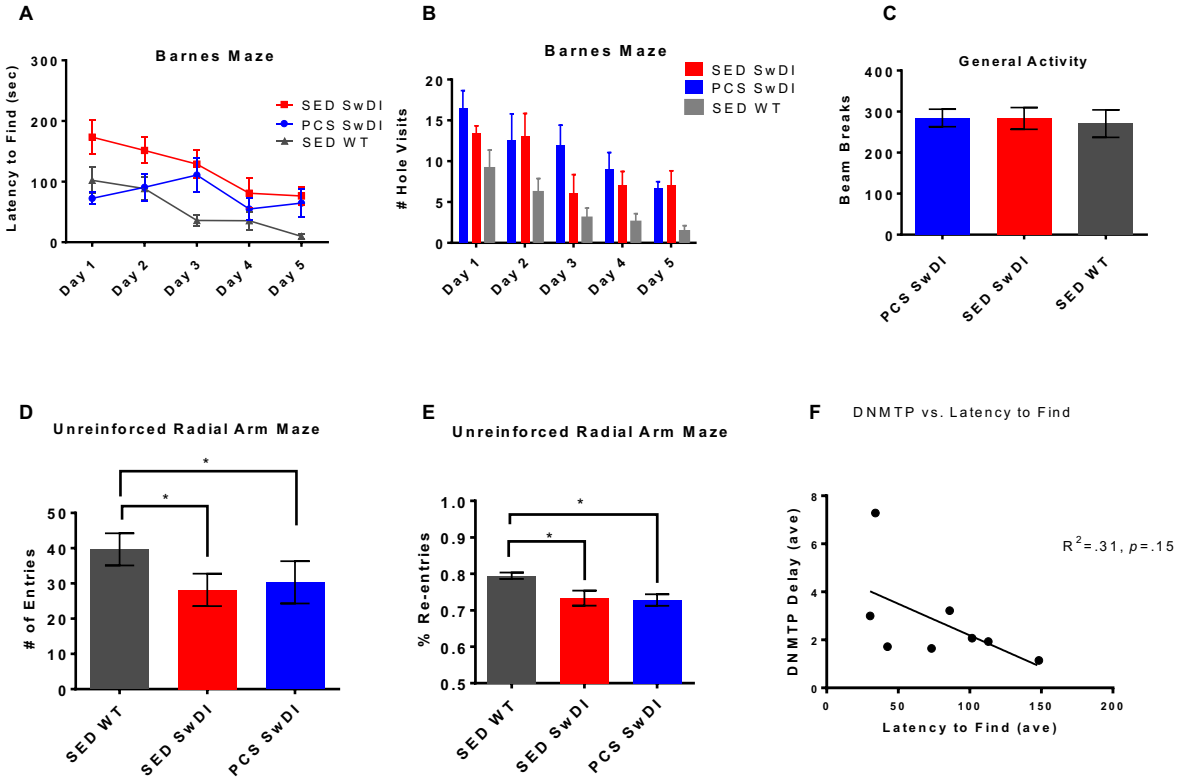


FIGURE 3

Graph shows total amount of Aβ in the following fractions: 40 soluble, 42 soluble, 40 membrane, 42 membrane, 40 insoluble and 42 insoluble There were no significant differences between any of the groups across the fractions. Error bars represent SEM.

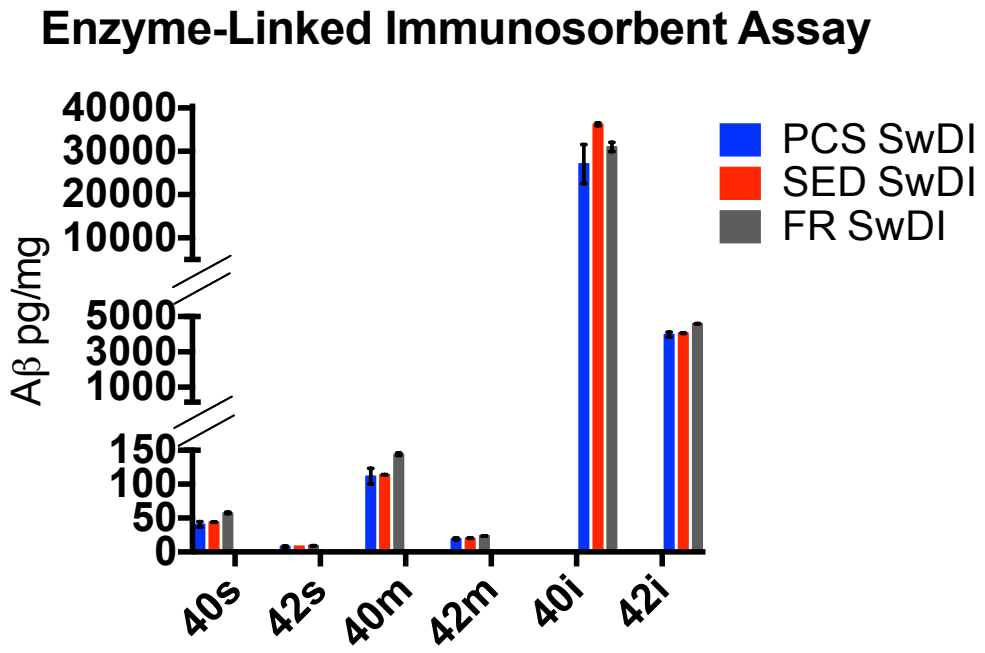


FIGURE 4

PCS Tg-SwDI and FR Tg-SwDI images of hippocampus (A,E,I,M;4x mag), subiculum (B,F,J,N; 10x mag), cortex (C,G,K,O;L 4x mag) and thalamus (D,H,L,P; 4x mag) stained with Thioflavin-s stain (Green) for fibrillar amyloid and 66.1 (Brown) stain for Total Aβ. Scale bars for 4x images set at 200µm, scale bar for 10x images set at 100µm.

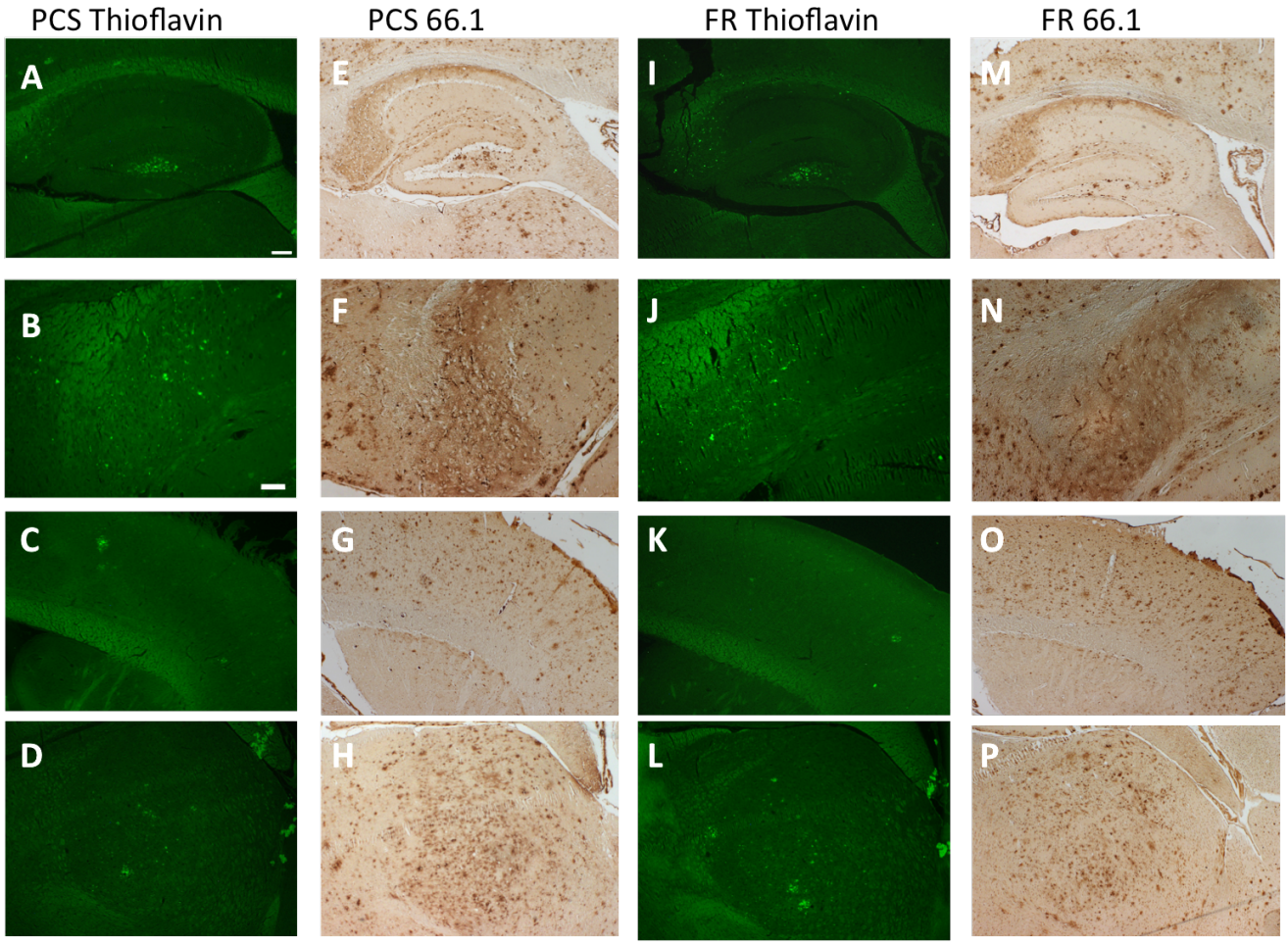


FIGURE 5

Double immunostaining of Tg-SwDI mouse. A) Collagen IV (Red) for vessels and Thioflavin-S (Green) for fibrillar amyloid. Capillaries coated by fibrillar amyloid deposits seen clearly. B) Collagen IV for vessels and 66.1 (Brown) for total Aβ. Diffuse Aβ primarily in parenchyma. 40x magnification - scale bar set at 50μm.

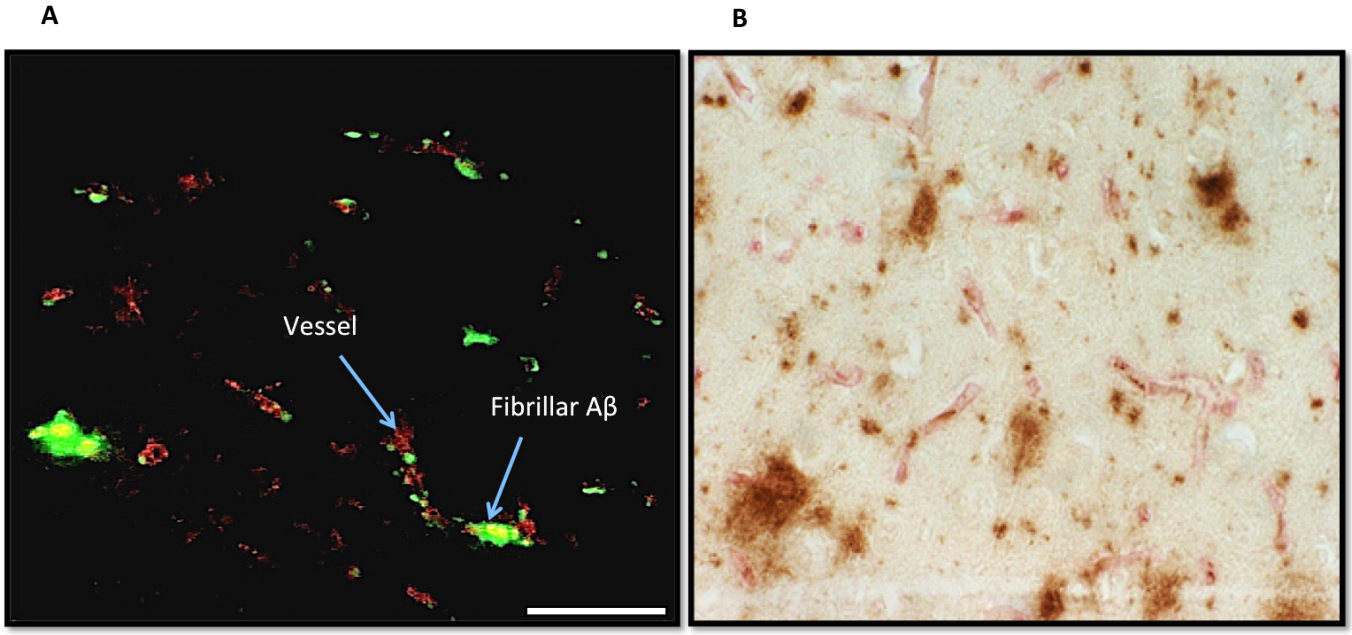


FIGURE 6

PCS Tg-SwDI and FR Tg-SwDI images of hippocampus (A,E; 4x mag), subiculum (B,F; 10x mag), cortex (C,G; 4x mag) and thalamus (D,H; 4x mag) stained with Keratan sulfate (Brown) antibody for activated microglia. Scale bar set at 200µm for 4x and 100µm for 10x.

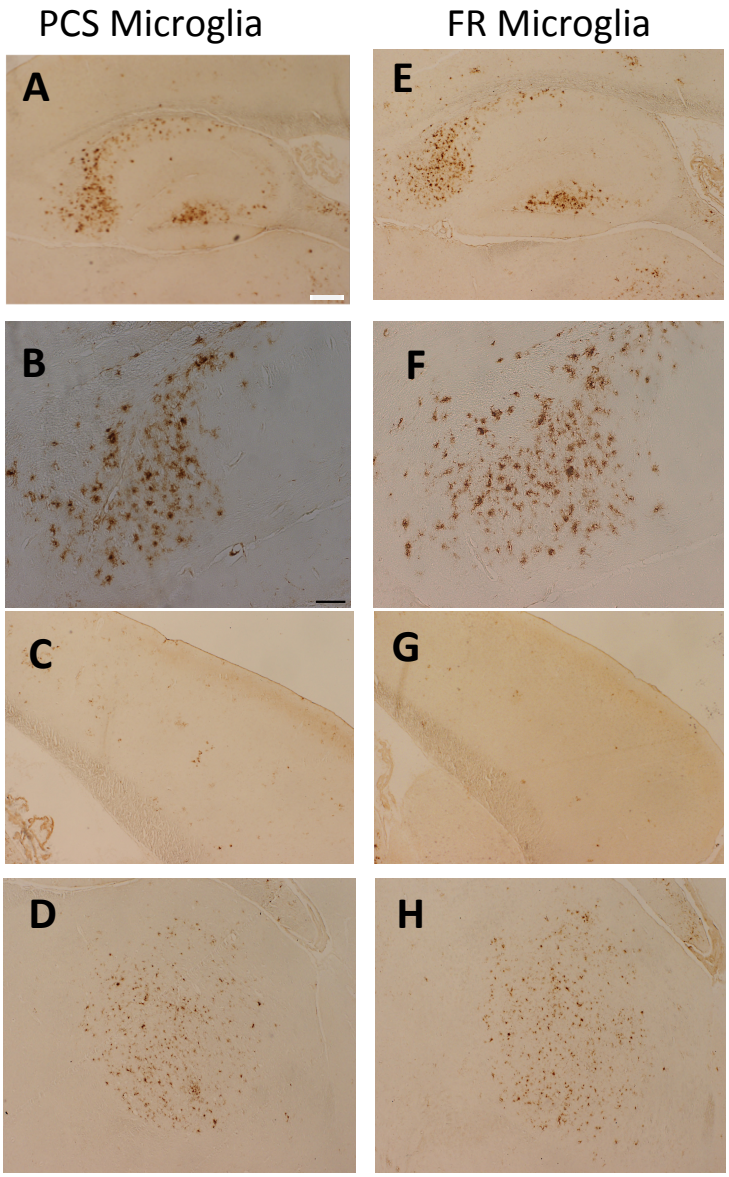


FIGURE 7

Graphs showing percent average area of positive immunostaining for individual Tg-SwDI mice in PCS (n=7), FR (n=4) or SED (n=6) groups. Regions of interest examined include thalamus, cortex, subiculum and hippocampus. A) Thioflavin-S stain for fibrillar amyloid, no statistically significant differences between any of the

groups in any of the regions (all t-tests, p>.05). B) 66.1 stain for total Aβ, no statistically significant differences between any of the groups in any of the regions, (all t-tests, p>.05). C) Keratan sulfate stain for activated microglia, no statistically significant differences across groups and regions (all t-tests, p>.05).

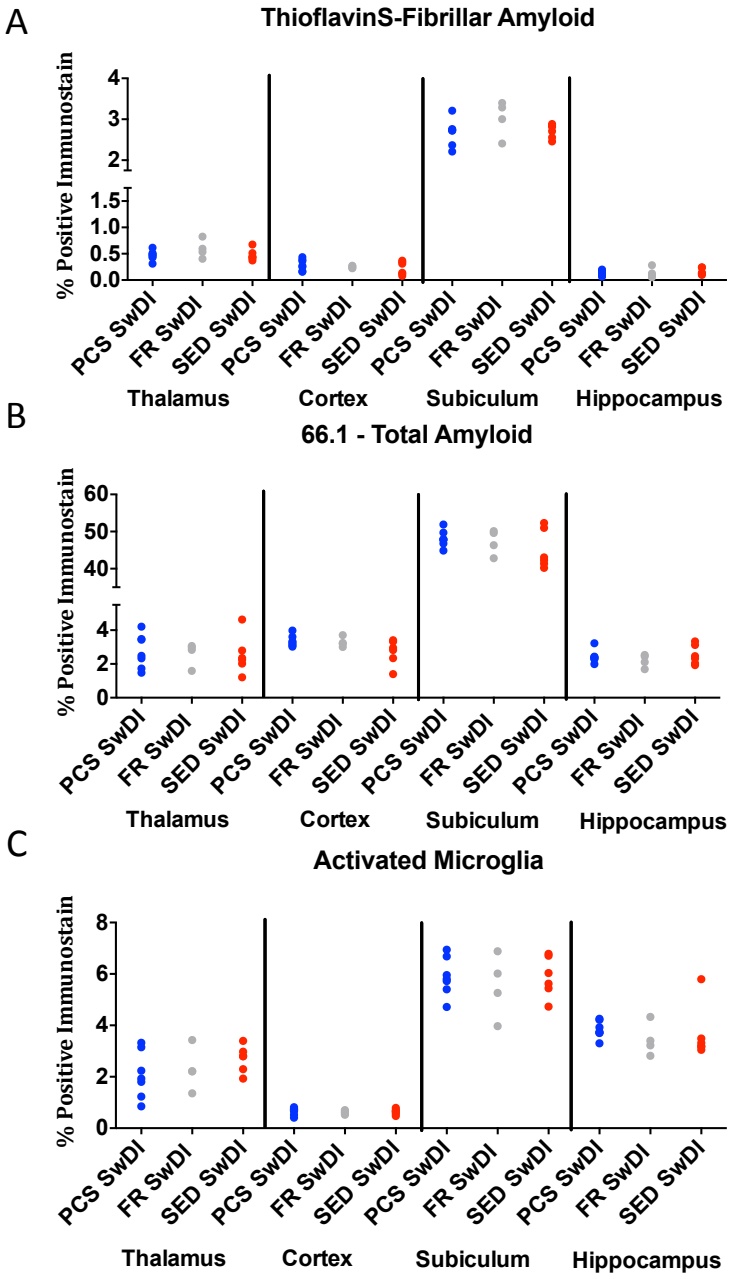


FIGURE 8

Correlation matrix showing Pearson correlation coefficients that represent relationships between behavioral data and performance on various intervention measures, behavioral data and specific measures of pathology, and intervention measures and measures of pathology. Marginally significant correlation between latency to find and average delay in DNMTP task highlighted in yellow.

	DNMTP	LTF	Soluble	Insoluble	Thiof. Thal	Thiof. Ctx	Thiof. Sub	Thiof. Hipp	66.1 Thal	66.1 Ctx	66.1 Sub	66.1 Hipp	Glia Thal	Glia Ctx	Glia Sub	Glia Hipp
DNMTP		-0.561	-0.015	0.156	-0.188	-0.211	-0.160	-0.103	0.520	0.462	0.078	0.604	-0.694	-0.049	0.438	0.096
LTF	-0.561		-0.388	-0.140	0.546	-0.325	0.221	-0.015	0.025	-0.193	-0.075	-0.552	0.438	0.578	0.095	-0.222
Soluble	-0.015	-0.388		0.219	-0.547	-0.317	-0.429	-0.655	0.118	0.173	-0.108	0.380	-0.139	0.082	0.182	0.170
Insoluble	0.156	-0.140	0.219		-0.334	0.423	-0.021	0.410	-0.589	-0.699	0.570	0.298	-0.424	0.042	-0.508	0.574
Th.Thal	-0.188	0.546	-0.547	-0.334		-0.176	-0.123	0.219	0.553	-0.288	-0.291	0.132	0.023	-0.266	0.261	-0.837
Th.Ctx	-0.211	-0.325	-0.317	0.423	-0.176		0.521	0.757	-0.660	-0.416	0.725	0.035	-0.391	-0.527	-0.409	0.411
Th.Sub	-0.160	0.221	-0.429	-0.021	-0.123	0.521		0.248	-0.415	0.230	0.752	-0.583	-0.117	0.215	0.243	0.447
Th.Hipp	-0.103	-0.015	-0.655	0.410	0.219	0.757	0.248		-0.415	-0.611	0.477	0.188	-0.409	-0.452	-0.538	0.171
66.1 Thal	0.520	0.025	0.118	-0.589	0.553	-0.660	-0.415	-0.415		0.395	-0.538	0.420	-0.150	-0.116	0.703	-0.755
66.1 Ctx	0.462	-0.193	0.173	-0.699	-0.288	-0.416	0.230	-0.611	0.395		-0.177	-0.291	0.129	0.327	0.639	0.046
66.1 Sub	0.078	-0.075	-0.108	0.570	-0.291	0.725	0.752	0.477	-0.538	-0.177		-0.047	-0.598	0.014	0.016	0.655
66.1 Hipp	0.604	-0.552	0.380	0.298	0.132	0.035	-0.583	0.188	0.420	-0.291	-0.047		-0.719	-0.594	0.028	-0.253
Glia Thal	-0.694	0.438	-0.139	-0.424	0.023	-0.391	-0.117	-0.409	-0.150	0.129	-0.598	-0.719		0.374	-0.228	-0.186
Glia Ctx	-0.049	0.578	0.082	0.042	-0.266	-0.527	0.215	-0.452	-0.116	0.327	0.014	-0.594	0.374		0.158	0.394
Glia Sub	0.438	0.095	0.182	-0.508	0.261	-0.409	0.243	-0.538	0.703	0.639	0.016	0.028	-0.228	0.158		-0.345
Glia Hipp	0.096	-0.222	0.170	0.574	-0.837	0.411	0.447	0.171	-0.755	0.046	0.655	-0.253	-0.186	0.394	-0.345	

CHAPTER IV: EFFECTS OF PCS INTERVENTION ON COGNITIVE FUNCTION AND PATHOLOGY IN A MURINE MODEL OF PARENCHYMAL AMYLOID PATHOLOGY

INTRODUCTION

A follow up to Study #1, the current study examined whether PCS would have an impact on cognitive function and neuropathology in a well-characterized transgenic mouse model of parenchymal amyloid. The goal of this study was to replicate the PCS intervention with a more commonly used model of AD, which would allow us to either extend the findings from the first study to another form of AD pathology, and/or to examine potential distinctions of the effects of PCS on two common pathological features and their associated symptoms.

MATERIALS AND METHODS

Subjects

A common pathology observed in transgenic mouse lines is an accumulation of Aß deposits in brain parenchyma. The Tg-5xFAD mouse line is an aggressive model of parenchymal Aß deposits, harboring three APP mutations (APP K670N/M671L [Swedish], I716V [Florida], V717I [London]) and 2 presenilin mutations (PS1 M146L, L286V) on a B6/SJL background under a Thy1 promoter (Oakley et al., 2006). Aß deposits begin accumulating at 2 months of age in cortex and subiculum, spreading to the hippocampus by 4 and 6 months of age, along with microglial and astroglial activation, and subsequent neuroinflammation (Oakley et al., 2006). These mice have also shown behavioral deficits, particularly in hippocampal dependent memory tasks (Kaczorowski, Sametsky, Shah, Vassar & Disterhoft, 2009; Xu et al., 2014), and have been particularly useful in studying the involvement of parenchymal fibrillar accumulation of Aß in AD.

Thirteen Tg-5xFAD mice (eight PCS, five FR, five male) were an average of three months of age at the start of each intervention period and were matched with seven C57BL/6 (wild type) controls (four PCS, three FR, four male). Mice were deprived of water for twenty-three hours/day, five days/week. They received water as a reinforcer for thirty minutes during their time in the operant chambers, and had thirty minutes free-access to water once their session was complete. This protocol was adapted from Blackshear et al. (2011). All mice had free access to water on Saturdays and Sundays, and body weights were taken weekly. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the SBU Institutional Animal Care and Use Committee.

REFER TO CHAPTER THREE FOR DESCRIPTION OF BEHAVIORAL TESTS AND BIOCHEMICAL ASSAYS

Statistical Analyses

All data were analyzed using StatView statistical software (SAS) or Microsoft Excel, and figures were generated in GraphPad Prism 6. One and two-way ANOVAs were used to analyze operant data, as well as data from the Unreinforced Radial Arm Maze and the DigiScan tasks. Two and three-way repeated measures ANOVA were used to analyze data from the Barnes Maze task. Student t-tests were used to assess differences in positive immunostaining as well as differences in total Aβ levels determined through ELISA. A series of Pearson correlations were used to examine preliminary relationships between behavioral data and performance on various intervention measures, behavioral data and specific measures of pathology, and intervention measures and measures of pathology. Significance level was set at $p = .05$.

As a follow up to the previous study, study #2 sought to investigate whether PCS would have an impact on cognitive function and neuropathology in a well-characterized, classic transgenic mouse model of parenchymal amyloid. While the results from study #1 suggest that the effects of a PCS intervention on the Tg-SwDI model of CAA are minimal, we hoped to replicate the intervention using a commonly used model of parenchymal amyloid, as it would allow us to either extend the findings to another form of AD pathology, or to see potential distinctions in how PCS impacts various pathological features and their associated symptomologies.

RESULTS

Operant Results

The Fixed Ratio (FR) task required that mice poke their nose into a lit nose port. The goal of this task was for mice to begin making the association between nose-pokes into lit ports and the delivery of a water reinforcer. The number of sessions required to reach criterion (a total of 15 or more responses in a single session) was measured. Two, one-way ANOVAs were performed to compare each group (PCS or FR) with their age-matched wild type control group. In the PCS group, the Tg-5xFAD mice required a slightly higher number of sessions to reach criterion (M=6.1, SD=.26) when compared to their age-matched wild types (M=5.7, SD=2.0). This difference was not statistically significant [$F(1,8)$=.058, p>.05.]. In the FR group, the Tg-5xFAD required slightly fewer sessions to reach criterion (M=6.2, SD=2.4) when compared to wild types (M=7.3, SD=5.7), but again there was no significant difference between the two [$F(1,6)$=.158, p>.05]. (Figure 9A).

Only data from the PCS group will be analyzed and reported from here on because the FR group remained on the FR schedule for the remainder of the 4-month intervention. One Tg-5xFAD mouse was unable to reach Alternation criterion, and therefore the data from that mouse will be excluded from the subsequent analyses. The Alternation task requires that mice generalize nose-pokes into both nose ports (on both the left and right side of the main chamber wall). Number of sessions required to reach criterion were again measured and analyzed using a one-way ANOVA. Tg-5xFAD mice required fewer sessions to reach criterion (M=8.8, SD=2.7) when compared to wild types (M=13.2, SD=6.8), but this difference was not statistically significant [$F(1,7)=1.8$, $p>.05$].(Figure 9B).

In the Light-Dark Discrimination task, mice are also asked to nose-poke into a lit port, but this time the ports alternate at random. An error was measured as a nose poke into an unlit nose port. Number of sessions required to reach criterion (a 90% correct response in one trial) was again measured and analyzed using a one-way ANOVA. Again we saw no statistically significant difference between the two groups [transgenic: M=4.4, SD=1; wild type: M=4.2, SD=2.87; $F(1,7)=.012$, $p>.05$]. (Figure 9C).

The Shuttle task requires that mice follow a specific sequence of events to completion, including an additional response in a lit port in the back of the chamber. Again we saw no differences in the number of sessions required to reach the criterion of 90% correct response in a single trial (transgenic M=1.6, SD=1.3; wild type M=1.2, SD=0.5; $F(1,7)=.240$, $p>.05$]. (Figure 9D).

The Non-Match to Position task has the same response sequence as the Shuttle schedule, except for the addition of a short-term memory component. After nose-poking the rear nose port, both front ports were illuminated and the mouse was required to first recall the port they poked

previously, and then poke the *opposite* nose port in order to receive a reinforcer. In this task, Tg-5xFAD mice required more sessions to reach criterion (M=34, SD=4.4) compared to wild types (M=25, SD=7.7), with a marginally significant difference between the two [$F(1,7)$=4.8, p=.0628]. (Figure 9E).

The last task of the intervention was the Delayed Non-Match to Position task. In this task, the delay between the first (front port) response and the second (back port) response varies, depending on the prior performance of the mouse. For example, if the mouse begins with a 3 second delay and reaches the appropriate criterion for that session, they will be moved to a 5 second delay the next day, and so on. All mice spent 28 days on this task. The average delays across sessions were calculated and potential differences between groups analyzed. There were no statistically significant differences between groups (transgenic M=2.74, SD=-0.8; wild type M=2.46, SD=.79, $F(1,7)$=.248, p>.05]. (Figure 9F). These results are discussed further in subsequent sections.

Behavior

Barnes Maze

A three-way repeated measures ANOVA, with experimental condition (PCS and FR), genotype (wild-type or Tg-5xFAD) and time set as factors, was first used to determine potential differences in spatial working memory performance and exploratory behavior in the Barnes Maze.

There was a trending main effect of genotype [$F(1,14) = 3.67$, p=.07], with Tg-5xFAD mice taking longer to locate the escape box over the 5 day period compared to wild types. (Figure 10A). Given the aggressive nature of the pathology in this mouse model, and the fact that

impairments in Barnes Maze learning driven by genotype have been previously reported (Xu et al., 2013), it is possible that this difference may have been more pronounced with a larger sample size. There was no significant main effect of group, or a significant genotype x group interaction, suggesting that the PCS intervention did not impact the latency to find measure in the Barnes Maze.

The number of hole-visits mice made prior to finding their assigned escape box was also analyzed. There was no significant main effect of genotype, or genotype x condition interaction, but there was a marginally significant main effect of condition [$F(1,14)=.064$, $p=.064$], with PCS mice making fewer hole-visits than FR mice (Figure 10B). However, a subsequent analysis that looked specifically at condition as a factor in Tg-5xFAD mice revealed no difference between PCS and FR groups, suggesting that the initial marginal difference seen was not entirely explained by the PCS intervention.

DigiScan

In order to investigate potential differences in general activity levels and limb strength, a two-way ANOVA was used to examine measures from the DigiScan test (Columbus Instruments, Columbus OH). There was no significant main effect of condition, genotype or a genotype x condition interaction, suggesting that neither the PCS intervention nor genotype had an influence on levels of general activity. (Figure 10C).

Enzyme-Linked Immunosorbent Assay (ELISA)

An ELISA was used to assess potential differences in whole-brain levels of Aβ between PCS and FR groups. Aβ 42-soluble, Aβ 42-insoluble and Aβ 42-membrane fractions were

examined with a student t-test. Wild type mice were not included in the analyses because they do not develop measurable levels of Aβ protein in the brain. As can be seen in Figure 3, there were no statistically significant differences in Aβ levels between any of the groups (all t-tests, $p>.05$) (Figure 11).

Given individual differences in both operant and Barnes Maze task performance, I next examined the relationship between average delay in DNMTP vs. levels of soluble and insoluble Aβ pathology. This may give us an indication of whether mice that were more successful at the short-term memory operant task (and thus had higher average delays) also had lower overall levels of Aβ pathology. There was no strong association between average delay in the DNMTP task and levels of soluble and insoluble Aβ ($r(3) = -0.09$, $p>.05$; $r(3) = -0.1$, $p>.05$, respectively). Second, Barnes Maze performance was examined as measured by the latency to find measure vs. soluble and insoluble levels of Aβ. This determined whether varying levels of pathology were related to Barnes Maze task performance. However, performance on the Barnes Maze and levels of soluble ($r(3),=.5$, $p>.05$) and insoluble Aβ levels ($r(3)=0.4$, $p>.05$) were not strongly correlated.

Immunohistochemistry and Immunoflourescence

ELISA gives a gross measure of whole-brain soluble and insoluble Aβ. In order to establish whether *regional differences* in quantity of amyloid plaque or activated microglia vary between PCS and FR groups, image analyses were used to examine hippocampal, subiculum, thalamic and cortical regions. These regions were of interest as they have been the focus of previous similar studies (Adlard et al., 2005; Jankowsky et al. 2003; Lazarov et al., 2005; Xu et al., 2014), and are among the most severely affected by AD (Alzheimer's Association, 2014).

ThioflavinS Stain of fibrillar amyloid

Independent-sample t-tests were conducted to compare potential differences in percent area occupied with thioflavin fluorescent stain of fibrillar amyloid in PCS and FR conditions. Four regions of interest (thalamus, cortex, hippocampus and subiculum) were selected because of their propensity to accumulate pathology in this particular mouse model and their vulnerability to degeneration in Alzheimer's disease. There was no statistically significant difference in percent positive stain between the PCS and FR groups in thalamus ($t(6)=0.7$, $p>.05$), cortex ($t(6)=1.19$, $p>.05$), subiculum ($t(5) =0.52$, $p>.05$) or hippocampus ($t(6)=0.8$, $p>.05$). (Figure 13A). These results suggest that the PCS intervention and did not seem to directly modify the level of amyloid pathology in these regions.

Total Aβ stain (66.1)

Similar to the thioflavin stain, independent-sample t-tests were conducted to compare potential differences in percent area occupied with a total Aβ stain in PCS, FR and SED conditions. There was no statistically significant difference in percent positive stain between the PCS and FR groups in thalamus ($t(6)=0.09$, $p>.05$), cortex ($t(6)=0.37$, $p>.05$), subiculum ($t(6)=0.016$, $p>.05$) or hippocampus ($t(6)=0.82$, $p>05$). (Figure 13B). Again, results lend more support to the finding that the PCS intervention and/or daily handling did not seem to directly modify the levels of both diffuse and fibrillar Aβ.

DISCUSSION

Operant Results

Similar to results from study #1, the results from the current operant intervention tell us that Tg-5xFAD mice and wild types were able to progress through the tasks and learn the progressively challenging rules at similar rates over the intervention period (Figure 9). This general matching of performance was useful as it allowed for similarities in training levels as we moved on to subsequent testing. We do see a marginally significant difference in the NMTP task, with Tg-5xFAD mice requiring more sessions to reach criterion. It is possible that this difference may have been more pronounced with a larger sample size. Given that this is the first short-term memory related task, and the aggressive nature of the pathology in the Tg-5xFAD, it is not surprising that they struggled to grasp the rules of this particular task. Interestingly, there were no differences between groups when measuring average delay in the DNMTP task. It is possible that even though the Tg-5xFAD took more time to master the basic rules of the NMTP task, once they had mastered the rule, they were then able to work through the slightly more challenging version at the same level as healthy controls. Another possibility is that given the low average delay for both groups (transgenic M=2.74, SD=.8; wild type M=2.46, SD=.79), the DNMTP task was too challenging for either group, and the data therefore reflect a floor effect.

The Tg-5xFAD mice performed comparably to Tg-SwDI on most operant tasks, except for requiring more sessions to master the Alternation task and fewer sessions to master the Shuttle task. This reflects distinctions in how different transgenic lines perform on particular operant tasks, something that has been examined thoroughly in a previous dissertation from our laboratory (Blackshear, 2009). It is possible that pathology with different spatial accumulation, as well as different rates of accumulation impacts the mouse's performance in tasks that require various abilities. Difficulty mastering the Alternation task, for instance, may reflect an

impairment in the mouse's ability to successfully transition *for the first time* to a task requiring the learning of a different rule. Impairment in the Shuttle task may indicate impaired performance in learning a specific sequence of relatively complex responses.

Behavioral Results

Similar to the Tg-SwDI results, the PCS intervention did not seem to impact cognitive function as measured by the Barnes Maze or exploratory behavior as measured by the DigiScan (Figure 10A,B&C). This tells us two things: First, it lends further support to the notion that isolated cognitive stimulation as an intervention may not generalize to other forms of cognitive abilities. Second, isolated cognitive stimulation does not seem to have an effect on two very common features of Alzheimer's pathology: parenchymal or cerebrovascular amyloid pathology.

Histological Results

The quantified levels of pathology reported here are similar to those reported in previous studies (e.g., Xu et al., 2014) confirming the validity of the mice used in the current study.

PCS did not change overall levels of Aβ pathology, as seen through ELISA, or regional differences in density of fibrillar Aβ or total Aβ in the thalamic, hippocampal, subicular or cortical regions (Figures 11, 12 & 13). Thus, the pathological results correspond to the behavioral results.

Conclusions

The current study was conducted for the purpose of replicating the novel PCS intervention in a classic mouse model of parenchymal amyloid pathology. Overall, results show that specific cognitive training failed to produce any benefits in general cognitive function, as measured by the Barnes Maze task. One major limitation of this study was the small sample size.

However, given the aggressive nature of the pathology in this particular model, and the findings from the previous study, it is likely that the intervention would have failed to produce measurable results had the sample size been larger. That being said, there are potential productive changes that could be made to the intervention timeline itself. One possibility is to start the intervention at an earlier age. In the current version, mice began the intervention at an age of ~three months, when pathology had already started to accumulate. It is possible that an earlier intervention, before any pathology and cognitive impairments were present, it would have been more effective at slowing the progression of the disease, or delaying the onset of pathology accumulation. It is also possible that benefits would have been more obvious had the mice remained on the short-term memory dependent DNMTP task for a longer period of time. In the 28 days allotted, the Tg-5xFAD mice were not able to work above an average of a three-second delay, either because they were not given enough time to master the task, or because they had reached their performance limit. It should also be noted, however, that extending the intervention would necessitate subsequent testing of cognitive abilities at around 8 or 9 months of age, when this model has widespread pathological damage and severe cognitive deficits (Oakley, 2006; Xu et al., 2014).

FIGURES: STUDY #2

FIGURE 9

Graphs showing average number of sessions required to reach criterion for individual training programs: A-E) 5XFAD and wild types performed comparably on the FR, Alternation, LDD and Shuttle tasks, respectively. F) No differences in average number of delays between groups in DNMTP task (p>.05) Error bars represent SEM.

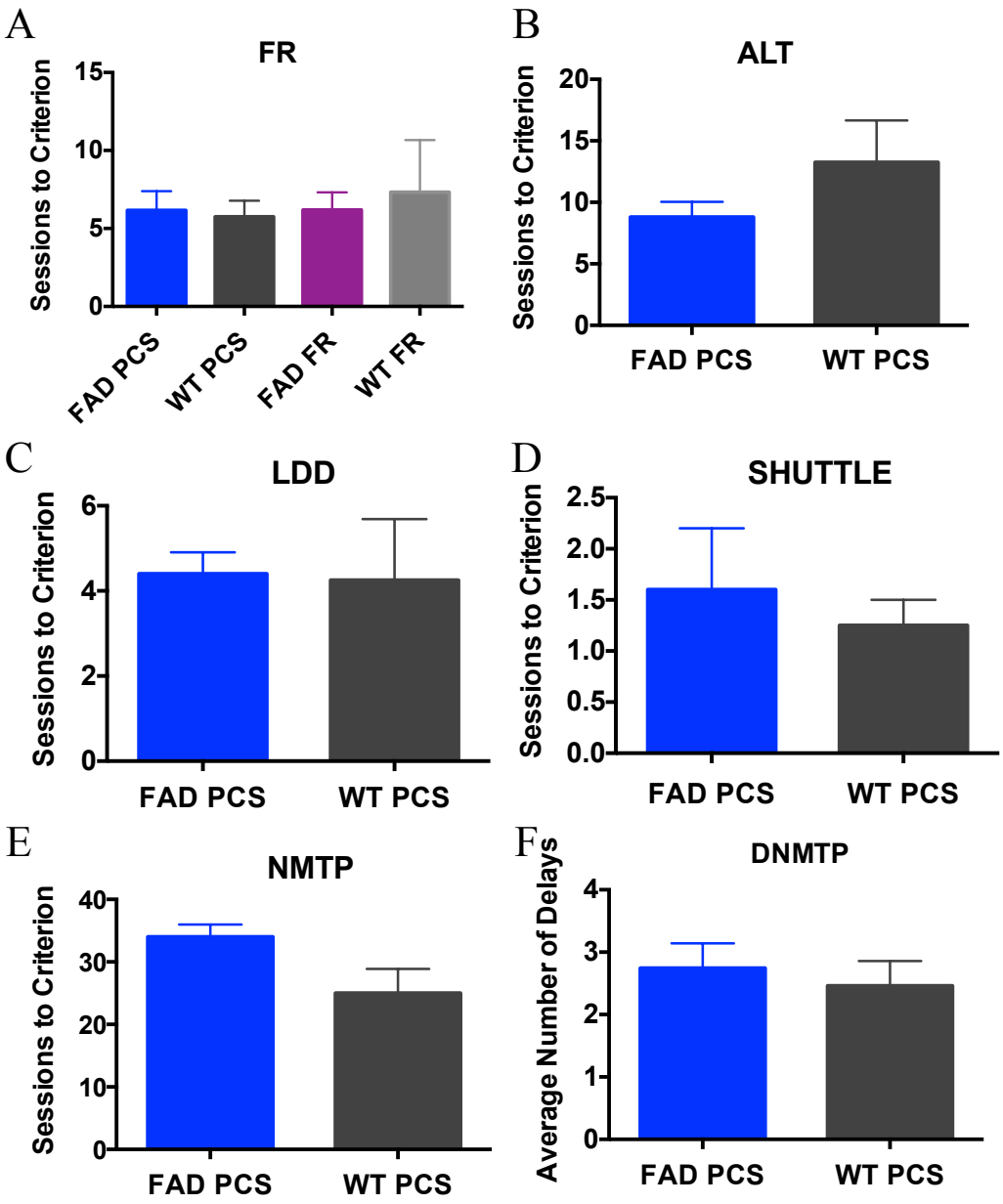


FIGURE 10

Graphs showing performance on series of behavioral measures, A) Latency to find escape box in Barnes Maze task, there was a trending main effect of genotype (Tg-5xFAD vs. wild type, p=.07), but no main effect of condition (PCS vs. Sedentary, p>.05), B) Number of hole visits in Barns Maze task, no significant main effect of genotype or condition (p>.05), C) Average number of beam breaks in a DigiScan test of general activity, no differences between groups (p>.05). Error bars represent SEM.

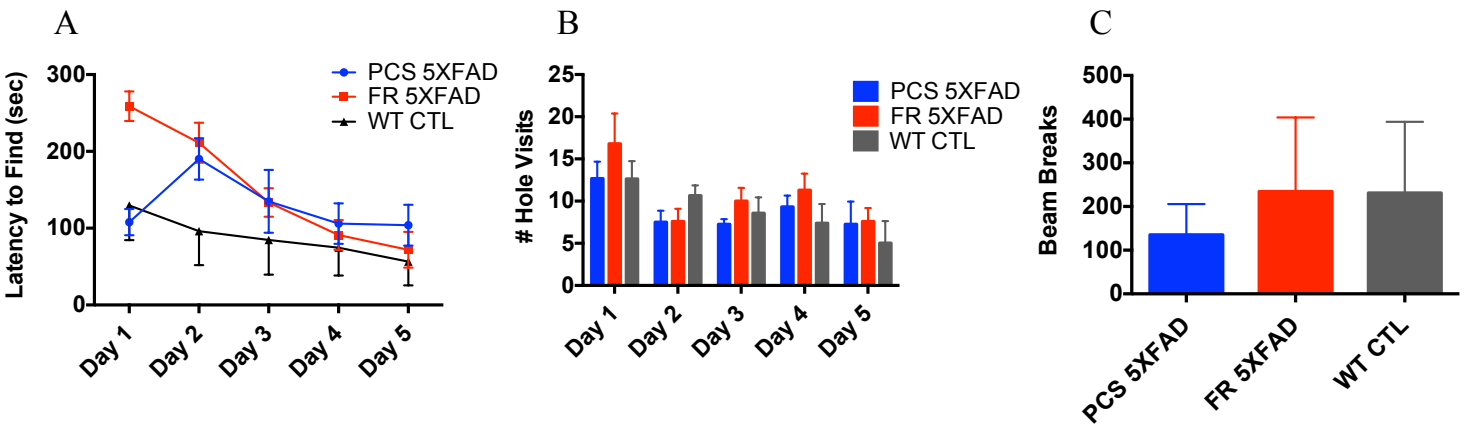


FIGURE 11

Graph shows total amount of Aβ in the following fractions: 40 soluble, 42 soluble, 40 insoluble and 42 insoluble There were no significant differences between any of the groups across the fractions. Error bars represent SEM.

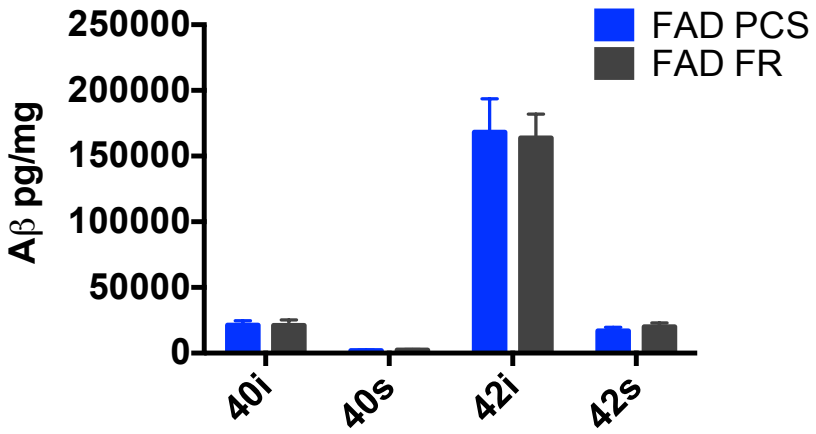


FIGURE 12

Tg-5xFAD images of hippocampus (A, E; 4x mag), subiculum (B,F; 10x mag), cortex (C;G, 4x mag) and thalamus (D,H; 4x mag) stained with thioflavin-s stain (Green) for fibrillar amyloid and 66.1 stain (Brown) for total Aβ. Scale bars for 4x images set at 200µm, scale bar for 10x images set at 100µm.

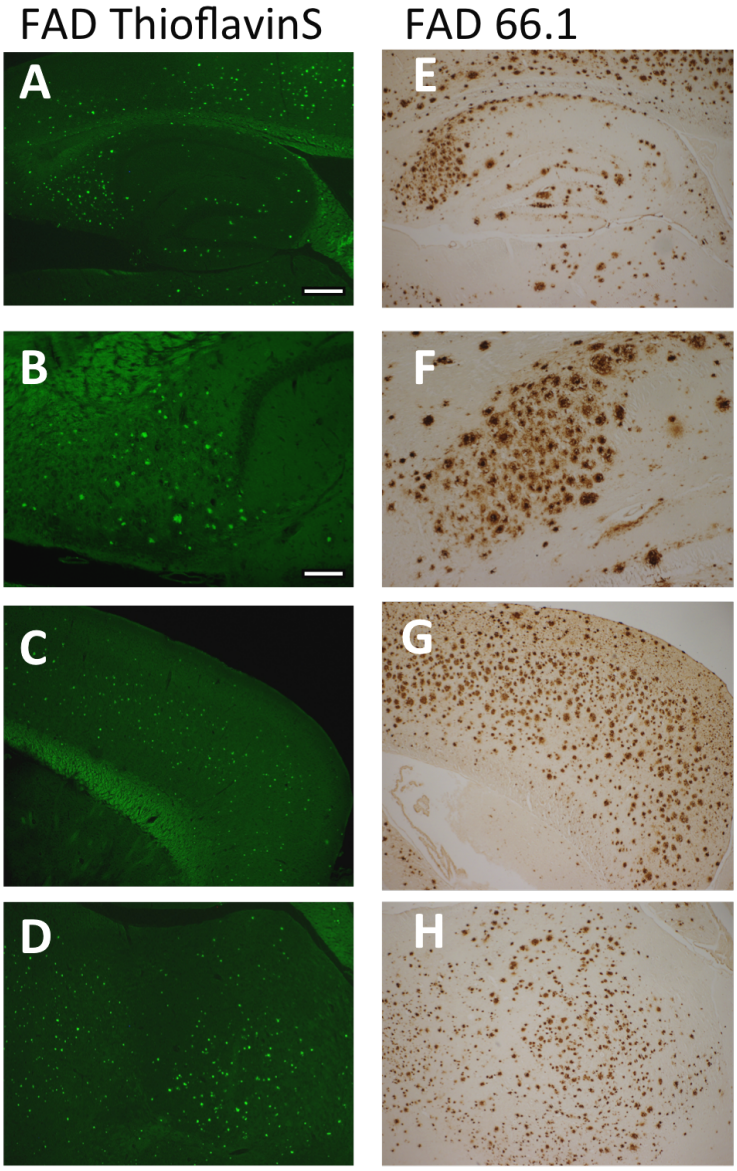
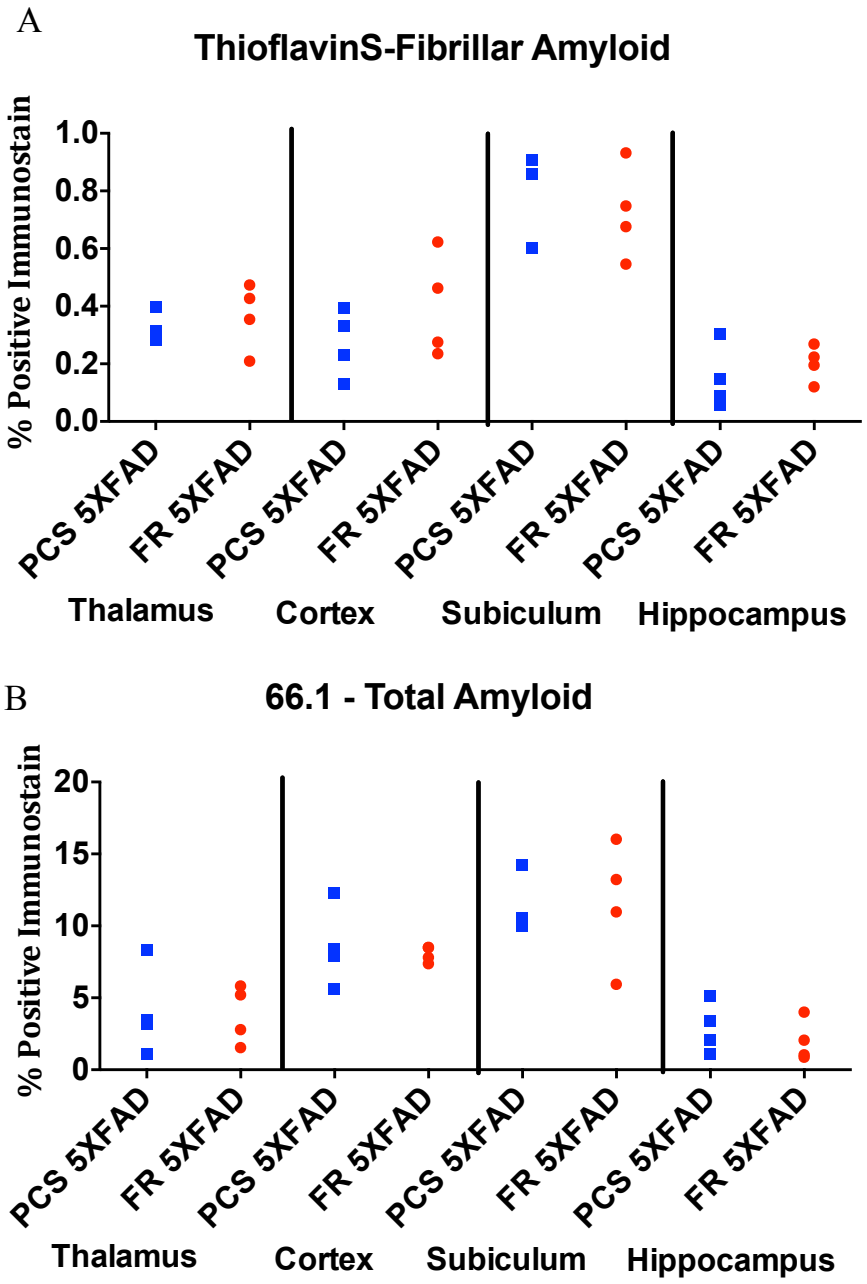


FIGURE 13

Graphs showing percent average area of positive immunostaining for individual Tg-5xFAD mice in PCS and FR groups (n=4 in each group). Regions of interest examined include thalamus, cortex, subiculum and hippocampus. A) Thioflavin-S stain for fibrillar amyloid, no statistically significant differences between any of the groups in any of the regions (all t-tests, p>.05). B) 66.1 stain for total Aβ, no statistically significant differences between any of the groups in any of the regions, (all t-tests, p>.05).



CHAPTER V: EFFECTS OF A VOLUNTARY AEROBIC EXERCISE INTERVENTION ON THE TG-SWDI MOUSE MODEL OF CAA: A PILOT STUDY

INTRODUCTION

A significant number of studies have reported benefits of exercise at the neuronal level and in cognitive function (e.g., Adlard et al., 2005; Liu-Ambrose et al., 2010; van Praag et al., 2005). Most of the reported studies have used transgenic mouse models that develop amyloid pathology in brain parenchyma. The current study sought to expand previous findings by determining whether 4-months of voluntary exercise would impact cognitive function and pathology in a Tg-SwDI mouse model of CAA. This study was designed to examine exercise as a central variable, thereby isolating it from other potential forms of stimulation and/or enrichment. Mice were individually housed in tub cages with an attached running wheel that was connected to rotation-counting software. A voluntary wheel-running intervention was chosen over forced running (e.g., treadmill) because it allowed for an environment where mice could choose how often to run, minimizing the potential confounding variables related to forced interventions (Leasure & Jones, 2008). Following the 4-month intervention, behavioral and histological changes were assessed.

MATERIALS AND METHODS

Subjects

Fourteen Tg-SwDI mice (eight RUN, six SED, all female) and seven C57B1/6 were an average of three months of age and weighed 20-30 grams at the start of the intervention period. All procedures were performed in accordance with the NIH Guide for the Care and Use of

Laboratory Animals and were approved by the SBU Institutional Animal Care and Use Committee.

Voluntary Exercise Intervention

Mice assigned to the voluntary exercise group were single-housed in a cage measuring 43x21x21cm equipped with a running wheel that measures 6.5 inch in diameter with a 3 inch-wide running platform. Number of rotations were recorded through a software system that responds to the closing of a switch produced by two magnets lining up – one magnet located directly on the wheel, and another on the outside of the cage. A group of sedentary control mice were single-housed in cages that lacked an attached running wheel. Mice had 24-hour access to their running wheels for approximately 16 weeks.

REFER TO CHAPTER THREE FOR DESCRIPTION OF BEHAVIORAL TESTS AND BIOCHEMICAL ASSAYS

Statistical Analyses

All data were analyzed using StatView statistical software (SAS) or Microsoft Excel, and figures were generated in GraphPad Prism 6. One and two-way ANOVAs were used to analyze operant data, as well as data from the Unreinforced Radial Arm Maze and the DigiScan tasks. One and two-way repeated measures ANOVA were used to analyze data from the Barnes Maze task. Student t-tests were used to assess differences in positive immunostaining as well as differences in total Aβ levels determined through ELISA. A series of Pearson correlations were used to examine preliminary relationships between behavioral data and performance on various

intervention measures, behavioral data and specific measures of pathology, and intervention measures and measures of pathology. Significance level was set at p =.05.

RESULTS

Intervention

Over the 4-month intervention, running animals showed a steady decrease in their average running distance. The average running distance in the first month was 7381 m/24-hours +/- 2422, 5297 m/24-hours +/- 2190 in the second month, 5049 m/24-hours +/- 2206 in the third month and 3812 m/24-hours +/- 1886. This drop over time may be attributed to the steady increase in pathology that develops as the mice age, as described in Davis et al. (2004).

Behavior

Barnes Maze

A two-way repeated measures ANOVA, with experimental condition (sedentary and exercise) as well as day set as the between-subject factors, was used to determine potential differences in spatial working memory performance and exploratory behavior in the Barnes Maze. There was a significant main effect of condition [$F(1,12)=6.415$, $p<.05$], suggesting that the exercise intervention improved performance on the latency to find measure of the Barnes Maze task. (Figure 15A). A series of one-way ANOVAs determined that there was a significant difference between groups only at day 2 [RUN M=92.5, SD= 44.648; SED M=151.5, SD=52.3; [$F(1,12) = 5.179$, $p<.05$], all other days did not reach statistical significance.

Because there is a possible dissociation between performance on the first and second trial in the Barnes Maze, given the differences in length of the inter-trial intervals (15 minutes vs. 24

hours), latency to find and hole visits on the two separate trials of each day of Barnes maze testing were analyzed separately using repeated measures ANOVA. The very first trial on the first day of testing were excluded from the analyses because the mice had not at this point been exposed to the escape position and their memories were presumably not yet being tested. Analyses determined that there was no main effect of condition for the 1st trial [$F(1,12)=1.126$, $p>.05$], but there was a significant 2nd trial main effect [$F(1,12)=4.604$, $p=.05$). (Figure 15B). One possibility is that exercise may have preferentially led to benefits in 'working' memory, or rather the ability to more easily recall the location of an escape box during a very short inter-trial delay (15minutes), as opposed to the longer delay (24 hours) of the 1st trial analyses.

The number of holes visited prior to finding the assigned escape box was also analyzed. There was no significant main effect of condition [$F(1,12)=.002$, $p>.05$] suggesting that the voluntary exercise intervention had no impact on modifying the number of holes entered prior to hole with escape box, or the animal's general exploratory behavior on the maze. (Figure 15C).

Unreinforced Radial Arm Maze

An unreinforced version of the 8-radial arm maze was used to investigate potential exploratory and behavioral differences between mice that are placed in a novel, complex environment. When measuring the total number of arm-entries made, a one-way ANOVA revealed that the Tg-SwDI-SED mice had significantly fewer number of entries when compared to wild type-SED mice [$F(1,10)=17.238$, $p=.002$], which suggests a phenotypic difference in the way that Tg-SwDI mice explore the maze. (See Figure 15D). Subsequent one-way ANOVAs showed that Tg-SwDI-RUN mice entered significantly more arms than the Tg-SwDI-SED [$F(1,12)=7.073$, $p=.0208$), and that there were no differences between the Tg-SwDI-RUN group

and the wild type-SED group [$F(1,13)=.065$, $p>.05$]. These results suggest that exercise may have preserved 'normal' exploratory behavior in this particular mouse line. Another series of one-way ANOVAs revealed that wild type-SED mice had significantly more re-entries compared to Tg-SwDI-SED mice, ($F(1,10)=7.274$, $p>.05$) and no statistically significant differences were found when comparing the wild type-SED group to the Tg-SwDI-RUN group [$F(1,12) = 2.491$, $p>.05$], lending further support to the finding that exercise preserves healthy exploratory behavior in an unreinforced radial arm maze task. (Figure 15E). These findings are examined further in the corresponding discussion section.

DigiScan

A DigiScan test was used to determine whether differences in levels of general activity might partially explain the differences seen in the Barnes maze and unreinforced radial arm maze tasks. There were no differences in horizontal behavior or assisted rearings found between the Tg-SwDI-RUN and Tg-SwDI-SED groups [$F(1,12) = .058$, $p>.05$; $F(1,12) = .03$, $p>.05$], between Tg-SwDI-RUN and wild type-SED [$F(1,12)=.046$, $p>.05$; $F(1,12)=.204$, $p>.05$], or between wild type-SED and Tg-SwDI-SED [$F(1,10)=.14$, $p>.05$; $F(1,10)=.063$, $p>.05$]. These results suggest that the differences found in both Barnes Maze and Radial Arm Maze tasks cannot be entirely explained by differences in general activity levels and limb strength. This lends further support to the theory that the exercise intervention had an influence on these endpoints. (See Figure 15F).

ELISA

Student t-tests were used to examine potential differences in Aβ 40-soluble, Aβ 40-insoluble, Aβ 40-membrane, Aβ 42-soluble, Aβ 42-insoluble and Aβ 42-membrane fractions between groups (RUN and Sedentary). Wild type mice were not included in the analyses since they do not develop measurable levels of the Aβ protein. As can be seen in Figure 16 there were no statistically significant differences in Aβ levels between any of the groups (all t-tests $p>.05$). (See Figure 16).

Levels of Aβ determined by ELISA were similar to levels reported in previous studies of Tg-SwDI mice (Xu et al., 2014).

Immunohistochemistry and Immunoflourescence

ELISA gives a gross measure of whole-brain soluble and insoluble Aβ. In order to establish whether *regional differences* in quantity of amyloid plaque, activated microglia or vessel density vary between RUN and Sedentary groups, image analyses were used to examine hippocampal, subiculum, thalamic and cortical regions. These regions were of interest as they have been the focus of previous similar studies (Adlard et al., 2005; Lazarov et al., 2005; Jankowsky et al. 2003; Xu et al., 2014), and are among the most severely affected by AD (Alzheimer's Association, 2014).

ThioflavinS Stain of fibrillar amyloid

Independent-sample t-tests were conducted to compare potential differences in percent area occupied with thioflavin fluorescent stain of fibrillar amyloid in RUN and Sedentary conditions. Four regions of interest (thalamus, cortex, hippocampus and subiculum) were

selected because of their propensity to accumulate pathology in this particular mouse model and their vulnerability to degeneration in Alzheimer's disease. There was no statistically significant difference in percent positive stain between the RUN and Sedentary groups in cortex ($t(11)=1.57$, $p>.05$), subiculum ($t(11)=1.514$, $p>.05$) or hippocampus ($t(11)=0.95$, $p>.05$). However, there was a marginally significant difference between groups when the thalamic regions were analyzed ($t(11)=2.03$, $p=.067$), but no differences in the average size of plaques ($t(11)=0.16$, $p>.05$).(Figure 19A). Results suggest that while 4-months of voluntary exercise did not seem to directly or indirectly impact the level of fibrillar amyloid pathology in cortex, hippocampus or subiculum, there may have been some reduction of fibrillar plaque density in the thalamic regions. This finding is examined further in the corresponding discussion section.

Total Aβ stain (66.1)

Similar to the Thioflavin stain, independent-sample t-tests were conducted to compare potential differences in percent area occupied with a total Aβ stain between RUN and Sedentary conditions. There was no statistically significant difference in percent positive stain between the RUN and Sedentary groups in thalamus ($t(11)=0.59$, $p>.05$), cortex ($t(11)=1.187$, $p>.05$), subiculum ($t(11)=1.5$, $p>.05$) or hippocampus ($t(11)=0.14$, $p>.05$). (Figure 19B). These results suggest that the RUN intervention did not modify density of total Aβ in the selected regions.

Activated Microglia Stain

It was shown previously that exercise interventions altered neuroinflammatory markers in a transgenic mouse model of AD (Parachikova, Nichol & Cotman, 2008). In order to examine whether there was an impact on activated microglia in these regions following the RUN

intervention, independent-sample t-tests were conducted to compare percent area occupied with positive stain in RUN and Sedentary conditions. Similar to the previous results from regional analyses, there were no statistically significant differences in positive staining of activated microglia between the RUN and Sedentary groups in the thalamic region ($t(11)=0.16$, $p>.05$) cortex ($t(11)=0.47$, $p>.05$), subiculum ($t(11)=0.2$, $p>.05$) or hippocampus ($t(11)=0.4$, $p>.05$). (Figure 19C).

Vessel Density

Previous reports in the literature have found increased microvessel density following exercise interventions in rats (Ding et al., 2004). In order to establish whether our running intervention led to an increase in vessel density, a collagen IV antibody was used to stain for brain microvessels. Running did not alter vessel density in the determined regions, as there were no differences between RUN and Sedentary groups in thalamus ($t(11)=1.292$, $p>.05$), cortex ($t(11)=0.311$, $p>.05$), subiculum ($t(11)=0.96$, $p>.05$) or hippocampus ($t(11)=0.02$, $p>.05$). (Figure 19D).

Correlations

A thorough series of correlations examining relationships between intervention measures, behavioral measures and pathological measures can be found in Figure 20. There are several relationships worth mentioning: First, there was a negative correlation between average number of rotations and total soluble Aβ, such that mice who ran more over the 4-month period also tended to have lower levels of soluble Aβ. This correlation was not statistically significant ($r(6)=-0.45$, $p=.25$), however it possible that with a larger sample size, this trend may have been

more pronounced. A weaker correlation was found between average number of rotations and levels of insoluble Aβ ($r(6)$=-0.02, p>.05). There were no significant relationships found between average number of rotations and other measures of pathology.

Second, given the marginal decrease in fibrillar amyloid in the thalamic region, determining whether increased vessel density was correlated with decreased levels of fibrillar amyloid was of particular interest. There was no relationship between the two; however, there was a significant negative correlation between vessel density in the thalamus and the latency to find measure in the Barnes Maze ($t(11)$=-0.7, p=.05), suggesting that those mice with higher levels of vessel density in thalamic regions spent less time locating their escape box in the Barnes Maze.

DISCUSSION

Eight, three-month-old Tg-SwDI mice were housed in a chamber with an attached exercise wheel that they had access to 24 hours/day for 4 months. A counting software program monitored the number of rotations the mice made per minute. Mice maintained a relatively stable running rate over the 4-month period, although it tapered off slightly over time (Figure 14). Following the intervention, mice were tested on a series of behavioral tasks that examined aspects of spatial memory and exploratory behaviors, and histological analyses were conducted to evaluate whether the intervention had an impact on levels of brain pathology. Results are discussed below.

Behavioral Results

Tg-SwDI-RUN mice spent significantly less time finding their escape box in the Barnes Maze task compared to Tg-SwDI-Sedentary mice (Figure 15A&B), indicating that there may have been some preservation of spatial memory function following the running intervention. This finding supports similar studies of other transgenic lines that show improvements in cognitive function following varying forms of cardiovascular interventions (e.g., Adlard et al., 2005; Garcia-Mesa et al., 2011; Ke et al., 2011; Nichol et al., 2007; Parachikova et al., 2008; Um et al., 2011; Yuede et al., 2009). Interestingly, mice seemed to show the clearest improvement in 2nd trial measures in particular, suggesting that the running intervention specifically ameliorated their working or spatial memory abilities. It is possible that this particular improvement can be attributed to alterations in hippocampal function following exercise interventions, a change that has been reported in previous studies (e.g., Cotman et al., 1995; Dietrich et al., 2008).

Tg-SwDI-RUN mice also exhibited healthier exploratory behavior (the term 'healthy' determined by wild type behavior) as measured by number of entries and re-entries into arms of the unreinforced 8-Radial Arm Maze. As can be seen in Figure 15D&E, Tg-SwDI-SED mice had relatively low numbers of entries and re-entries while wild type mice entered arms much more often. This may be due to a phenotypic difference in the way the Tg-SwDI mice explore the maze, perhaps indicating a level of caution or disorientation that emerges when mice are placed in a novel environment. Exercise seems to reverse this; Tg-SwDI-RUN mice had a significantly higher number of entries and re-entries into arms when compared to Tg-SwDI-SED. Because disorientation in extrapersonal space is a common symptom in humans with AD (Henderson et al., 1989; Monacelli et al., 2003), understanding if and how exercise alleviates such symptoms could be extremely useful from a health care standpoint.

Because of the high levels of activity required for the intervention, measuring general activity levels between groups was essential. Exercise may have increased fitness and energy levels overall, which raises the possibility that the differences we see in the Barnes Maze and URAM were simply due to increased speed or higher levels of overall activity. The DigiScan test allowed us to measure general activity by calculating the number of movements the mice made quantified by the number of photobeam breaks in the DigiScan chamber. Interestingly, there were no differences in overall activity levels (Figure 15F), suggesting that the effects we found in the Barnes Maze and URAM were likely not being driven by a variation in overall activity levels. However, it is important to note that the actual speed during the Barnes Maze and URAM was not reported. There were no differences found in assisted rearings or unassisted rearings, indicating that limb strength was also not a likely driving factor (data not shown).

ELISA & Histology

Histological analyses were conducted subsequent to behavioral testing in order to determine whether the running intervention had any measurable effect on vascular amyloid pathology in the Tg-SwDI-RUN mice. An enzyme-linked immunosorbent assay (ELISA) revealed no differences in gross levels of whole-brain Aβ pathology between groups (Figure 16) indicating that running did not alter levels of whole-brain Aβ as determined by the ELISA. Improvements in cognitive function, despite steady or increased levels of Aβ pathology, have been reported in previous studies using environmental enrichment paradigms that contain running wheels (e.g., Arendash et al., 2004; Jankowsky et al., 2003).

Measuring total brain Aβ levels through an ELISA assay may be diluting potential changes in pathology in specific regions of the brain. To address this limitation, regional

differences in quantity of amyloid plaque, activated microglia and vessel density were used to examine hippocampal, subiculum, thalamic and cortical regions. These regions were of interest as they have been the focus of previous studies (Adlard et al., 2005; Jankowsky et al. 2003; Lazarov et al., 2005; Xu et al., 2014), and are among the most severely affected by AD (Alzheimer's Association, 2014). Overall, there were no statistically significant differences in density of fibrillar amyloid, total levels of Aβ or activated microglia (Figure 19). There was a marginally significant difference in thalamic fibrillary amyloid density between Tg-SwDI-RUN and Tg-SwDI-SED groups (Figure 19) which may be indicative of differences in rates of sensory processing seen in the behavioral tests. While the Barnes Maze is ostensibly a task that measures spatial memory abilities, it is also possible that improvements in the latency to find measure were driven by an improvement in the mouse's ability to process sensory information more efficiently. One possibility is that this modification may have been related to decreases in levels of fibrillar amyloid pathology in thalamic regions.

Previous studies have reported increased microvessel density following exercise interventions in rats (Ding et al., 2004). Surprisingly, as can be seen in Figure 19, there were no differences in microvessel density in the chosen regions. One possible explanation for this finding is that the levels of fibrillar amyloid deposited within and surrounding vessel overwhelmed vessel restructuring or angiogenesis from taking place. It is also possible that while vessel density did not increase, running led to improved functioning of extant vessels. This may have potentially resulted in improved clearance of toxic substances via perivascular pathways, which may have in turn played a role in the improved cognitive function found.

Relationships found between variables

There was a negative, marginally significant correlation between average number of rotations and total soluble Aβ, such that mice who ran more over the 4-month period also tended to have lower levels of soluble Aβ. This relationship suggests that higher levels of running may have reduced levels of soluble Aβ, which may have been an underlying driving force in preserving cognition. It is also possible, however, that the direction goes the opposite way, such that mice with lower levels of soluble Aβ at baseline were more cognitively lucid and willing to run more often. There were no significant relationships found between average number of rotations and other measures of pathology.

Given the marginal decrease in fibrillar amyloid in the thalamic region, it was of particular interest to determine whether increased vessel density was correlated with decreased levels of fibrillar amyloid. There was no relationship between the two. However, there was a significant negative correlation between vessel density in the thalamus and the latency to find measure in the Barnes Maze, suggesting that those mice with higher levels of vessel density in thalamic regions spent less time locating their escape box. It is possible that higher levels of vessel density in thalamic regions may have led to improved cognitive function despite stable levels of fibrillar amyloid, a finding that has potential implications for the role of the thalamus in sensory processing in the Barnes Maze.

Conclusions

A 4-month voluntary exercise intervention led to improvements in aspects of spatial working memory in the Barnes Maze task and healthier exploratory behaviors in the URAM, despite steady levels of whole-brain Aβ, regional fibrillar amyloid, total Aβ, activated microglia

and vessel density. While the thorough investigation of whole-brain and regional levels of Aβ pathology and signs of neuroinflammation and angiogenesis revealed no obvious differences between groups (see correlation matrix: Figure 20), it is very possible that the improvements in behavioral performance were driven by changes at the neural level. For example, several studies by Henriette van Praag (1999; 2005) reported that running interventions increase neurogenesis in the dentate gyrus in aged mice. Others have reported increased levels of brain-derived neurotrophic factor (BDNF), a protein that supports survival of neuronal subtypes and is involved in synaptic efficiency and other forms of plasticity (Cotman et al., 2002; McAllister, 1999; Neeper et al., 1996; Oliff et al., 1998; Schinder & Poo, 2000). It is also possible, considering the particular feature of AD expressed by the Tg-SwDI, that there were increases in blood flow following running interventions, which may have played a role in driving cognitive changes.

FIGURES: STUDY #3

FIGURE 14

Figure showing average number of rotations over the 4 month intervention period. Error bars represent SEM.

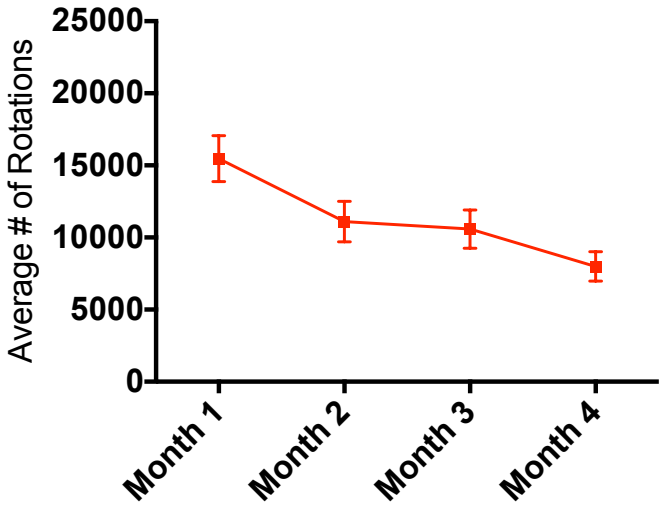


FIGURE 15

Figures illustrating results from a series of behavioral measures. A) Latency to find on the Barnes Maze. There was a main effect of condition, with exercised Tg-SwDI mice spending less time locating their escape platform. Post-hoc tests revealed a significant difference between sedentary Tg-SwDI and RUN Tg-SwDI at day 2 (p<.05). B) A similar difference was found when only the second trial of each day was included in the analysis. A repeated measures ANOVA again revealed a main effect of condition and post-hoc tests showed significance at day 2 (p<.05). C) Number of hole-visits made prior to locating escape position. There was no main effect of condition. D) The unreinforced Radial Arm Maze task revealed a main effect of condition, with sedentary Tg-SwDI entering fewer arms than RUN Tg-SwDI. E) Percent re-entries in the unreinforced Radial Arm Maze showed a significant difference between sedentary wild-type and sedentary Tg-SwDI mice, with wild-type mice spending more time re-entering arms. There were no differences between sedentary wild-type and Tg-SwDI RUN mice. F) A DigiScan test was used to measure levels of general activity. This analysis revealed no differences between genotypes or conditions (p>.05). Error bars represent SEM.

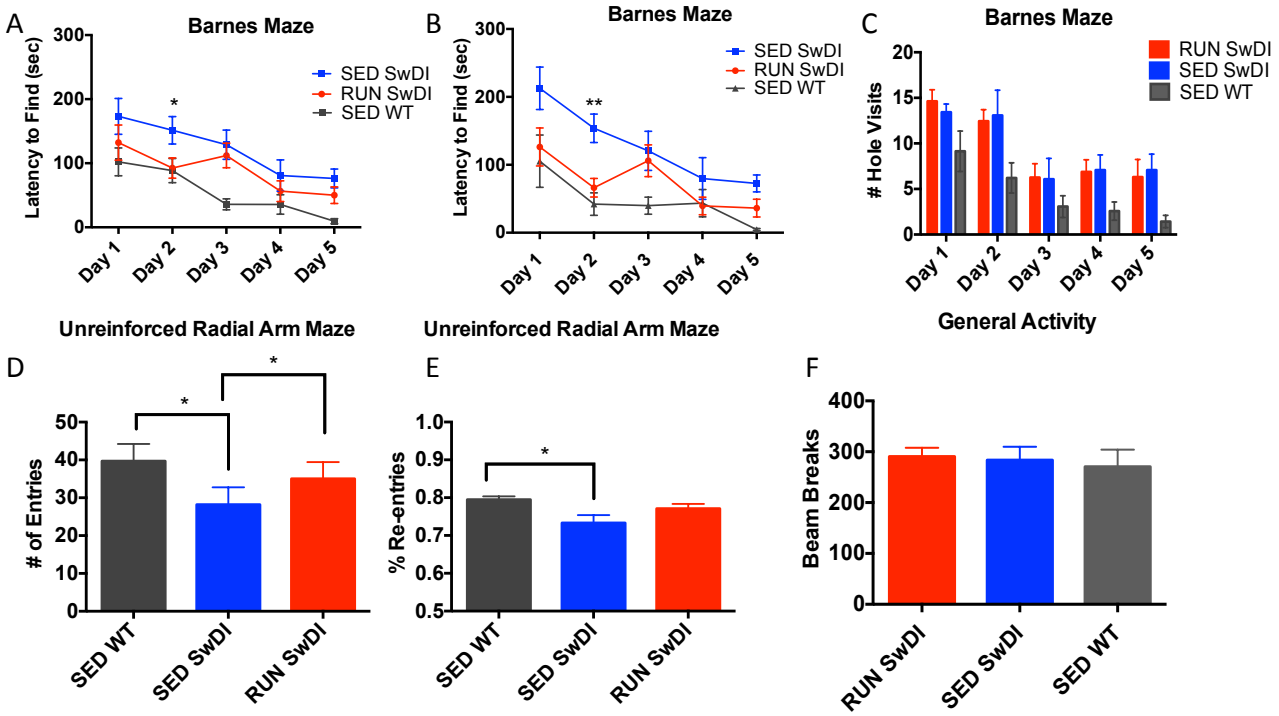


FIGURE 16

Graph shows total amount of Aβ in the following fractions: 40 soluble, 42 soluble, 40 membrane, 42 membrane, 40 insoluble and 42 insoluble There were no significant differences between RUN and SED groups across the fractions. Error bars represent SEM.

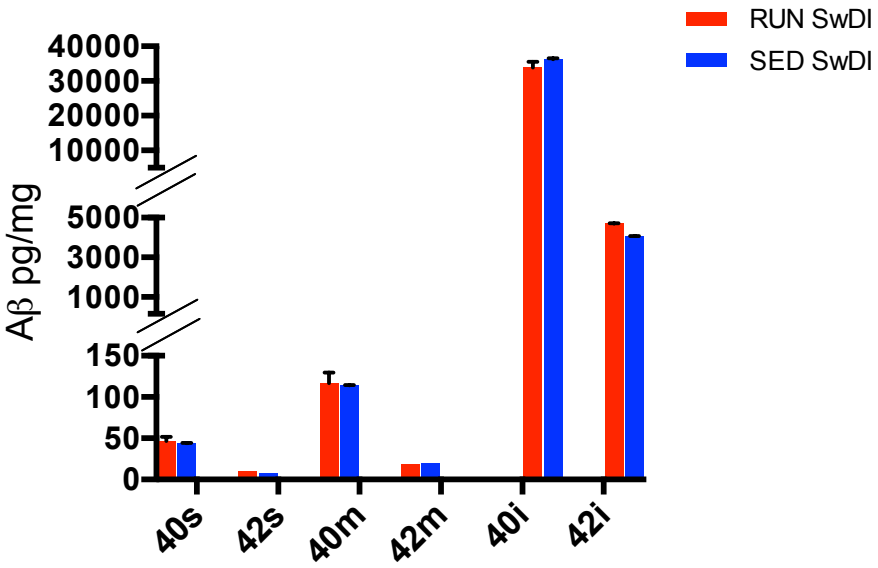


FIGURE 17

RUN Tg-SwDI and SED Tg-SwDI images of hippocampus (A,E,I,M;4x mag), subiculum (B,F,J,N; 10x mag), cortex (C,G,K,O;L 4x mag) and thalamus (D,H,L,P; 4x mag) stained with Thioflavin-s stain (Green) for fibrillar amyloid and 66.1 (Brown) stain for Total Aβ. Scale bars for 4x images set at 200µm, scale bar for 10x images set at 100µm.

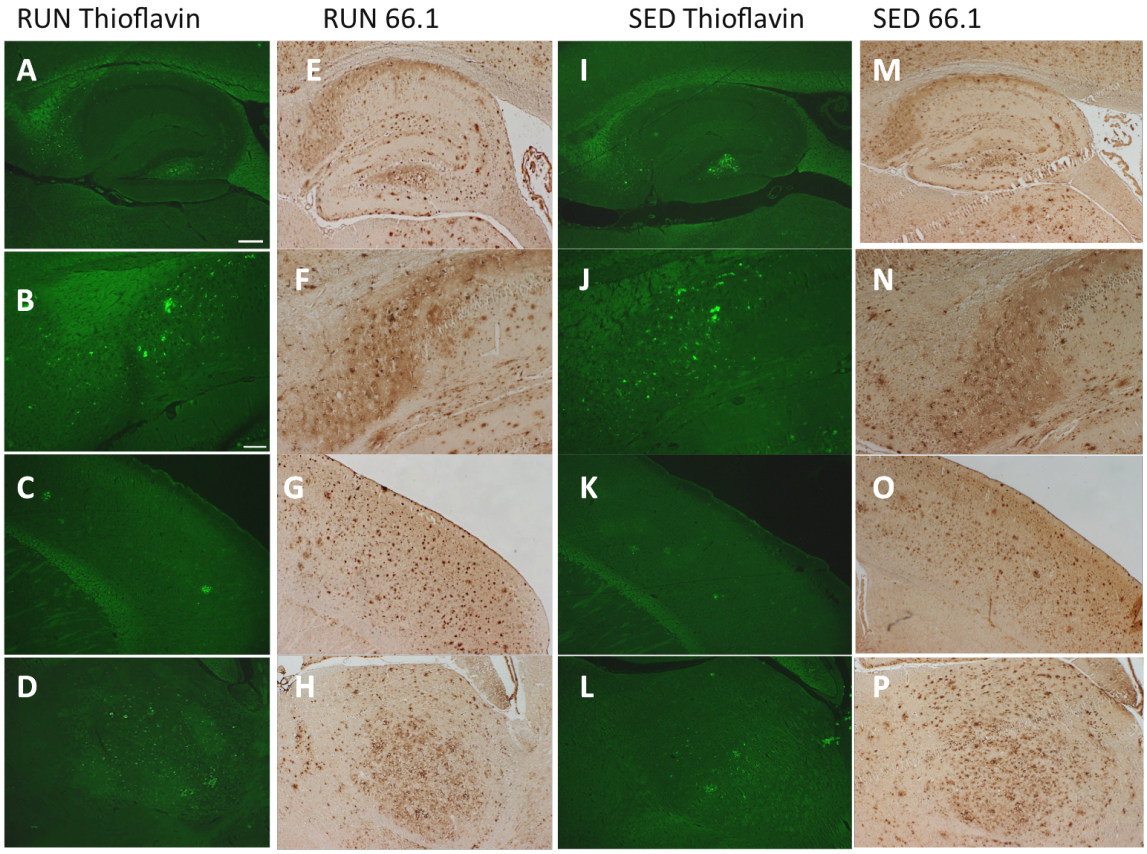


FIGURE 18

RUN Tg-SwDI and SED Tg-SwDI images of hippocampus (A,E; 4x mag), subiculum (B,F; 10x mag), cortex (C,G; 4x mag) and thalamus (D,H; 4x mag) stained with Keratan sulfate (Brown) antibody for activated microglia. Scale bar set at 200µm for 4x and 100µm for 10x.

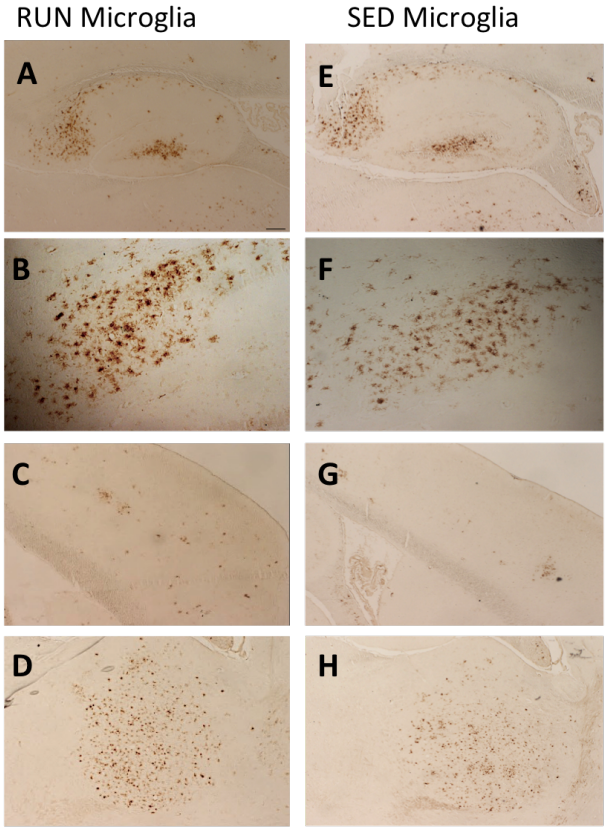
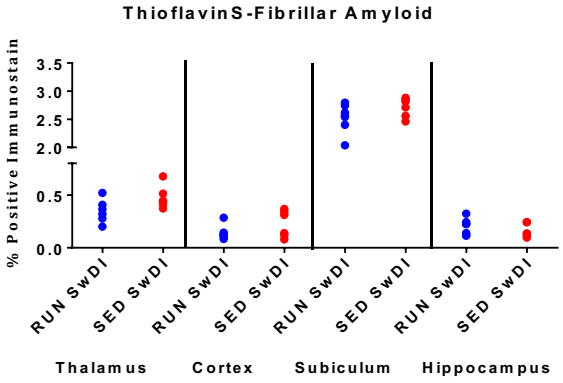
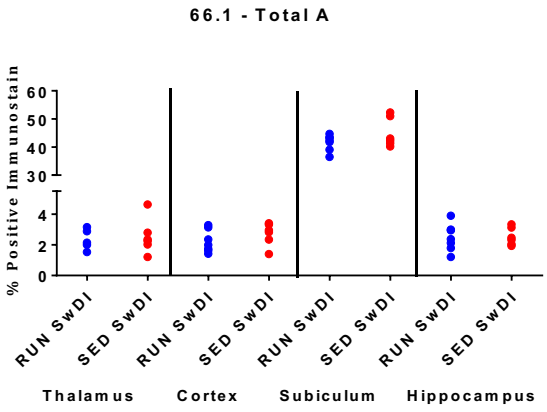
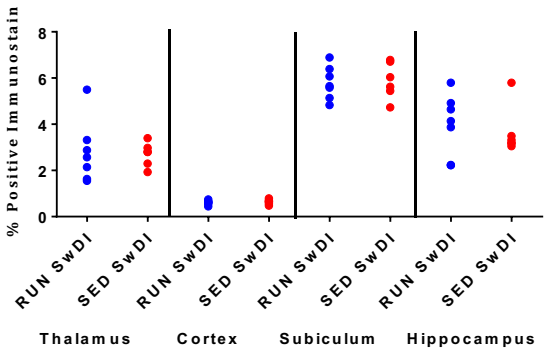


FIGURE 19

Graphs showing percent average area of positive immunostaining for individual Tg-SwDI mice in RUN and SED groups. Regions of interest examined include thalamus, cortex, subiculum and hippocampus. A) Thioflavin-S stain) for fibrillar amyloid, no statistically significant differences between any of the groups in any of the regions (all t-tests, p>.05). B) 66.1 stain for total Aβ, no statistically significant differences between any of the groups in any of the regions, (all t-tests, p>.05). C) Keratan sulfate stain for activated microglia, no statistically significant differences across groups and regions (all t-tests, p>.05). D) There were no differences in vessel density across group (all t-tests, p>.05).


ThioflavinS-Fibrillar Amyloid
% Positive Immunostain
3.5
3.0
2.5
2.0
0.5
0.0
RUN SwDI
SED SwDI
Thalamus
Cortex
Subiculum
Hippocampus


66.1 - Total A
% Positive Immunostain
60
50
40
30
4
2
0
RUN SwDI
SED SwDI
Thalamus
Cortex
Subiculum
Hippocampus


Activated Microglia
% Positive Immunostain
8
6
4
2
0
RUN SwDI
SED SwDI
Thalamus
Cortex
Subiculum
Hippocampus

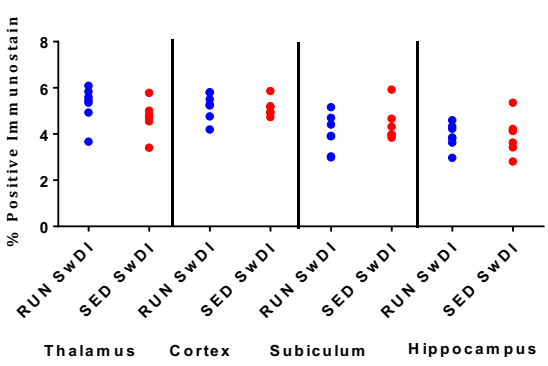

Vessel Density
% Positive Immunostain
8
6
4
2
0
RUN SwDI
SED SwDI
Thalamus
Cortex
Subiculum
Hippocampus

FIGURE 20

Correlation matrix showing Pearson correlation coefficients that represent relationships between behavioral data and performance on various intervention measures, behavioral data and specific measures of pathology, and intervention measures and measures of pathology in RUN mice.

	Rotations	LTF	Soluble	Insoluble	Thiof.Thal	Thiof. Ctx	Thiof. Sub	Thiof. Hipp	66.1 Thal	66.1 Ctx	66.1 Sub	66.1 Hipp	Glia Thal	Glia Ctx	Glia Sub	Glia Hipp	%VessThal	%VessCtx	%VessSub	%VessHipp
Rotations		0.063	-0.456	-0.028	0.229	0.261	-0.372	-0.245	-0.108	-0.256	-0.222	0.452	-0.408	-0.689	0.037	0.108	-0.293	-0.687	-0.359	-0.337
LTF	0.063		-0.294	0.581	-0.289	0.608	-0.414	0.228	-0.054	-0.557	-0.168	0.564	0.575	0.164	-0.542	0.583	-0.742	-0.313	0.305	0.061
Soluble	-0.456	-0.294		-0.284	0.225	-0.434	0.835	-0.174	-0.210	0.710	0.165	0.112	0.417	0.422	0.004	-0.657	0.444	0.829	0.182	0.255
Insoluble	-0.028	0.581	-0.284		-0.370	0.860	-0.586	0.001	0.035	-0.471	0.193	0.222	0.080	0.443	0.324	0.481	-0.646	-0.349	-0.455	-0.321
Thiof. Thal	0.229	-0.289	0.225	-0.370		-0.032	-0.013	-0.250	0.402	0.375	-0.318	0.371	-0.241	-0.450	0.104	-0.175	0.011	0.126	-0.063	0.642
Thiof. Ctx	0.261	0.608	-0.434	0.860	-0.032		-0.819	-0.255	0.332	-0.418	0.156	0.377	-0.176	0.159	0.284	0.454	-0.892	-0.663	-0.481	-0.117
Thiof. Sub	-0.372	-0.414	0.835	-0.586	-0.013	-0.819		0.049	-0.420	0.612	0.060	-0.125	0.440	0.222	-0.249	-0.652	0.733	0.833	0.434	0.108
Thiof. Hipp	-0.245	0.228	-0.174	0.001	-0.250	-0.255	0.049		-0.612	-0.674	-0.774	0.144	0.534	-0.323	-0.378	0.705	0.360	0.317	0.294	-0.160
66.1 Thal	-0.108	-0.054	-0.210	0.035	0.402	0.332	-0.420	-0.612		0.383	0.474	-0.302	-0.495	0.215	0.188	-0.215	-0.478	-0.324	-0.007	0.632
66.1 Ctx	-0.256	-0.557	0.710	-0.471	0.375	-0.418	0.612	-0.674	0.383		0.566	-0.298	-0.156	0.384	0.154	-0.950	0.286	0.445	0.133	0.453
66.1 Sub	-0.222	-0.168	0.165	0.193	-0.318	0.156	0.060	-0.774	0.474	0.566		-0.553	-0.342	0.750	0.361	-0.642	-0.222	-0.154	-0.170	-0.051
66.1 Hipp	0.452	0.564	0.112	0.222	0.371	0.377	-0.125	0.144	-0.302	-0.298	-0.553		0.436	-0.347	-0.315	0.341	-0.385	-0.068	0.011	0.085
Glia Thal	-0.408	0.575	0.417	0.080	-0.241	-0.176	0.440	0.534	-0.495	-0.156	-0.342	0.436		0.252	-0.718	0.201	0.052	0.553	0.668	0.180
Glia Ctx	-0.689	0.164	0.422	0.443	-0.450	0.159	0.222	-0.323	0.215	0.384	0.750	-0.347	0.252		0.122	-0.366	-0.172	0.276	0.092	0.069
Glia Sub	0.037	-0.542	0.004	0.324	0.104	0.284	-0.249	-0.378	0.188	0.154	0.361	-0.315	-0.718	0.122		-0.172	0.079	-0.157	-0.912	-0.375
Glia Hipp	0.108	0.583	-0.657	0.481	-0.175	0.454	-0.652	0.705	-0.215	-0.950	-0.642	0.341	0.201	-0.366	-0.172		-0.327	-0.340	-0.060	-0.190
%VessThal	-0.293	-0.742	0.444	-0.646	0.011	-0.892	0.733	0.360	-0.478	0.286	-0.222	-0.385	0.052	-0.172	0.079	-0.327		0.698	0.109	-0.135
%VessCtx	-0.687	-0.313	0.829	-0.349	0.126	-0.663	0.833	0.317	-0.324	0.445	-0.154	-0.068	0.553	0.276	-0.157	-0.340	0.698		0.398	0.307
%VessSub	-0.359	0.305	0.182	-0.455	-0.063	-0.481	0.434	0.294	-0.007	0.133	-0.170	0.011	0.668	0.092	-0.912	-0.060	0.109	0.398		0.567
%VessHipp	-0.337	0.061	0.255	-0.321	0.642	-0.117	0.108	-0.160	0.632	0.453	-0.051	0.085	0.180	0.069	-0.375	-0.190	-0.135	0.307	0.567	

CHAPTER VI: COMPARISON OF DIFFERENT DOSES OF VOLUNTARY EXERCISE ON COGNITIVE AND NON-COGNITIVE MEASURES IN A TRANSGENIC MURINE MODEL OF CAA

INTRODUCTION

There is robust evidence in both the animal and human AD literature that cardiovascular exercise has benefits for overall health as well as cognitive function (Berkman et al., 1993; Laurin et al., 2001; Nichol et al., 2007). These findings have been so encouraging that regular exercise is often recommended to patients with AD and to those at risk for developing AD (Alzheimer's Association Report, 2014). However, while it is generally accepted that exercise is beneficial, the precise dose of exercise needed to maximize health benefits remains unclear. In previous studies in rodents, exercise has typically been presented as a treatment that is compared only with a sedentary control condition, and does not assume that the intervention may have potential dose-response properties. Until now, no study has systematically compared the effects of *different amounts* of exercise on cognitive decline and overall health in AD.

Although the advantages of using rodent models to design highly controlled studies is clear, there are also potential pitfalls to using behavioral measures in rodents that should be addressed. When it comes to examining aspects learning and memory, for example, animal researchers must often make assumptions about the validity of the memory tests that they employ. Differences in behavior profiles of mice, that may be contingent upon certain intervention conditions or genetic backgrounds, may influence the behavioral phenotype expressed through various behavioral measures. These need to be taken into account as one interprets behavioral results.

MATERIALS AND METHODS

Subjects

Seventy-nine female mice made up the subject pool in the study. The breakdowns into conditions were as follows:

Group	N	Strain	Treatment
1	8 (1 deceased)	Tg-SwDI	Voluntary Wheel Running (12 hour access)
2	10 (1 deceased)	C57	Voluntary Wheel Running (12 hour access)
3	8 (1 deceased)	Tg-SwDI	Voluntary Wheel Running (3 hour access)
4	11	C57	Voluntary Wheel Running (3 hour access)
5	11	Tg-SwDI	Voluntary Wheel Running (1 hour access)
6	9	C57	Voluntary Wheel Running (1 hour access)
7	10	Tg-SwDI	Sedentary
8	12	C57	Sedentary

Mice were between 1.5-3 months of age at the beginning of the intervention. Given the range of ages in this study, ages were distributed evenly across conditions. All procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the SBU Institutional Animal Care and Use Committee.

Intervention

All mice weighed 14.6-22.5 grams at the start of testing and had unlimited access to food and water throughout the intervention period. Six days/week (Monday-Saturday), mice were gently removed from their home cages and placed into a chamber measuring 43x21x21cm with an attached running wheel for the allotted amount of time (either one, three or twelve hours). The

chambers were equipped with a running wheel that measured 6.5 inch in diameter with a 3 inch-wide running platform. Number of rotations were recorded through a software system that responds to the closing of a switch produced by two magnets lining up – one magnet located directly on the wheel, and another positioned in parallel on the outside of the cage. A group of sedentary control mice were single-housed in home cages. The intervention lasted a total of 16 weeks.

Additional Behavioral Assessments

In addition to the battery of behavioral tasks described previously, the following behavioral tests were included into the present study. Crawley's three-chamber social approach test was used as a measure of sociability and social anxiety. The Open Field was used as a measure of spontaneous exploratory behavior as well as anxiety-like behavior. The Light/Dark transition test was used to examine anxiety-like behavior, and the Rota-Rod test assessed basic coordination and motor function.

Open Field

In the open field test, mice were individually tested in a square enclosure that measured 60 cm by 60 cm. Using the AnyMaze^{tm} software, regularly spaced grid lines were created to form a 4x4 grid square with each box measured to 15cm by 15cm. The Any-maze software allows researchers to record various measures in a field of view, which expands the previous limitations of human recording. The open field apparatus was used to examine time immobile, assisted and unassisted rearings, and time spent in center of apparatus vs. periphery. Each mouse was placed in the same starting location in one corner of box and was run for 300 seconds. The

AnyMaze[tm] software was set up to systematically collect the data. The rearing measurements were manually entered into the software by the experimenter.

Three Chamber Social Interaction Task

Crawley's three-chamber social approach test was used as a measure of general sociability and social anxiety (Crawley, 2004). The box was a 18.5inch x 8.25inch rectangle divided into three sections. Side chambers measure 8.25inch x 6.5inch and the middle chamber measures at 8.25inch x 5.5 inches. For habituation purposes, each mouse was placed in the center chamber of the empty apparatus for 5 minutes prior to the task to avoid any extraneous variables of the apparatus itself to create an effect. Each of the outer chambers had a metal cup-like cylinder, one of which housed a conspecific. This wire-metal cup allows for air exchange but prevents physical contact between the animal on the inside and outside. On the task day, a novel conspecific was placed inside the cylinder of one chamber while the cylinder on the opposite chamber was left empty. The test mouse began in the middle chamber and freely explored the three sections for 300 seconds. The Any-maze software was again implemented to record and compare the time spent interacting with each cup.

Rota-Rod

The Rotarod apparatus (Medical Associates Incorporated [model ENV-575M] is a mechanized 30cm long rod divided into five 6cm sections by 4 plastic white discs. The speed of rotation increased from 4rpm to 40rpm through the course of five minutes. Mice were run four at a time on the different sections of the rod. The time that it took each mouse to fall off the apparatus was recorded along with the corresponding speed at that time. Each mouse was tested

for three consecutive trials and each trial ended when all mice had fallen off the rod or the five-minute trial had ended.

Light/Dark Box

The light-dark box is a 43cm x 21cm x 21cm plexiglass container divided into two parts. The 'dark' section is made of black plexiglass with a cover and was 14cm long while the "light" part is 29cm made with clear plexiglass with no cover. These sections were separated by a black plexiglass wall that has a 5cm x 5cm hole cut into the middle to allow for transitions. Each mouse was placed into the dark part of the box and allowed five minutes to either stay in the dark or transition into the light. A transition was defined as the mouse passing the entirety of its body into a different section of the box. The number of transitions, time spent in the light section, and time spent in the dark section were recorded

Statistical Analyses

All data were analyzed using StatView statistical software (SAS) or Microsoft Excel, and figures were generated in GraphPad Prism 6. One-way ANOVAs and t-tests were used to analyze intervention data. One and two-way ANOVAs were used to analyze the Unreinforced Radial Arm Maze, Open Field and Light Dark Box. Two and three-way repeated measures ANOVAs were used to analyze data from the Barnes Maze task and Social Interaction task. Pearson correlations were used to examine preliminary relationships between behavioral data and performance on various intervention measures. Significance level was set at $p = .05$.

RESULTS

Intervention

One-hour exercise mice ran an average of 744 meters +/- 277; 3-hour mice ran an average of 2198 meters+/-243; and 12-hour mice ran an average of 5428 meters +/- 925. These distances remained similar across groups when each genotype was taken into account separately. The mice in each condition also maintained a steady rate of running over the four-month intervention period (refer to Figures 21B&C). This similar rate of running over the 16 week intervention was useful as it allowed for similarities in training levels as we moved on to subsequent cognitive testing.

One interesting question to consider is whether data collected from the intervention can reveal anything about potential differences in running patterns. Of particular interest is the question of whether there is an adjustment of running rate that is contingent upon the duration period of each session. A qualitative examination of running patterns in rotations/minute (Figure 22) would in fact suggest a difference between conditions. In order to quantify this difference, the average number of rotations/hour for each genotype (C57, Tg-SwDI) in each condition (one, three, twelve-hour) was calculated. The first five days of training was taken into account because after the fifth intervention day, the running rate from each condition clusters around the mean. Interestingly, there was a linear trend found in the one hour condition (C57: $y=146.32x + 813.79$, $R^2=0.837$, Tg-SwDI: $y=230.51x + 489.04$, $R^2=0.63$), the three hour condition (C57: $y=153.13x + 717.2$, $R^2=0.78$, Tg-SwDI: $y=195.53x + 710.79$, $R^2=0.72$), but not the twelve hour condition (C57: $y=4.1308x+816.51$, $R^2=0.00131$, Tg-SwDI: $y=7.44x + 739$, $R^2=0.0221$) (Refer to Figure 23). What these data suggest is that limiting the time mice have with the running wheel increases the rate of running in a linear fashion over the first several days of the intervention. The slopes

here represent the learning curve between conditions. Mice are, presumably, adapting to the shorter run duration by increasing the amount of running that they do in the allotted period of time.

These differences between running volume and intensity were also illustrated by examining the average number of breaks that mice take per minute. Twelve-hour mice took significantly more breaks/minute when compared to both the one-hour group ($t(10)= 8.077$, $p<.0001$), and three-hour group ($t\ (10)=5.98$, $p=.0001$) (Figure 24).

These data give insight into specific duration-dependent running patterns that mice engage in when given access to an exercise wheel. Taking these differences into account may prove useful in studies that use voluntary wheel running as a form of intervention, considering that they likely have implications for the outcome of various behavioral measures. For instance, instead of simply using 'average number of rotations' as a gross measure of intervention performance, measures of volume and intensity such as running duration, number of breaks and rate of learning at the beginning of training may allow for more fine-grained analysis of the quality of running each mouse receives.

Behavior

Given the complexity of the study design, the subsequent results section was written using the following formula as a guide, with the intention of presenting the results from each behavioral measure in clear and thorough way.

A general ANOVA including all variables that allows for an overview of significant main effects and interactions in the study.

In order to establish a baseline for what would be considered 'normal' Tg-SwDI and wild-type behavior, one way ANOVAs conducted to compare potential differences in behavior between these two groups

One and two-way ANOVAs conducted exclusively on wild-type animals across all experimental conditions. Although not directly related to our interest in the effects of exercise on the Tg-SwDI mouse model of CAA, this set of analyses can establish what behavioral outcomes following exercise interventions look like in 'healthy' mice.

One and two-way ANOVAs conducted specifically on Tg-SwDI mice across all experimental conditions. These analyses can reveal the effects of different amounts of exercise on a mouse model of CAA pathology and cognitive decline.

Barnes Maze

Latency to Find

Although the 'latency to find' measure is used extensively to detect alterations in spatial memory abilities, it is particularly susceptible to influence by other factors, predominantly varying levels of anxiety and motivation to escape. This caveat should be kept in mind as the following statistical analyses are considered.

A three-way repeated measures ANOVA, with experimental condition (1HR, 3HR, 12HR, Sedentary) genotype (wild-type and Tg-SwDI) and day set as factors, was first used to determine potential differences in aspects of spatial working memory performance and exploratory behavior in the Barnes Maze task. There was a significant main effect of genotype ($F(1,70) = 13.349$, $p=.0005$), indicating that wild-type mice took less time moving to their escape positions, experimental condition ($F(3,70)=4.602$, $p=.0054$), indicating that running animals on average took longer to move to their escape position, and day ($F(4,70)=15.5$, $p=.0001$), suggesting a possible learning effect on latency to find measures. There were no significant genotype x condition or day x condition interactions, but there was a significant genotype x day interaction ($p<.05$), suggesting that the effects of genotype on latency to find performance depended on the trial day.

A one-way repeated measures ANOVA that looked only at wild-type sedentary mice vs. Tg-SwDI sedentary mice was conducted in order to establish a baseline of behavior between the two genotypes. There was no significant main effect of genotype, but there was a significant main effect of day ($p<.05$), indicating a learning effect of latency to find in both groups.

A subsequent two-way repeated measures ANOVA including only wild-type mice did not show a main effect of condition but did show a main effect of day ($F(4,144)=32.46$, $p<.05$),

again suggesting that all mice took less time to locate their escape position in later trials, and a significant day x condition interaction ($F(12,144) = 2.14$, $p<.05$), suggesting that the effect of condition on latency to find in wild-type mice changed depending on the day. Fisher's post-hoc comparison revealed a significant difference between 12HR vs. SED groups and 3HR vs. SED groups on day 1 and 12HR vs. SED groups on day 2, with sedentary groups taking less time to find their escape position.

A two-way repeated measures ANOVA including only Tg-SwDI in the analysis revealed a significant main effect of condition ($F(3,34)=2.934$, $p<.05$). Fisher's post-hoc comparison revealed differences between 1HR vs. SED, 3HR vs. SED and 12HR vs. SED, with sedentary mice taking less time to locate their escape platform. There was also a significant main effect of day ($F(4,136) = 6.76$, $p<.05$), and no condition x day interaction ($p>.05$).

Hole Visits

A three-way repeated measures ANOVA, with experimental condition (1HR, 3HR, 12HR, Sedentary), genotype (wild-type or Tg-SwDI) and day set as factors, was first used to determine potential differences in the number of hole visits mice made prior to locating the escape box. As explained previously, hole-visits can be considered 'errors' made prior to finding the 'goal,' but can also be thought of as measures of exploratory behavior on the maze platform. This analysis revealed no main effect of genotype, but a significant day x genotype interaction ($F(4,70)=3.12$, $p<.05$), indicating that the effect of genotype on number of hole visits depended on the trial day. There was also a main effect of experimental condition ($F(1,70)=2.977$, $p<.05$), suggesting that exercised animals overall made more hole visits, and day ($F(3,70) = 105.5$, $p=.0001$), indicating a reduction in the number of hole visits over time.

A one-way repeated measures ANOVA including only wild-type and transgenic sedentary conditions revealed no significant differences between the number of hole-visits made between these two groups. There was a significant day x genotype interaction, however, suggesting that the effect of genotype on hole visits depended on the day. Fisher's post-hoc comparison showed that Tg-SwDI sedentary mice made more hole-visits on day 4 ($p<.05$).

A two-way repeated measures ANOVA including only wild-type mice revealed no main effect of condition and no condition x day interaction, suggesting that wild-type mice in all conditions made, on average, the same number of hole visits before finding their escape box.

A two-way repeated measures ANOVA including only Tg-SwDI mice again revealed no main effect of condition or a condition x day interaction.

Mean Speed

Mean speed, calculated in meters/second, was also examined using a three-way repeated measures ANOVA. There was a significant main effect of genotype ($F(1,70)=25.5$, $p<.0001$), with wild-type mice recorded as being faster on average, experimental condition ($F(3,70)=16.5$, $p<.0001$), with sedentary mice being faster on average, and day ($F(4,70)=16.5$, $p<.0001$), suggesting that all mice became faster on average over time. There was no significant genotype x condition interaction ($p>.05$), but there were significant condition x day and genotype x day interactions (all $p<.05$), suggesting that the effect of condition on speed as well as the effect of genotype on speed depended on the trial day.

A two-way ANOVA including only sedentary groups from each genotype revealed a significant main effect of genotype ($F(1,20)=4.43$,$p<.05$), indicating that wild-type mice were recorded as moving around the maze faster than Tg-SwDI, and day ($F(4,80) = 6.8$, $p<.05$) again

showing an increase in speed in later trial days. There was no significant genotype x day interaction ($p>.05$).

A subsequent two-way ANOVA including only wild-type mice revealed a significant main effect of condition ($F(3,36)=6.392$, $p<.05$). Fisher's post-hoc comparisons showed significant differences between 1HR and SED mice ($p<.05$), 12HR and SED mice ($p<.05$) and 3hr and SED mice ($p<.05$), with sedentary mice moving faster. There were no significant differences between 1HR, 3HR or 12HR groups.

When only Tg-SwDI were taken into account, a two-way ANOVA showed a significant main effect of condition ($F(3,34)=15.064$, $p<.0001$). Fisher's post-hoc comparisons showed a similar trend: there were significant differences between the 1HR and SED conditions, 3HR and SED conditions and 12HR and SED conditions (all $p<.05$). None of the other conditions (1HR vs. 3HR vs. 12HR) were statistically significant, again showing that exercise lead to a reduction in speed on this particular task.

Path Efficiency

A three-way repeated measures ANOVA that included genotype, experimental condition and day as factors revealed a significant main effect of genotype ($F(1,70)=5.05$, $p<.05$), indicating that wild-type mice took more direct routes to their escape box, and day ($F(4,70)=88.3$, $p<.0001$), suggesting a learning effect in the path efficiency measure. There was a significant genotype x day interaction ($F(4,70)=4.42$, $p<.05$), suggesting that the effect of genotype on path efficiency depended on which trial day the mice were on. No main effect of experimental condition, a genotype x condition interaction, or day x condition interaction ($p>.05$) was found.

RotaRod

The Rota-rod test measured coordination and limb strength in order to determine whether there were differences in these measures across genotypes and conditions.

Time spent on rod

A two-way ANOVA with genotype and condition set as factors revealed a significant main effect of experimental condition ($F(1,71)=3.02$, $p<.05$), such that exercised mice spent more time on the rod, and no genotype x condition interaction ($p>.05$).

A one-way ANOVA including only sedentary groups revealed no main effect of genotype, and a one-way ANOVA including only wild-type mice showed no main effect of experimental condition ($p>.05$).

However, a one-way ANOVA including only Tg-SwDI mice revealed a marginal main effect of experimental condition ($p=.08$), with Fisher's post-hoc comparisons showing significant differences between 12HR vs. sedentary mice ($p=.01$). This indicates that high levels of exercise improved motor coordination in Tg-SwDI mice. These results also rule out the role of motor deficits as potentially driving differences seen in various measures (e.g., Latency to Find in the Barnes Maze and average speed in the URAM).

Unreinforced Radial Arm Maze

An unreinforced version of the 8-radial arm maze was used to investigate potential differences in exploration between mice that are placed in a novel, complex environment.

Total number of arms entered

When measuring the total number of arm-entries made, a two-way ANOVA revealed no main effect of genotype, condition or a genotype x condition interaction ($p>.05$), suggesting that overall activity levels were consistent across genotypes and conditions in this task, and that, on average, all mice explored the same number of arms. The same pattern was found when only sedentary mice, only wild-type mice and only Tg-SwDI mice were included into the analysis.

Percent re-entry

Another two-way ANOVA showed a similar pattern when looking at percent re-entry, with no main effect of genotype, experimental condition or genotype x condition interaction ($p>.05$).

Average speed

A two-way ANOVA examining average speed did reveal a main effect of genotype ($F(1,71)=44.83$, $p<.0001$), with wild-type mice recorded as moving faster on average, as well as experimental condition ($F(3,71)=11.965$, $p<.0001$), with sedentary mice recorded as moving faster, and no interaction between the two ($p>.05$).

There was a main effect of genotype when only sedentary wild-type and Tg-SwDI mice were included ($F(1,20)=18.5$, $p<.05$), again with wild-type mice recorded as moving faster as they explored the URAM.

A one-way ANOVA taking only wild-type mice into account found again that running reduced average speed in this task ($F(3,37)=6.2$, $p<.05$). Fisher's post-hoc comparisons revealed differences between 1HR vs. SED, 3HR vs. SED and 12HR vs. SED, with sedentary mice recorded as faster (all $p<.05$).

The same pattern was found when only Tg-SwDI were included ($F(3,34)=6.9$, $p<.05$). Fisher's post hoc comparisons again revealed that significant differences between 1HR vs. SED mice, 12HR vs. SED mice and 3HR vs. SED mice, indicating that exercise reduced speed in the URAM test.

These results suggest that although there were not obvious differences between any of the groups in their level of exploration, exercise seems to have led to a reduction in speed in both wild-type and Tg-SwDI mice.

Open Field

In order to further explore differences found in the Barnes maze and URAM tasks, a series of tests designed to measure aspects of anxiety-like behavior in mice were used.

Time spent in Center

A two-way ANOVA was used to examine time spent in the bright center of the open field apparatus compared to the darker periphery. Here, we find no significant main effect of genotype ($F(1,71)=.010$, $p>.05$), but there was a significant main effect of experimental condition ($F(3,71)=2.804$, $p<.05$), indicating that exercised mice spent more time in the center than sedentary mice.

A one-way ANOVA looking exclusively at sedentary mice found a significant main effect of genotype ($F(1,20)=10.019$, $p<.05$), with sedentary Tg-SwDI spending more time on the periphery than sedentary wild-type mice.

A subsequent one-way ANOVA taking only wild-type mice into account revealed no main effect of condition ($F(3,37)=.654$, $p>.05$), which most likely drove the reported lack of main effect of genotype in the initial analysis, given the subsequent analysis in Tg-SwDI mice.

Including only Tg-SwDI revealed a marginal main effect of condition ($F(3,34)=2.313$, $p=.09$). Fisher's post-hoc analyses show significant differences between the 1HR group vs. SED group and 12HR vs. SED group ($p<.05$) such that Tg-SwDI mice from the running groups spent significantly more time in the center of the open field compared to sedentary mice. Taken together, these results suggest that Tg-SwDI mice in the 1HR and 12HR running conditions spent more time in the center, exhibiting fewer anxiety-like behaviors, compared to Tg-SwDI SED mice, despite levels of general activity and interest in exploratory behavior remaining constant between all groups (e.g., RAM total arms data).

Light/Dark Transition Test

The Light/Dark box was used to examine anxiety-like behavior in mice, operationalized by how much time mice spent in the bright open part of the apparatus vs. the smaller, dark portion. It also allowed for an assessment of interest in exploring a potentially fear-inducing novel environment, measured by the number of transitions into the light.

Time in Light

A two-way ANOVA that included genotype and condition as factors revealed no main effect of genotype and no genotype x condition interaction. There was a significant main effect of condition ($F(3,71)=3.9$, $p<.05$), with exercised groups spending more time in the light than sedentary mice.

A one-way ANOVA including only sedentary wild-types and sedentary Tg-SwDI revealed a significant main effect of genotype ($F(1,20)=6.28$, $p<.05$), with Tg-SwDI sedentary mice spending less time in the light.

A one-way ANOVA looking exclusively at wild-type mice found no main effect of experimental condition ($p>.05$)

A one-way ANOVA including only Tg-SwDI mice found a significant main effect of condition ($F(3,34)=3.6$, $p<.05$), with post-hoc tests revealing differences between 1HR vs. SED, 12HR vs. SED and 3HR vs. SED (all $p<.05$), with sedentary mice spending less time in light than exercised mice. These results suggest that the anxiety-like behavior seen in sedentary Tg-SwDI is mitigated by low, moderate and high doses of exercise.

Transitions into Light

A two-way ANOVA with 'number of transitions into light' set as the dependent variable showed no main effect of genotype, experimental condition or a genotype x condition interaction (all $p>.05$). This result suggests that all mice had, on average, the same general interest in exploring a novel, potentially fear-inducing environment.

When only sedentary mice were examined, there was no main effect of genotype ($p>.05$). A one-way ANOVA including only wild-type mice showed no main effect of condition ($p>.05$). When only Tg-SwDI were included, there was a main effect of condition ($F(3,34)=2.8$,$p<.05$). A Fisher's post-hoc comparison showed differences between 12HR vs. SED mice, indicating that 12HR mice made more transitions into the light than sedentary mice, suggesting that high levels of exercise may have increased exploratory behavior in a novel, potentially fear-inducing environment.

Three-Chamber Social Approach

A three-chamber social approach test was next used in order to determine whether the mitigating effects of exercise on anxiety in Tg-SwDI would generalize beyond open spaces and bright lights (e.g., Barnes Maze, Open Field, Light/Dark box) to other forms of anxiety, including anxiety in a social situation.

Mice were first habituated to the empty test environment for 5 minutes. There was no side preference detected during habituation (data not shown). Mice were then tested on general levels of sociability, operationalized by the amount of time spent interacting with an empty cup vs. a stranger mouse.

A three-way repeated measures ANOVA, with time spent with novel mouse vs. cup as a repeated measurement, and genotype, condition and side as between factors, revealed no main effect of genotype, but a significant main effect of side ($p<.05$), with all mice spending more time with the novel mouse than with the empty cup.

A two-way repeated measures ANOVA looking only at sedentary mice showed no main effect of genotype ($p>.05$), but a significant main effect of side ($F(1,18)=21.1$, $p<.05$), with both groups spending more time with the stranger mouse than with the cup.

A two-way repeated measures ANOVA including only wild-types revealed no main effect of condition, but again a main effect of side, with all wild-type animals preferring to interact with the stranger mouse than with the empty cup ($p<.05$). The same analysis done on Tg-SwDI showed a similar pattern: no main effect of condition, but a significant main effect of side ($F(1,34)=40.08$, $p<.05$). These results suggest that there were no differences between

genotypes or experimental conditions, and that the anxiety-like behavior found previously in Tg-SwDI does not generalize to social conditions.

DISCUSSION

The goal of the present study was to determine whether different amounts of exercise are related to distinct behavioral alterations in the Tg-SwDI mouse model of CAA. Mice in this study were assigned to a specific intervention condition where they were given free access to an exercise wheel for 1 hour, 3 hours or 12 hour/day. A group of mice in a sedentary condition remained in their home cage for the duration of the intervention. Following the intervention, a thorough battery of behavioral assessments was completed in order to investigate potential exercise-induced behavioral alterations. The present study revealed the following general conclusions that will be expanded upon below:

- Mice that are given different amounts of time with an exercise wheel will adjust the intensity of their running early on in the intervention, fundamentally changing the quality of running that they receive to a higher level of intensity
- Exercise resulted in apparent benefits including improved coordination and reduced anxiety-like behavior, but the effects of exercise on learning and memory were less clear.
- In most cases, the Tg-SwDI mice demonstrated equal sensitivity to the behavioral effects of the exercise manipulation

Intervention

Data collected from the 4-month intervention period point to interesting differences in the quality of running that mice received in each condition. For example, Figure 23 illustrates clear differences in learning curves between the groups during the initial five days of the intervention. Mice in the 1 and 3-hour conditions adjust to the shorter allotted time that they are given by increasing their running rate. Figure 24 further illustrates this difference by presenting a comparison between the average breaks/minute that mice in each condition took. The lower-dose exercise groups took fewer breaks/minute compared to the higher-dose exercise group (this can also be seen across a sixty-minute timeline in Figure 22), with mice in the lower-dose conditions ramping up the intensity of their running in order to compensate for the smaller volume allotted. Of interest is whether high intensity may compensate for lower volume (or higher volume for low intensity) when it comes to certain behavioral outcomes. The behavioral data, in which we saw general exercise-related benefits, would indicate that this may be the case: while we do see a dose-response pattern in the Rota-rod test, with 12HR Tg-SwDI mice seemingly the best coordinated, there were no clear dose-response patterns found in any of the other behavioral measures. Therefore, we see that exercise in general seems to lead to certain general benefits, and that high doses of exercise seem comparable to low across most of these measures. One interpretation from the clinical standpoint would be to recommend that only small doses of regular exercise are necessary in order to achieve health benefits. There is some recent evidence to support this interpretation (e.g., Beddhu et al., 2015; Hupin et al., 2015), although these studies are typically observational and focus on a particular non-representative subgroup. Another interpretation is that high-intensity workouts are equally as beneficial as moderate volume, low-intensity workouts (Gibala et al., 2012; Little et al., 2010). According to Gibala et

al. (2012), low-volume high intensity workouts, defined as brief bursts of vigorous activity followed by short periods of rest, lead to physiological changes similar to traditional high volume exercise regimens, including improved metabolism and cardiovascular function.

Interestingly, we do see a dose-response effect on a particular measure within the 12-hour Tg-SwDI condition (Figure 27D). Here, higher levels of running were correlated with reduced anxiety-like behavior in the Light/Dark test. This relationship was not seen in wild-type mice or in the other conditions in the Tg-SwDI. One possibility for why this relationship is not seen in the lower-dose conditions may again relate to differences in running intensity. It is possible that within high volume/moderate-low intensity running, such as the kind that the 12-hour mice received, more is better than less when it comes to achieving anxiolytic effects, while the variation of low volume/high intensity running, such as the kind mice in the 1 and 3-hour conditions received, does not significantly change behavioral outcomes.

Behavioral Results

Contrary to the study hypothesis, we found that exercise reduced performance in the Barnes Maze test. Exercised mice spent more time locating their escape box over the 5 day period (Figure 25A), made more hole visits on their way to their escape box (Figure 25B) and were generally slower than wild-type or sedentary Tg-SwDI mice (Figure 25C). If one interprets these results under the assumption that the Barnes Maze is a pure test of spatial memory, we might conclude that exercise exacerbated or induced memory impairment in Tg-SwDI mice. The mechanisms related to this alteration in performance may have involved further damage to an already vulnerable vasculature. It has been reported that damage to the vasculature, through neuroinflammatory processes, may be the initial factor that results in the cascade of pathological

features in AD (Marchesi et al., 2011). Amyloid begins to build up due to lack of proper perivascular clearance, which in turn leads to further vessel deterioration. One interpretation is that exercise exacerbated this positive feedback loop, increasing vascular damage through a deleterious imbalance between oxidative stress and antioxidants in the brain. This may have been particularly pronounced in this mouse model, given that it develops high amounts of amyloid and neuroinflammation around microvessels (Davis, 2004) and may therefore be particularly vulnerable to changes in oxygen metabolism. However, aside from a few studies reporting the negative effects of excessive exercise (e.g., O'Keefe et al., 2012; Schultz et al., 2007), there is substantial evidence in the literature indicating that exercise, in fact, facilitates recovery from oxidative stress (e.g., Radak et al., 2007), induces angiogenesis in the cerebral vasculature (e.g., Ding et al. 2006) and reduces plaque load while improving aspects of memory in transgenic mouse models of AD (e.g., Adlard et al., 2005; Yuede et al., 2009). A closer examination of the standard measures used in the Barnes Maze test, as well as placing these results in the context of reported outcomes from other behavioral measures, provides a different interpretation of these initial findings.

Exercised Tg-SwDI mice had increased performance on the rota-rod, a reliable test of coordination and limb strength (Bogo et al., 1981; Figure 26A), thereby ruling out motor deficits as potentially driving the reduced performance found in the Barnes Maze. There were no differences in the number of arm entries and re-entries made in the unreinforced Radial Arm Maze (Figure 26C and 26D), suggesting that sedentary and exercised mice were equally motivated to explore a novel environment, although exercised Tg-SwDI mice were again reported as having navigated the apparatus more slowly (Figure 26B).

The Light/Dark transition test and Open Field were used to examine measures of anxiety-like and spontaneous exploratory behavior across genotypes and conditions (Bourin & Hascoet, 2003; Crawley & Goodwin, 1980, Crawley, 1985). In the Light/Dark test, measures of transitions from dark into light as well as time spent in the light compartment were used as an index of anxiety-like behavior in rodents. As can be seen in Figures 27A and 27C, a significant difference between sedentary wild-type and sedentary Tg-SwDI mice suggests that CAA-related pathology leads to increased anxiety-like behavior in Tg-SwDI mice. Sedentary Tg-SwDI mice spent significantly more time in the periphery of the Open Field and the dark portions of the Light/Dark box when compared to wild-type sedentary mice. As the graph illustrates, exercise seems to restore the healthy phenotype in Tg-SwDI mice, with significant main effects of exercise specific to Tg-SwDI animals reported.

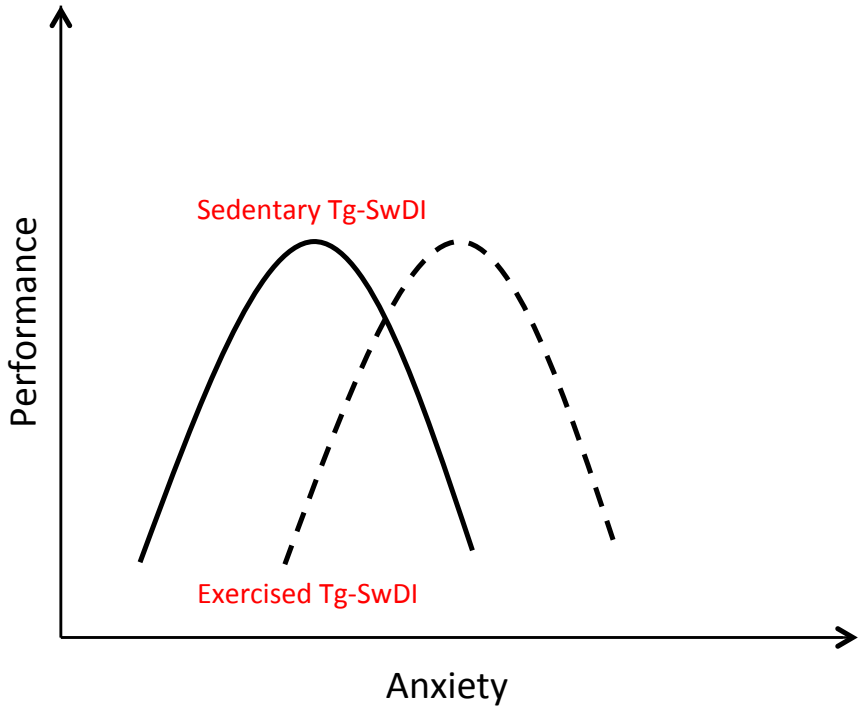
There are multiple studies linking exercise with reduced anxiety-like behavior in rodents (Binder et al., 2004; Duman et al. 2008; Fulk et al., 2004; Salam et al., 2009; Santos-Soto et al., 2013) including those that have demonstrated changes at the synaptic level, and alterations in noradrenergic and serotonergic systems (Broocks et al., 2001; Chaouloff et al., 1997; Morishima et al., 2006) which are thought to play a role in depression and anxiety in humans. One study found that exercise was associated with decreased fear reactivity (Schruers et al., 2002), which may provide support for the slowed movements reported in mice in the present exercised condition (Figures 23C & 24B). It is possible that the reduction in anxiety-like behavior was related to the difference in daily environmental stimulation that mice received across conditions. Exercised animals, by receiving daily sensory feedback from their running chamber and wheel, may have been more resilient to facing new challenging and less reactive to anxiety-provoking environments. Importantly, human studies have also linked exercise to a reduction in anxiety

(Broocks et al., 1998; Hassmen et al., 2000), reporting greater well-being and fewer symptoms of anxiety in populations that exercise regularly. Other longitudinal and cross-sectional studies in humans have reported high levels of anxiety as a predictor of cognitive decline (Sinoff & Werner, 2003) have suggested that anxiety increases our risk of cognitive decline by reducing our cognitive resources (Wetherell et al., 2002), and have reported that anxiety-like symptoms in patients with AD are related to poorer cognition (Ferretti et al., 2001). Interestingly, volume reductions of both the hippocampus and amygdala in MRI studies have been shown to predict dementia in healthy elderly adults (den Heijer et al., 2006). A recent study by Mah et al. (2014) showed that anxiety severity increased rates of conversion to AD in individuals with mild cognitive impairment (MCI), independent of depression. Taken together, these findings suggest that treatment of anxiety symptoms may have benefits for cognition and slowing the progression of cognitive decline, and that exercise may be a way of contributing to that treatment.

The three-chamber social approach test revealed that all mice, across genotypes and conditions, preferred to interact with a novel conspecific than an empty cup (Figure 28). This suggests that the anxiety-like behavior seen in brightly lit spaces did not generalize to situations that are social in nature.

The results from these behavioral studies demand a re-examination of Barnes Maze measures that are commonly reported in the literature, with particular attention paid to the sensitivity of these measures to behavioral changes following exercise interventions. It is possible that the anxiety-driven motivation the Barnes Maze relies on can be illustrated with an inverted U-shaped function (below), which illustrates that certain moderate levels of anxiety are directly related to a degree of motivation to escape (and therefore, performance), while low or extremely high levels of anxiety may either impair memory or simply override memory

processes. This basic idea of the inverted U-shape function as related to stress and performance has been reported previously (Baldi & Bucherlli, 2005). One possibility is that exercise, via mechanisms that lead to a reduction in anxiety-like behavior, leads to a shift in the inverted-U shaped curve, such that higher levels of anxiety would incentivize exercised mice to escape and, in turn, lead to higher performance.



Current rodent behavioral paradigms used to assess memory

Do other existing behavioral paradigms provide a better alternative for studying effects of exercise interventions? Many studies have chosen to use tests like the Morris Water Maze and Radial Water Maze in order to show improvements in memory following exercise interventions in rodents (eg. Adlard, 2005; Nichol et al., 2007; Praag et al., 2005). One potential issue with water-based tasks relates to the aforementioned relationship between anxiety and performance.

Water-based tests have been shown to increase corticosterone in mice (Harrison et al., 2009), suggesting that stress responses may be interfering with interpretations of reported alterations in learning and memory. Also of concern is the ability to disentangle certain basic motor changes following exercise interventions from alterations in memory. Because exercise improves general fitness and coordination (Figure 26A), it is often difficult to tease apart whether improvements reported in any motor-based test are due to changes in memory or simply an artifact of improvements in overall limb strength and/or coordination. These potentially confounding factors should be taken into account when results from motor-based tests such as the Morris Water Maze are reported in the literature.

In the present study, exercise was shown to improve coordination and reduce anxiety-like behavior. It is possible that exercise, via these mechanisms and others, improves learning and memory or slows cognitive decline. However, in order to make inferences about alterations in memory following exercise interventions, appropriate tests of memory must first be developed and tailored to particular subject groups, taking into account intervention-induced physiological and behavioral changes that may alter the sensitivity of the memory measure.

Future Directions

In order to examine the exercise-related effects on pathology, a series of histological measures can be completed. These may include the examination of a wide variety of CAA-related changes, including the analysis of cerebral Aß peptides through ELISA, immunohistochemical analyses that look at regional differences in neuroinflammation, vessel density and different forms of amyloid pathology throughout the brain. Regression analyses could then be used in order to examine whether certain biochemical variables predict behavioral

alterations. Pilot data from study III provides a first-look into what we might expect from the histological analyses in the present study. The pilot study showed no differences in gross levels of pathology (Figure 16), or regional differences in fibrillary amyloid, activated microglia or vessel density (Figures 17,18&19) indicating that exercise-related changes in behavior were not a result of any alterations in these measures. However, it should be noted that the measures used can be broadened to include other analyses that may be related to exercise-induced behavioral changes. These are discussed below.

It may also be interesting to examine areas of the brain that are potentially associated with anxiety and fear-induced responses in the exercised and sedentary Tg-SwDI, including specific regions of the amygdala and hippocampus that have been shown previously to relate to anxiety-like behavior in mice (Felix-Ortiz et al., 2013). These regions have not been studied in the Tg-SwDI in this context, and it would be interesting to examine potential regional biochemical and structural changes following our intervention.

Examining both vessel morphology and levels of oligomeric Aß through dot-blot analysis throughout the brain and relating these to behavioral changes may also be illuminating, as higher levels of oligomeric Aß are thought to correlate reliably with cognitive decline (Cleary et al., 2005; Koffie et al., 2011; Selkoe, 2002). In addition, analyzing levels of BDNF and neurogenesis in hippocampus and other related regions would confirm previous studies that have shown increased levels of both following exercise interventions (Adlard et al., 2005 & Vaynman et al., 2004).

Limitations

Potential alterations to future study designs may include the addition of a 'locked wheel' condition, where mice are matched for handling but are otherwise sedentary. This would allow us to begin ruling out differences in handling as a potential factor that could influence the behavioral changes reported, although the lack of differences between mice in the PCS and sedentary conditions in study I would suggest that being transferred from one's home cage to a novel environment each day does not lead to measurable behavioral changes.

One other possibility is to replace voluntary wheel running with a fixed treadmill intervention. This would allow for strict control over the amount of exercise mice receive and therefore would establish a uniform running baseline going into behavioral testing. However, one drawback of using a treadmill is the possibility of introducing confounding stress variables that may be brought on by forced movement (Leasure & Jones, 2008).

Conclusions

The present study revealed that a 16-week exercise intervention in a Tg-SwDI mouse model of CAA pathology leads to improvements in coordination and a reduction in anxiety-like behavior across multiple measures. It also revealed a general slowing of speed during the exploration of a novel environment in exercise animals. The Barnes Maze was used in an attempt to determine whether exercise led to changes in learning and memory. However, reported reduced levels of anxiety-like behavior most likely confounded the Barnes Maze results, the memory-sensitive measures of which are contingent upon mice experiencing a certain moderate level of anxiety.

The results from this study encourage a more nuanced interpretation of reported exercise-related effects on learning and memory. While it has been well established that exercise has general health benefits, the effects on memory and cognitive decline are less clear, urging the field to become more sensitive to potential confounds and limitations of behavioral studies in rodents.

FIGURES: STUDY #4

FIGURE 21

The following graphs show A) the average distance run (meters/hour) by C57 and Tg-SwDI from 1HR, 3HR and 12HR running conditions, calculated by averaging the total number of meters run over the 4 month intervention period and dividing by the corresponding number of allotted hours (1,3 or 12). B&C) Average number of meters run by C57 and Tg-SwDI, broken up into each month of the intervention. All mice maintained a relatively stable running rate over the entire intervention period. Error bars represent SEM.

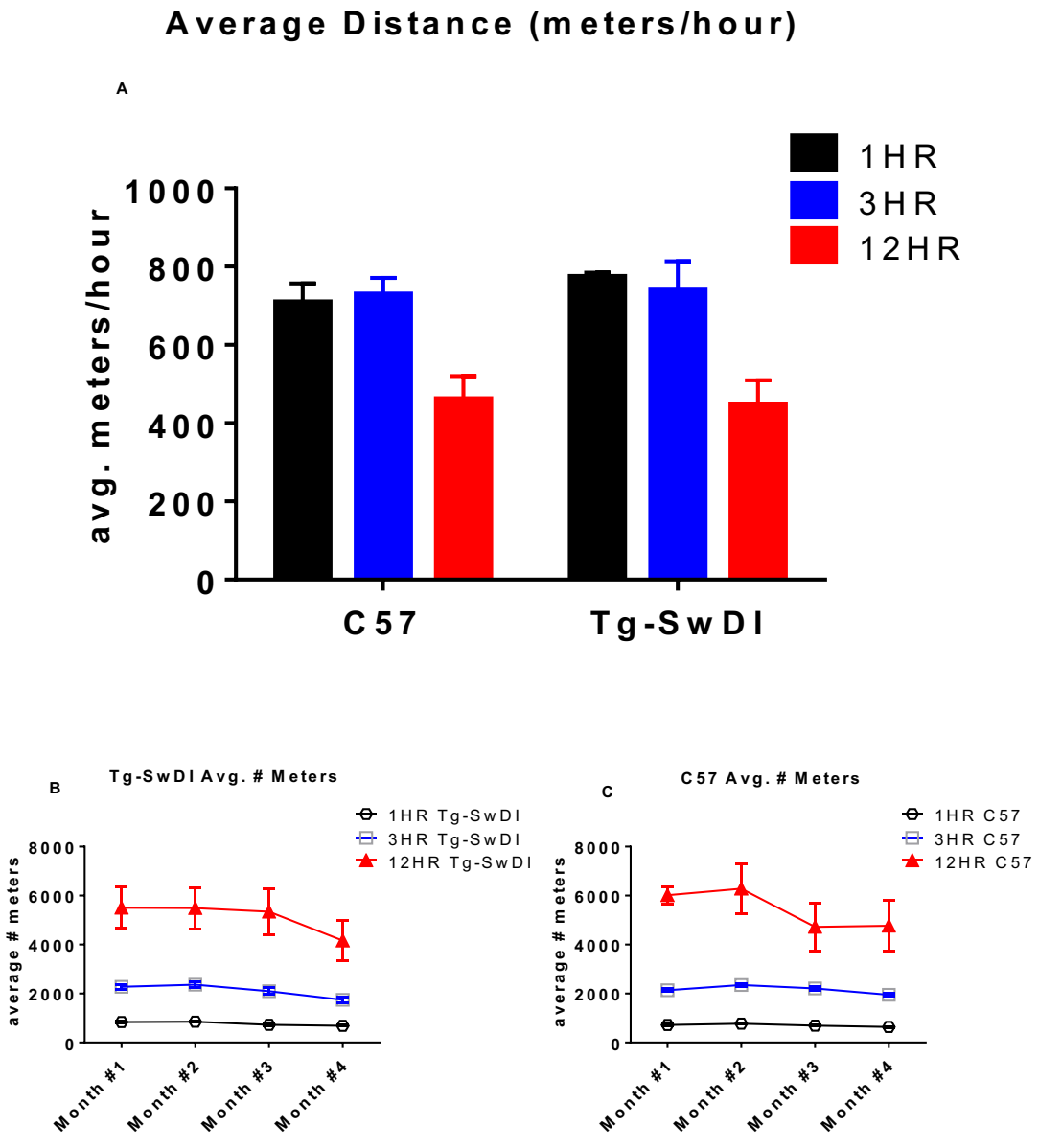


FIGURE 22

Graphs depicting typical running patterns in rotations/minute of 1HR (A) and 12HR (B) Tg-SwDI mice. The first hour of wheel access for both groups was used. Day 60 was used to represent a typical data set because it occurred at a mid-way point of the intervention.

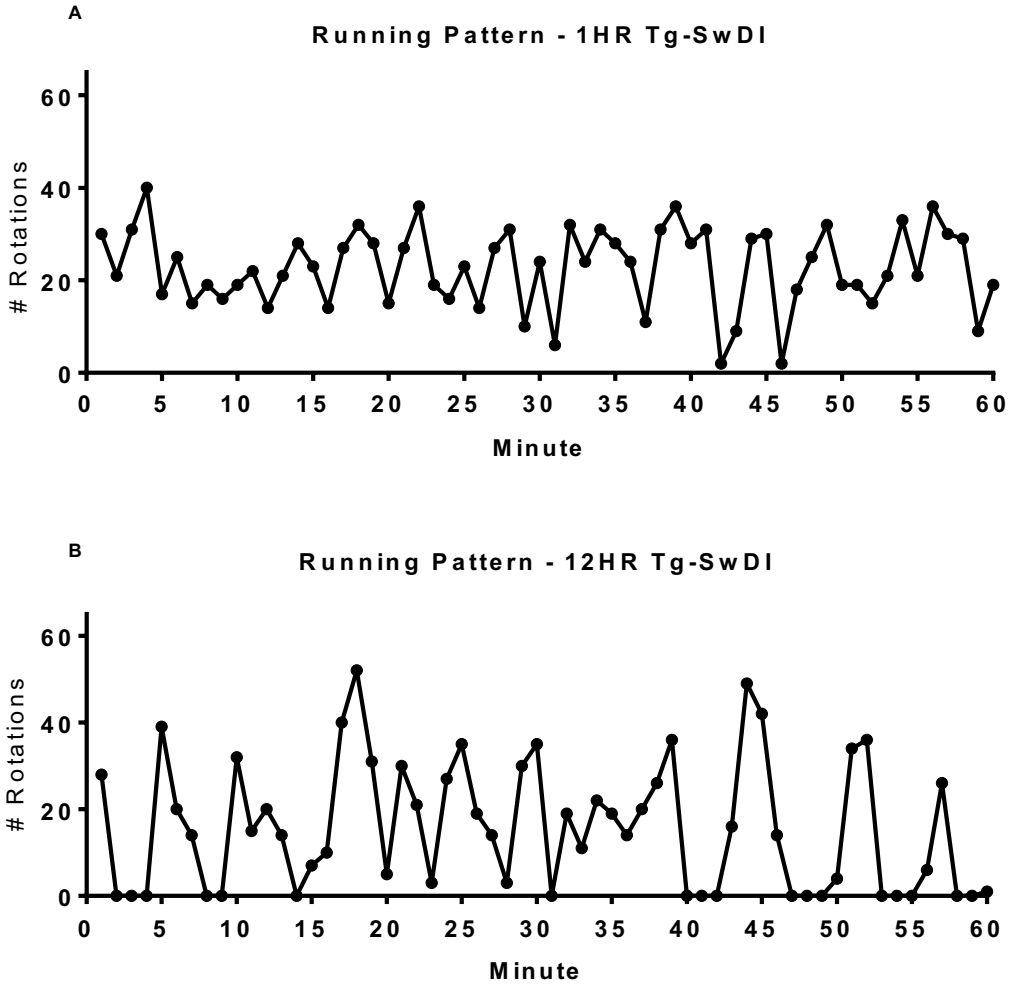


FIGURE 23

The following graphs represent the number of rotations/hour during the first five days of the running intervention for both Tg-SwDI and C57 mice in 1HR, 3HR and 12HR conditions. The five day period was selected because it represents the period before running volume from all groups begins to cluster around the mean. The slopes exhibit the different learning curves between the 1HR/3HR and 12HR groups.

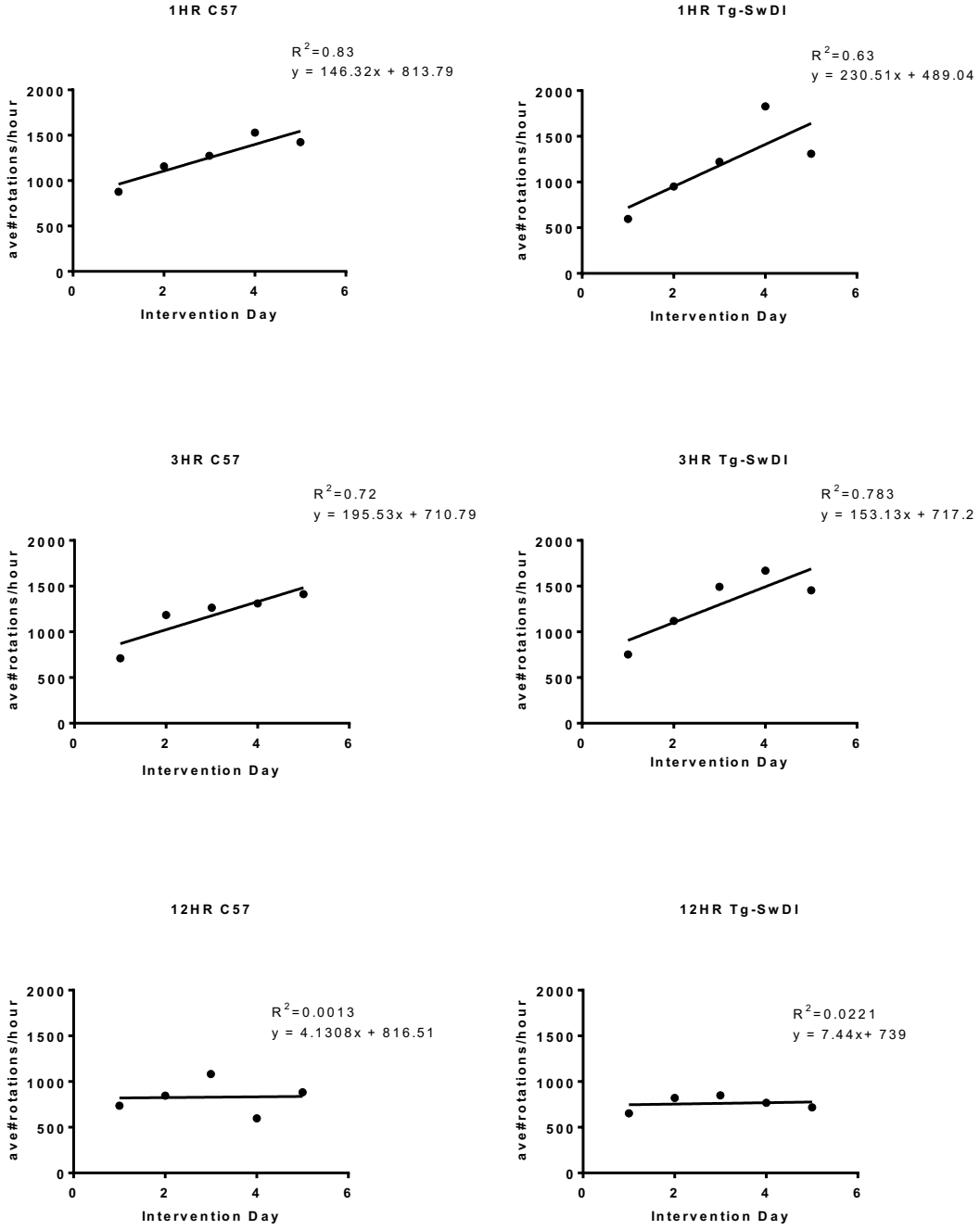


FIGURE 24

The following preliminary figure illustrates the number of breaks/min taken by C57 and Tg-SwDI in 1HR, 3HR and 12HR conditions. Each condition includes data from six mice that were averaged across three intervention days. 12HR mice took significantly more breaks (defined by when the number of rotations hit 'zero') when compared to both 1HR and 3HR mice. This trend remains constant between genotypes. Error bars represent SEM.

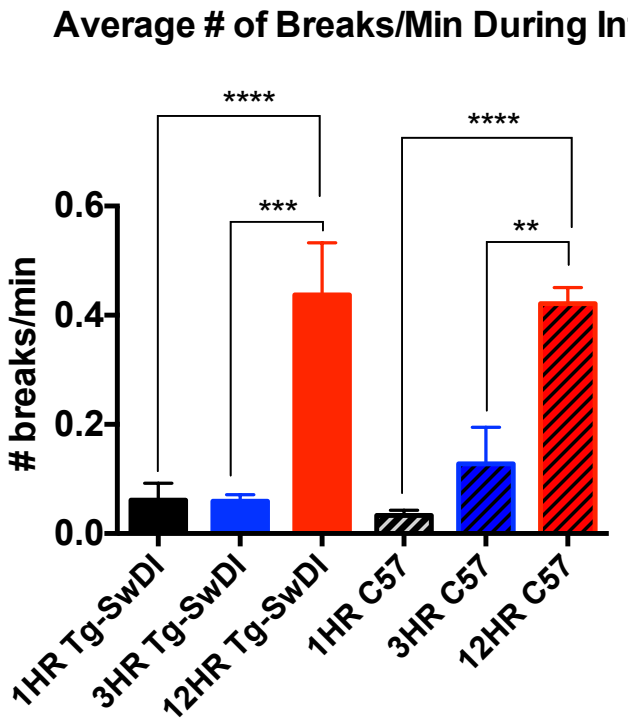


FIGURE 25

Graphs showing performance of Tg-SwDI in 1HR, 3HR, 12HR groups and both Tg-SwDI and C57 sedentary groups on the Barnes Maze test. A) In the latency to find measure there were significant main effects of genotype and experimental condition, with wild-type and sedentary mice spending the least amount of time locating their escape platform (all p<.05). There were no significant differences between exercise conditions. B) Analyses on the number of hole-visits made prior to locating the escape box showed a significant main effect of condition, with exercise mice making more hole-visits prior to locating escape box. C) Average speed (calculated in meters/second) revealed a main effect of genotype and condition, with wild-type and sedentary mice moving at a higher speed. There was also a significant day x condition interaction. D) Path efficiency (1=most efficient route to escape box) showed a main effect of genotype. Error bars represent SEM.

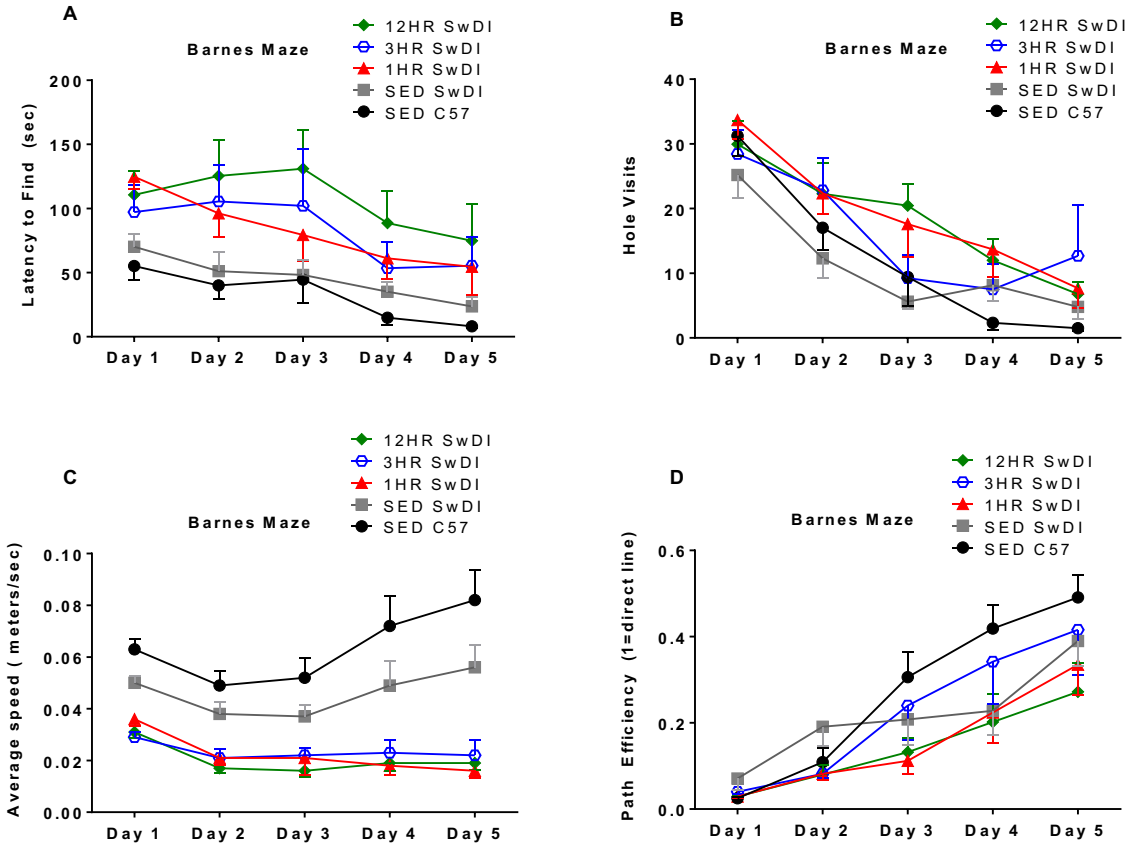


FIGURE 26

The following graphs represent performance on a series of behavioral measures. A) Time spent on rotating rod revealed a main effect of experimental condition, with exercised animals spending more time on rod than sedentary animals. Post-hoc test revealed differences between 12HR and SED Tg-SwDI mice. B) Average speed (meters/second) in the unreinforced Radial Arm Maze. There was a main effect of genotype and condition (all p<.05). C) There were no differences in total number of arms entered across any of the groups (p>.05). D) There were no differences in percent re-entries across conditions and genotypes (p>.05). Error bars represent SEM.

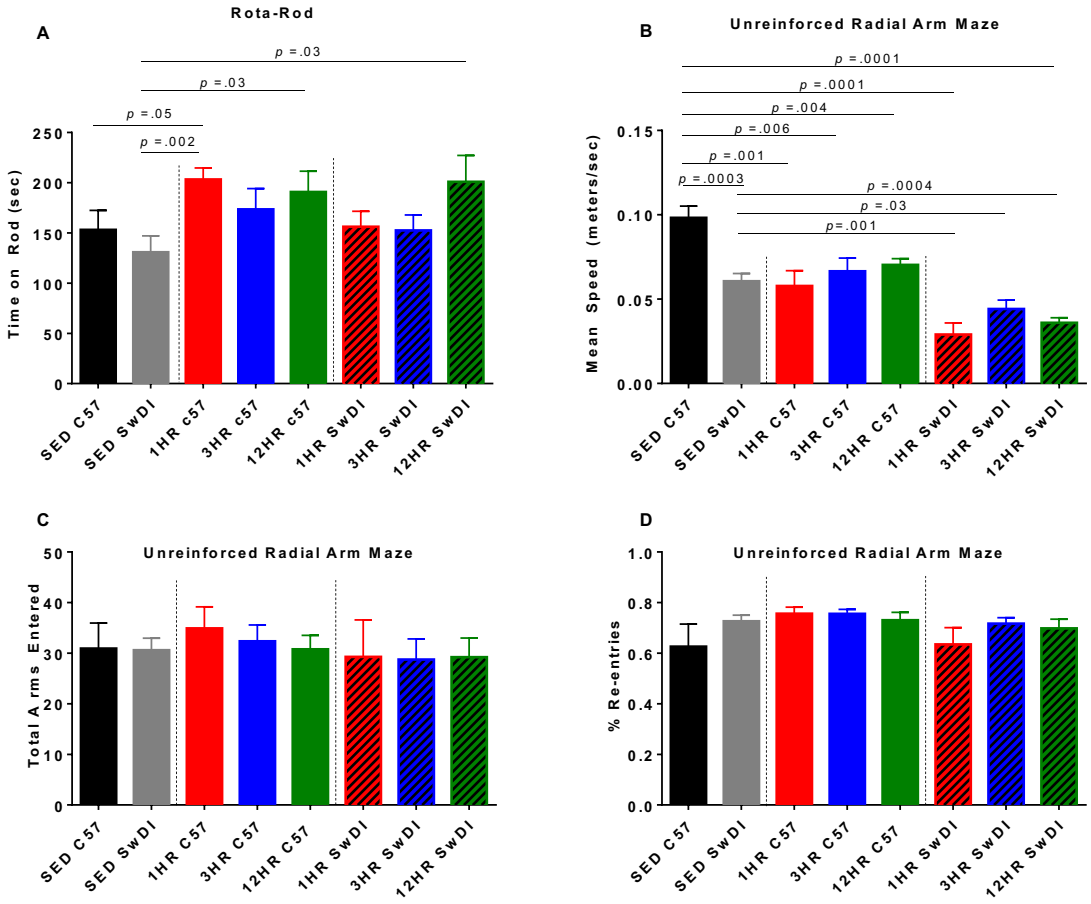


FIGURE 27

Graphs representing performance on a series of behavioral measures. A) Light-dark box test of anxiety-like behavior and exploration. In the 'time in light' measure, there was a main effect of condition, with exercised animals spending significantly more time in the light part of the apparatus ($p<.05$). B) Number of transitions into light. Here, there were no significant main effects or interactions reported ($p>.05$). C) 'Time in center' measure in the open field revealed a main effect of experimental condition, with exercised mice spending more time in the center of the open field vs. periphery ($p<.05$). D) Correlation illustrating the number of rotations made by the 12HR Tg-SwDI mice vs. time spent in the light part of the light/dark box. There was a significant positive correlation, indicating that higher levels of exercise in this group are related to lower levels of anxiety-like behavior. Error bars for all graphs represent SEM.

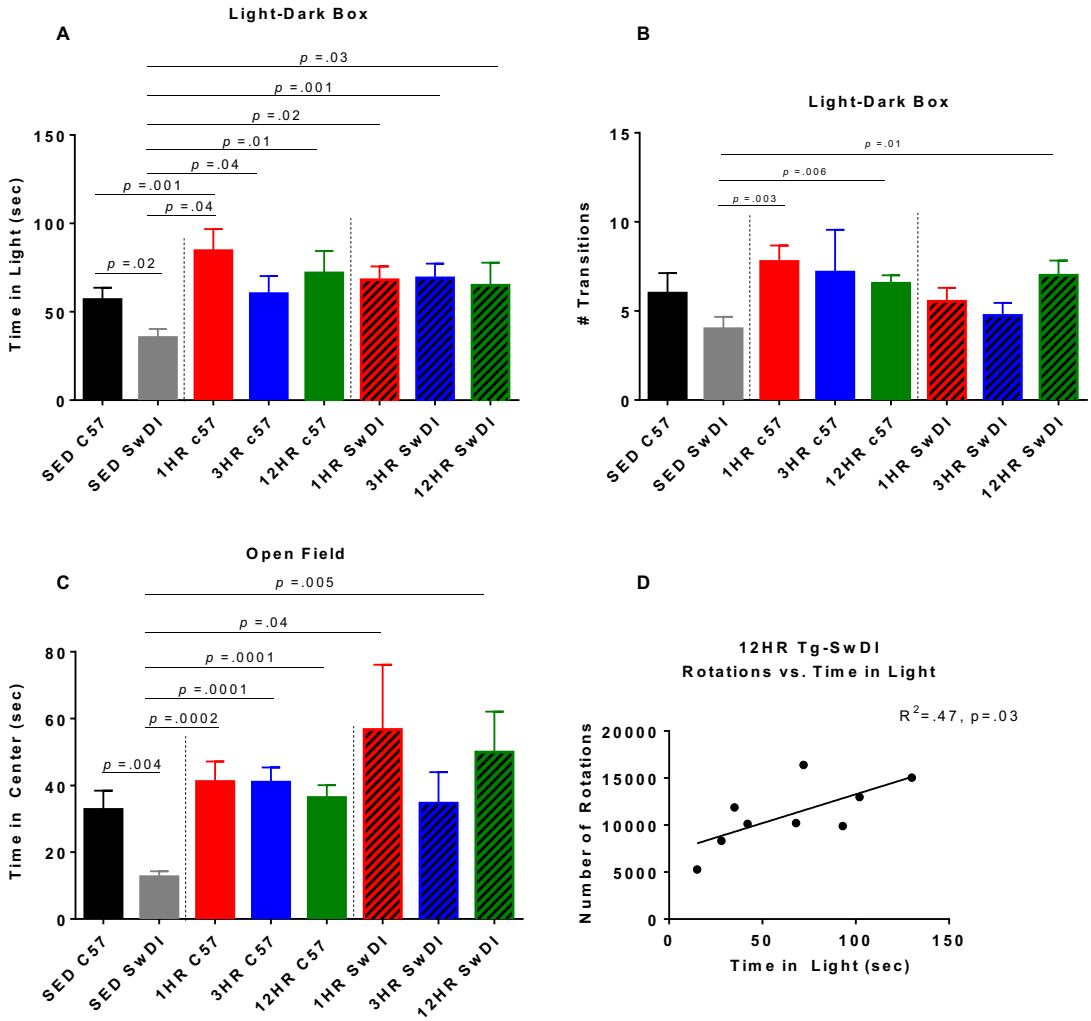


FIGURE 28

The figure illustrates the results of a three-chamber test of sociability and social anxiety. A repeated measures ANOVA revealed a significant main effect of side, with mice in all genotypes and experimental conditions spending significantly more time with the stranger mouse than with the empty cup ($p<.05$).

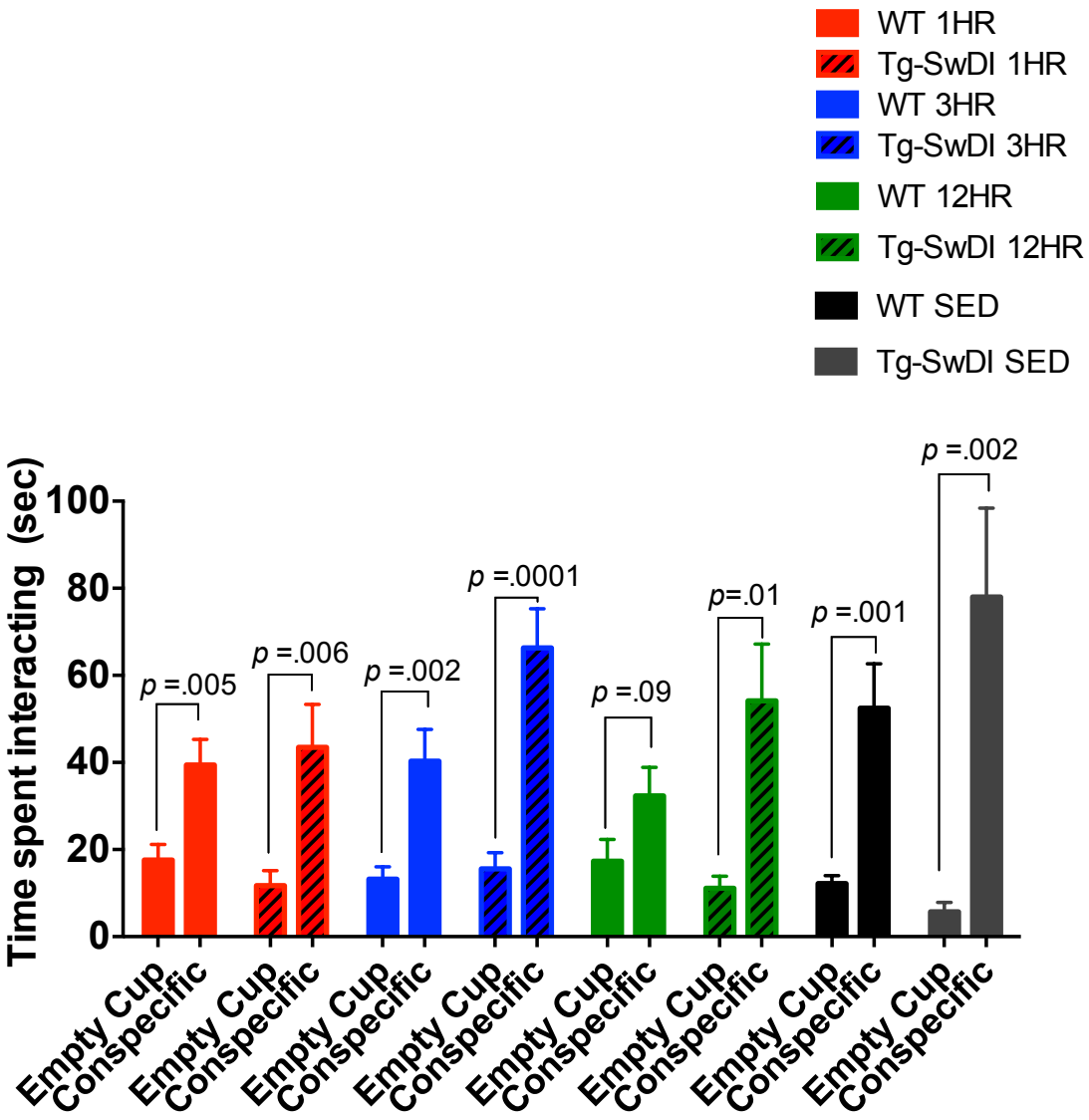


TABLE 1

A summary of each study, including a break-down of important characteristics of each mouse line used, behavioral tests and results, and results of neuropathology and histology.

Tg line	Hum. transgene	**Sex**	Intervention/ Duration	**Behavioral task, age tested (mo)**	Results	**Neuropathology in experimental vs. control**
TgSwDI	Swedish Dutch Iowa	**Female**	PCS 16 weeks	**Barnes Maze URAM DigiScan 7mo**	No significant differences in BM, URAM or DigiScan	**Aß40 and Aß42 levels stable Activated microglia levels stable**
5XFAD	Swedish Florida London PSEN (L286V& M146L)	**Female & Male**	PCS 16 weeks	**Barnes Maze DigiScan 7mo**	No significant differences in BM or DigiScan	**Aß40 and Aß42 levels stable Activated microglia levels stable**
TgSwDI	Swedish Dutch Iowa	**Female**	Vol. Exercise 16 weeks	**Barnes Maze URAM DigiScan 7mo**	Significant differences in BM and URAM No differences in DigiScan	**Ab40 and Ab42 levels stable Activated microglia levels stable %Vessel density stable**
TgSwDI	Swedish Dutch Iowa	**Female**	Vol. Exercise Dose-response 16 weeks	**Barnes Maze Rota-Rod Open Field Light/Dark Box URAM Social Approach 5-7mo**	Significant differences in BM, Rota-Rod, Light/Dark box, Open Field No significant differences in URAM, Social Approach	**TBD**

Legend
BM = Barnes Maze
URAM = Unreinforced Radial Arm Maze
PCS = Progressive Cognitive Stimulation

TABLE 2

A summary of results from the series of behavioral tests used in study IV.

Behavioral Test/Parameter	Result		Dose-response pattern? (y/n)
Barnes Maze Aspects of spatial memory/Exploratory behavior	Latency to Find: SED	↑	**Latency to Find: No** **Hole-Visits: No** **Average Speed: No**
	Hole-Visits: SED	↑	
	Average Speed: SED	↑	
Rota-Rod Coordination/ Limb Strength	Time on Rod: RUN	↑	**Time on Rod: Yes -12HR spent longest time on rod vs. 1HR and 3HR.**
Unreinforced Radial Arm Maze Spontaneous exploratory behavior	Total Arms Entered:	↔	**Total Arms Entered: No** **%Re-entry: No** **Mean Speed: No**
	%Re-entry:	↔	
	Mean Speed: SED	↑	
Open Field Anxiety-like behavior	Time in Center: RUN	↑	**Time in Center: No**
Light/Dark Box Anxiety-like behavior/exploration	Time in Light: RUN	↑	**Time in Light: No**
	#Transitions: RUN	↑	**#Transitions: No**
Social Approach Sociability/Social Anxiety	Sociability	↔	**Sociability: No**

Legend
↑ Performance increase
↔ No difference
SED = Sedentary Tg-SwDI
RUN = Exercised Tg-SwDI

CHAPTER VII: GENERAL DISCUSSION

The four studies described in this document used murine model systems to clarify our understanding of how lifestyle interventions alter behavior and neuropathology. These studies are timely given the extent of public, clinical and commercial interest in the potential utility of specific lifestyle changes for the prevention and treatment of Alzheimer's-related disease (Ballard, 2011; Barnes & Yaffe, 2011; Stanford Center on Longevity, 2014). More so, these studies also yielded methodological advances and insights as to limitations by systematically isolating and studying each factor independently, yet in a manner from which comparisons might be made. The principal mouse line used in these studies, the Tg-SwDI, was chosen because it models cerebral amyloid angiopathy (CAA). CAA is a disease in which amyloid accumulates around the cerebral vasculature; an intricate network of vessels in charge of providing oxygen and glucose to the brain, and clearing toxic wastes through perivascular drainage systems, among other essential functions (Zlokovic, 2005). In using the Tg-SwDI line, these studies provided for the first time an assessment of lifestyle factors on CAA symptomology. More so, damage to the cerebrovasculature is a common feature of AD and is closely related to cognitive decline (Nicoll, 2004; Xu et al., 2014). Therefore, the cerebrovascular system is an attractive target for symptom improvement and might reasonably be affected by present interventions.

Studies #1 and #2 focused on isolated cognitive stimulation in the form of 'cognitive training;' the currently popular phenomenon grounded in the theory that specific, isolated 'brain training' may allow for a generalized improvement in cognitive function (Ballard, 2011). We tested this theory by implementing a highly controlled, isolated cognitive training intervention that required mice to progress through a series of increasingly challenging operant tasks. The mice advanced through these tasks at their own rate, were constantly working at their limits, and were introduced to new cognitive challenges. These features are present in most commercialized

cognitive training regimens (e.g., Lumosity) as well as regimens being tested in research and clinical settings (e.g., Rosen et al., 2011). Our studies found no changes in memory measures, general activity or levels of pathology or neuroinflammation following the intervention, suggesting that even a substantial amount of cognitive-specific training does very little to improve AD-related symptoms and pathology in the Tg-SwDI. These results suggest that positive results reported in human interventions may not be the direct result of stimulation of specific cognitive domains, especially given the less intensive manipulations implemented in clinical settings. Given the popularity of brain-training programs such as those offered by the company Lumosity (70 million users currently enrolled, according to their website http://www.lumosity.com), and the number of individuals presumably being reassured by their claims about reducing cognitive slowing and forgetfulness, it is becoming important to provide clear and reliable evidence about the benefits (or lack thereof) of cognitive training programs. However, our study does not provide compelling scientific evidence to suggest that cognitive-specific training reduces cognitive decline. Indeed, many of the studies that brain-training companies cite as support for efficacy contain methodological weaknesses, such as small sample sizes and report benefits not sustained over time (e.g., Hardy et al., 2011; Kesler et al., 2012). Clearly in light of these concerns and the present findings, large-scale studies, replicated at multiple sites, must be done before the efficacy of training regimens can be considered established.

Studies #3 and #4 examined cardiovascular exercise interventions in isolation. Study #3, a pilot study, provided histology and preliminary behavioral results following a 4-month intervention in Tg-SwDI mice. Here, the running group showed shorter latency to find the hidden escape box in the Barnes Maze but no differences in levels of amyloid pathology,

neuroinflammation and vessel density in various brain regions related to AD pathology and symptomology. The subsequent large-scale study (study #4), again examined the effects of a 4-month cardiovascular intervention, but added to its design the systematic comparison of different amounts of exercise, and focused on completing an extensive post-intervention behavioral characterization. This study showed performance patterns in the running group usually thought to represent impairments in the Barnes Maze task, though were more likely a function of reduced anxiety-like behavior in these mice. It also revealed an improvement in coordination and overall limb strength in running animals. Additionally, while it was initially hypothesized that exercise would have produced benefits in a dose-response fashion across multiple behavioral measures, a clear benefit of one condition vs. another was not seen, with the exception of the Rota-Rod task. Mice in the 12HR condition performed significantly better on this task (Figure 26A), suggesting that longer-distance running provides benefits for coordination and limb strength in the Tg-SwDI mouse. However, in regards to the lack of dose-response patterns across the remaining measures (Barnes Maze, Light Dark Box, Open Field and Unreinforced Radial Arm Maze) one possibility is that the quality of running mice received in each condition may have influenced the lack of reported dose-response patterns. For instance, high intensity running in the 1 and 3-hour exercised groups may have compensated for lack of volume, while high volume running in the 12-hour condition may have compensated for lower levels of intensity. This may suggest that both are beneficial, a comparison that has been discussed previously in the human literature (e.g., Gibala, 2014).

One concern to address is the discrepancy between several behavioral outcomes in studies #3 and #4. One possibility is that study #3 was statistically underpowered, and the differences in performance seen in the Barnes Maze and URAM were simply an artifact of a low

sample size. Another possibility emerges when the basic differences in study design are examined. While study #4 included in its design two separate environments: a home cage and daily transfer into a chamber with a running wheel, mice in study #3 used the running chamber as their home-cage. This brings up the possibility of whether being introduced to two separate environments every day and/or the physical removal of mice from one environment to the next can lead to a fundamental change in behavior. One way to partially rule out a handling effect in study #4 would be to re-visit and make comparisons with the design and behavioral outcomes of study #1. Here, mice were also transferred from home cages into a novel environment (in this case, operant chambers) daily, but there were no statistical differences between sedentary Tg-SwDI and PCS Tg-SwDI in any of the behavioral measures. This would suggest that the differences reported between conditions in study #4 are likely directly related to the exercise itself, though why the exercise occurring in a non-home cage setting would be important is unknown.

Behavioral results from study #4 demonstrate the importance of designing intervention studies that include multiple control groups and a wide array of behavioral assessments. The wild-type mice distributed throughout each condition allowed for a systematic comparison between the effects of exercise on particular CAA-related behavioral deficits and the general effects of exercise on healthy mice. Taken in isolation, without the addition of wild-type control groups, one might conclude that there were beneficial effects of exercise across multiple measures in a particular model, instead of examining the effects of exercise more generally. In addition, the wide array of behavioral assessments contributed to the development of a comprehensive story that allowed for a broader examination of exercise-related behavioral changes.

Health and Clinical Implications

Although studies 1&2 reported minimal improvements following cognitive-specific training, the message from these studies should not be that individuals should steer clear of brain-training all together. The message, rather, is that individuals should be able to make careful, systematic decisions about how they choose to spend their time improving their brain health, and should be able to base those decisions off clear results that are scientifically grounded. The epidemiological literature supporting benefits of physical exercise and being socially active, for instance, suggests that combinations of both physical, social and cognitive factors may combine to promote and protect cognitive reserve (Stern, 2009), whereas individuals spending time doing brain-training games may not be spending it in the most productive manner if not engaging in these other, potentially more beneficial, activities.

The most striking effect from study #4 was that daily running sessions, both of high intensity and high volume, seem to change the way that mice with CAA-related pathology acquire and integrate stimuli from spatial environments. We may explain this change as a reduction in anxiety-like behavior or as remediation of sensory slowing, which may itself have indirect clinical benefits, described previously (Ferretti et al., 2001; Mah et al. 2014; Sinoff & Werner, 2003; Wetherell et al., 2002), although this would have to be explored systematically in future studies.

Perhaps the most striking final conclusion is about the resistance of the pathology in the transgenic animals to any change, positive or negative, to two highly intensive training regimens. This is a sobering reflection on the stubbornness of Aβ pathology, though may also be somewhat

artifactual to the aggressive, early emerging pathology produced by the familial gene combinations that are a convenient feature of the mouse models.

Future Directions

Along with cognitive-training and exercise, dietary and nutritional factors are also likely to play a role in prevention and risk of AD (e.g., Bayer-Carter et al., 2011; Bowen et al., 2002; Carrillo et al., 2013; Engelhart et al., 2002; Morris, 2009). Future studies may choose to examine the role of low vs. high fat diets and food-derived antioxidants in ameliorating CAA pathology and symptomology. Importantly, once the effects of isolated lifestyle factors are established across multiple models of AD and multiple behavioral and pathological measures, it will then be prudent to begin systematically combining factors. This would allow for a thorough examination of potential additive effects of various lifestyle factors.

In conclusion, this set of experiments explored the role of two important lifestyle interventions in a Tg-SwDI mouse model of CAA. Ultimately, cognitive-training had minimal effects on behavior and pathology. These results concur with the scant evidence in the human literature that support cognitive-specific interventions as a means to improve general cognitive function (e.g., Noack et al., 2014; Redick et al., 2013). Cardiovascular exercise seemed to have profound effects on reducing what appears to be anxiety-like behavior or sensory slowing in the Tg-SwDI, along with improving coordination and limb strength. Selective changes to cognitive-performance following the intervention were less clear, which points to the importance of developing appropriately sensitive behavioral measures when testing exercise-induced changes in learning and memory. Looking forward, it will be prudent to examine the sustained effects of

exercise interventions at different ages and degrees of pathology in this model in order to begin developing clinical guidelines for an at risk population.

REFERENCES

Adlard, P. A., Perreau, V. M., Pop, V. & Cotman, C. W. (2005a). Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *JNeuro, 25*(17), 4217–21

Ahlskog, J.E., Geda, Y.E., Graff-Radford, N.R., Petersen, R.C. (2011). Physical exercise as a preventative or disease-modifying treatment of dementia and brain aging. *Mayo Clinic Proceedings. 86(9),* 876-884.

Alzheimer's Association (2014). http://www.alz.org/alzheimers_disease_facts_and_figures.asp

Arendash, G. W., Garcia, C. A. M. F., Costa, D. A., Cracchiolo, J. R., Wefes, I. M., & Potter, H. (2004). Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable b-amyloid deposition. *Neuroreport, 15*(1), 51–54.

Attems, J. (2005) Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms. *Acta Neuropathol*, *110*, 345-359.

Attems J, Jellinger KA (2004) Only cerebral capillary amyloid angiopathy correlates with Alzheimer pathology—a pilot study. *Acta Neuropathol 107*, 83-90.

Baker, L.D., Frank, L.L., Foster-Schubert, K., Green, P.S., Wilkinson, C.W., McTiernan, A., Plymate, S.R., Fishel, M.A., Watson, G.S., Cholerton, B.A., Duncan, G.E., Mehta, P.D., and Craft, S. (2010) Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *Arch. Neurol. 67,* 71-79.

Baldi, E. & Bucherelli, C. (2005). The Inverted "U-Shaped" Dose-Effect Relationships in Learning and Memory: Modulation of Arousal and Consolidation. *Nonlinearity in Biology, Toxicology, and Medicine, 3,* 9-21.

Ball, K., Berch., D.B., Helmers, K.F. et al. (2002). Effects of cognitive training interventions with older adults—a randomized controlled trial. *JAMA. 288,* 2271–2281.

Ballard, C., Khan, Z., Clack, H., & Corbett, A. (2011). Nonpharmacological treatment of Alzheimer disease. *Canadian Journal of Psychiatry. Revue Canadienne de Psychiatrie*, *56*(10), 589–95.

Barnes DE, Yaffe K. (2011). The projected effect of risk factor reduction on Alzheimer's disease prevalence. *Lancet Neurol*: 819–28.

Bartus R, Dean R, Beer B, Lippa A. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science 217,* 408–14.

Bartus, R. T. (2000). On neurodegenerative diseases, models, and other treatment strategies: Lessons learned and lessons forgotten a generation following the cholinergic hypothesis. *Experimental Neurology, 163,* 495–529.

Bayer-Carter, J. L., Green, P. S., Montine, T. J., VanFossen, B., Baker, L. D., Watson, G. S., … Craft, S. (2011). Diet intervention and cerebrospinal fluid biomarkers in amnestic mild cognitive impairment. *Archives of Neurology*, *68*(6), 743–52.

Becker, J.T., Mintun, M.A., Aleva, K., Wiseman, M.B., Nichols, T., & DeKosky, S.T. (1996). Compensatory reallocation of brain resources supporting verbal episodic memory in Alzheimer's disease. *Neurology, 46,* 692–700.

Beddhu, S., Wei, G., Marcus, R.L., Chonchol, M., Greene, T. (2015). Light-Intensity Physical Activities and Mortality in the United States General Population and CKD Subpopulation. *Clinical Journal of the American Society of Nephrology, doi:* 10.2215/CJN.08410814.

Bennett D.A., Schneider, J.A., Arvanitakis, Z., Kelly, J.F., Aggarwal, N.T., Shah, R.C. & Wilson, R.S. (2006). Neuropathology of older persons without cognitive impairments from two community-based studies. *Neurology, 66,* 1837-1844.

Berkman, L.F. et al. (1993). High, usual and impaired functioning in community-dwelling older men and women: findings from the MacArthur Foundation Research Network on Successful Aging. *J. Clin. Epidemiol. 46,* 1129–1140.

Binder, E., Droste, S.K., Ohl, F. & Johannes, M.H.M. Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice. *Behavioral Brain Research, 155(2),* 197-206.

Black, J. E., Isaacs, K. R., Anderson, B. J., Alcantara, A.A., & Greenough, W. T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(14), 5568–72.

Blackshear, A. L., Xu, W., Anderson, M., Xu, F., Previti, M. Lou, Van Nostrand, W. E., & Robinson, J. K. (2011). A novel operant testing regimen for multi-construct cognitive characterization of a murine model of Alzheimer's amyloid-related behavioral impairment. *Neurobiology of Learning and Memory*, *96*(3), 443–451

Blomquist, K.B. and Danner, F. (1987). Effects of physical conditioning on information-processing efficiency. *Percept. Mot. Skills 65,* 175–186 4

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H., DelTredici, K (2006). Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol (Berl) 112*, 389–404.

Broocks A., Bandelow B., Pekrun G. et al. Comparison of aerobic exercise, clomipramine, and placebo in the treatment of panic disorder. (1998). 10-week exercise program outperformed placebo at post-treatment in anxiety reduction. *Am. J. Psychiatry. 155(5), 6*03–609.

Broocks A., Meyer T., Gleiter C.H. et al. (2001). Effect of aerobic exercise on behavioral and neuroendocrine responses to meta-chlorophenylpiperazine and to ipsapirone in untrained healthy subjects. *Psychopharmacology (Berl.)* 155(3):234–241.

Bruel-Jungerman, E., Davis, S., Rampon, C., Laroche, S. (2006). Long-term potentiation enhances neurogenesis in the adult dentate gyrus. *The Journal of Neuroscience, 26,* 5888-5893.

Bogo, V., Hill, T.A. & Young, R.W. (1981). Comparison of accelerod and rotarod sensitivity in detecting ethanol- and acrylamide-induced performance decrement in rats: review of experimental considerations of rotating rod systems. *Neurotoxicology (2*), 765–87.

Bowen, D. J., & Beresford, S. a a. (2002). Dietary interventions to prevent disease. *Annual Review of Public Health*, *23*(88), 255–86. doi:10.1146/annurev.publhealth.23.100901.140555

Binder, E., Droste, S.K., Ohl, F., Reul, J.M.H.M. (2004) Regular voluntary exercise reduces anxiety-related behavior and impulsiveness in mice. *Behavioural Brain Research*, *155(2),* 197-206.

Carrillo, M. C., Brashear, H. R., Logovinsky, V., Ryan, J. M., Feldman, H. H., Siemers, E. R., … Sperling, R. a. (2013). Can we prevent Alzheimer's disease? Secondary "prevention" trials in Alzheimer's disease. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association*, *9*(2), 123–131.e1.

Chaouloff F. Effects of acute physical exercise on central serotonergic systems. (1997). *Med. Sci. Sports Exerc. 29(1)*, 58–62.

Cleary, J.P., Walsh, D.M., Hofmeister, J.J., Shankar, G.M., Kuskowski, M.A., Selkoe, D.J., Ashe, K.H. (2005). Natural oligomers of the amyloid- β protein specifically disrupt cognitive function. *Nature Neuroscience, 8,* 79-85.

Cotman C.W., Berchtold N.C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci, 25,* 295– 301.

Cotman, C. W. & Berchtold, N. C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends in neurosciences*, *25*(6), 295–301.

Cummings, J.L., Morstorf, T., Zhong, K. (2014). Alzheimer's disease drug-development pipeline: few candidates, frequent failures. *Alzheimer's Research and Therapy, (6)*, 37.

Davies P., Maloney A.J. (1976). Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet, 2*, 1403.

Davis, J., Xu, F., Deane, R., Romanov, G., Previti, M. Lou, Zeigler, K., Van Nostrand, W. E. (2004). Early-onset and robust cerebral microvascular accumulation of amyloid beta-protein in transgenic mice expressing low levels of a vasculotropic Dutch/Iowa mutant form of amyloid beta-protein precursor. *The Journal of Biological Chemistry*, *279*(19), 20296–306.

den Heijer T, Geerlings MI, Hoebeek FE, et al. (2006). Use of hippocampal and amygdalar volumes on magnetic resonance imaging to predict dementia in cognitively intact elderly people. *Arch Gen Psychiatry, 63,* 57–62.

Deutsch, G., Halsey, J.H., & Harrell, L.E. (1993). Exaggerated cortical blood flow reactivity in early Alzheimer's disease dur- ing successful task performance. *Journal of Clinical and Experimental Neuropsychology, 15*, 71.

Domnitz, S. B., Robbins, E. M., Hoang, A. W., Garcia-Alloza, M., Hyman, B. T., Rebeck, G. W.

& Frosch, M. P. (2005). Progression of cerebral amyloid angiopathy in transgenic mouse models of Alzheimer disease. *Journal of Neuropathology and Experimental Neurology*, *64*(7), 588–94.

Diamond, M. C., Krech, D., & Rosenzweig, M. R. (1964). the Effects of an Enriched Environment on the Histology of the Rat Cerebral Cortex. *The Journal of Comparative Neurology*, *123*, 111–20.

Dietrich, M.O., Andrews, Z.B., Horvath, T.L., 2008. Exercise-induced synaptogenesis in the hippocampus is dependent on UCP2-regulated mitochondrial adaptation. *J. Neurosci. 28,* 10766–10771

Ding, Y., Li, J., Luan, X., Ding, Y.H., Lai, Q., Rafols, J.A., Phillis, J.W., Clark, J.C., Diaz, F.G. (2004). Exercise pre-conditioning reduces brain damage in ischemic rats that may be associated with regional angiogenesis and cellular overexpression of neurotrophin. *Neuroscience, 124,* 583-591.

Duman, C.H., Schlesinger, L., Russell, D.S., Duman, R.S. (2008). Voluntary Exercise Produces Antidepressant and Anxiolytic Behavioral Effects in Mice. *Brain Res, (1199),* 148-158.

Elder, G. A., Sosa, M. A. G., & Gasperi, R. De. (2010). Transgenic Mouse Models of Alzheimer ' s Disease, *Mount Sinai Journal of Medicine, 77,* 69-81.

Engelhart, M. J., Geerlings, M. I., Ruitenberg, A., van Swieten, J. C., Hofman, A., Witteman, J. C. M., & Breteler, M. M. B. (2002). Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA : The Journal of the American Medical Association*, *287*(24), 3223–9.

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L. & Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences of the United States of America*,

108(7), 3017–22.

Esquivel G., Schruers K., Kuipers H., Griez E. (2002). The effects of acute exercise and high lactate levels on 35% CO2 challenge in healthy volunteers. *Acta Psychiatr. Scand*. *106(5),* 394–397.

Ferretti L, McCurry SM, Logsdon R et al. (2001). Anxiety and Alzheimer's disease. *J Geriatr Psychiatry Neurol, 14,* 52–58.

Feliz-Ortiz, A.C., Beyeler, A., Seo, C., Leppla, C.A., Wildes, C.P., Tye, K.M. (2013). BLA to vHPC Inputs Modulate Anxiety-Related Behaviors. *Neuron, 79(4),* 658-664.

Fulk, L.J., Stock, H.S., Lynn, A., Marshall, J., Wilson, M.A., Hand, G.A. (2004). Chronic Physical Exercise Reduces Anxiety-Like Behavior in Rats. *International Journal of Sports Medicine*, *25(1)*, 78-82.

Fratiglioni, L., Paillard-Borg, S. & Winblad, B. (2004). An active and socially integrated lifestyle in late life might protect against dementia. *Lancet Neurol 3*, 343–53.

Garcia-Mesa, Y., Lopez-Ramos, J.C., Gimenez-Llort, L., Revilla, S., Guerra, R., Gruart, A., Laferla, F.M., Cristofol, R., Delgado-Garcia, J.M., Sanfeliu, C., (2011). Physical exercise protects against Alzheimer's disease in 3xTg-AD mice. *J. Alzheimer's Dis. 24,* 421-454.

Geda, Y.E., Silber, T.C., Roberts, R.O., Knopman, D.S., Christianson, T.J., Pankratz, V.S., Boeve, B.F., Tangalos, E.G., and Petersen, R.C. (2012). Computer activities, physical exercise, aging, and mild cognitive impairment: a population-based study. *Mayo Clin. Proc*. 87, 437-442.

Gobbo O.L. & O'Mara S.M. (2004) Impact of enriched-environment housing on brain-derived neurotrophic factor and on cognitive performance after a transient global ischemia. *Behav Brain Res 152,* 231–241

Grabowski, T.J., Cho, H.S., Vonsattel, J.P.G., Rebeck, G.W., and Greenberg, S.M. (2001) Novel

amyloid precursor protein mutation in an Iowa family with dementia and severe cerebral amyloid angiopathy. *Ann. Neurol. 49,* 697-705.

Henderson, V.W., Mack, W., White Williams, B. (1989). Spatial disorientation in Alzheimer's disease. *Arch Neurol, 46,* 391-394.

Holloway, R. L., Jr. (1966) Dendritic branching: some preliminary results of training and complexity in rat visual cortex. *Brain Res. 2,* 393–396.

Hardy, J & Selkoe, D (2002). The amyloid hypothesis and Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* 297*,* 353.

Hebb, D. O. The effects of early experience on problem- solving at maturity. *Am. Psychol. 2,* 306–307 (1947).

Hernandez SS, Coelho FG, Gobbi S, Stella F. (2010). Effects of physical activity on cognitive functions, balance and risk of falls in elderly patients with Alzheimer's dementia. *Rev Bras Fisioter 14*, 68–74.

Hupin, D., Roche, F., Gremeaux, V. et al. Relation between physical activity and morbi-mortality of elderly people: the Proof cohort study. Presented at EuroPRevent 2015, Lisbon.

Jankowsky, J. L., Xu, G., Fromholt, D., Gonzales, V., & Borchelt, D. R. (2003). Environmental enrichment exacerbates amyloid plaque formation in a transgenic mouse model of Alzheimer disease. *Journal of Neuropathology and Experimental Neurology*, *62*(12), 1220–7.

Jankowsky, J. L., Melnikova, T., Fadale, D. J., Xu, G. M., Slunt, H. H., Gonzales, V., Younkin, L. H. et al. (2005). Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's disease. *The Journal of neuroscience : the official journal of the*

Society for Neuroscience, *25*(21), 5217–24.

Jellinger, K.A. (2002) Alzheimer's disease and cerebrovascular pathology: an update. *J. Neural. Trasm*. *109*, 813-836.

Karp, A. (2004). Relation of Education and Occupation-based Socioeconomic Status to Incident Alzheimer's Disease. *American Journal of Epidemiology*, *159*(2), 175–183.

Katzman R., Terry R., DeTeresa R. et al. (1998). Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann Neurol, 23,* 138–144.

Katzman, R. (1993). Education and the prevalence of dementia and Alzheimer's disease. *Neurology, 43(1),* 13-20.

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature*.

Kim, M.H. & Leem, Y.H. (2014). Chronic exercise improves repeated restraint stress-induced anxiety and depression through 5HT1A receptor and cAMP signaling in hippocampus. *Journal of Exercise Nutrition and Biochemistry, 18(1),* 97-104.

Koffie, R.M., Hyman, B.T., Spires-Jones, T.L. (2011). Synapses Gone Cold. *Molecular Neurodegeneration*, *6,* 63.

Laurin, D. et al. (2001). Physical activity and risk of cognitive impairment and dementia in elderly persons. *Arch. Neurol. 58*, 498–504

Lautenschlager, N.T., Cox, K.L., Flicker, L., Foster, J.K., van Bockxmeer, F.M., Xiao, J., Greenop, K.R., and Almeida, O.P. (2008) Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. *JAMA, 30*, 1027-1037.

Lazarov, O., Robinson, J., Tang, Y.-P., Hairston, I. S., Korade-Mirnics, Z., Lee, V. M.-Y., Hersh, L. B. et al. (2005). Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. *Cell*, *120*(5), 701–13.

Leggio, M. G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., & Petrosini, L. (2005). Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. *Behavioural Brain Research*, *163*(1), 78–90.

Leasure, J.L. & Jones, M. (2008). Forced and Voluntary Exercise Differentially Affect Brain and Behavior. *Neuroscience 156,* 456-465.

Leppla, C.P. & Wildes, K.M. (2013). BLA to vHPC Inputs Modulate Anxiety-Related Behaviors. *Cell*, *79(4),* 658-664.

Levy, E., Carman, M.D., Fernandez-Madrid, I.J., Power, M.D., Lieberburg, I., van Duinen, S.G., Bots, G.T.A.M., Luyendijk, W., and Frangione, B. (1990) Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* 248, 1124-1126.

Little, J.P., Safdar, A.S., Wilkin, G.P., Tarnopolsky, M. & Gibala, M. (2010). A practical model of low-volume high-intensity interval training induces mitochondrial biogenesis in human skeletal muscle: potential mechanisms. *The Journal of Physiology*, *588(6),* 1011-1022.

Liu-Ambrose, T., Eng, J.J., Boyd, L.A., Jacova, C., Davis, J.C., Bryan, S., Lee, P., Brasher, P., and Hsiung, G.Y. (2010) Promotion of the mind through exercise (PROMoTE): a proof-of-concept randomized controlled trial of aerobic exercise training in older adults with vascular cognitive impairment. *BMC Neurol. 10*, 14.

Lue, L.F., Kuo ,Y.M., Roher, A.E., Brachova, L., Shen, Y., Sue, L. et al. (1999). Soluble

amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol 155,* 853–862.

Mah, L., Binns, M.A., Steffens, D.C. (2014). Anxiety Symptoms in Amnestic Mild Cognitive Impairment Are Associated with Medial Temporal Atrophy and Predict Conversion to Alzheimer's Disease. *Am J Geriatric Psychiatry, 23(5),* 466-476.

Marchesi, V. T. (2011). Alzheimer's dementia begins as a disease of small blood vessels, damaged by oxidative-induced inflammation and dysregulated amyloid metabolism: implications for early detection and therapy. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *25*(1), 5–13.

Masters, C. L., & Selkoe, D. J. (2012). Biochemistry of Amyloid β-Protein and Amyloid Deposits in Alzheimer Disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(6), a006262.

McAllister, A.K. et al. (1999) Neurotrophins and synaptic plasticity. *Annu. Rev*. *Neurosci*. *22*, 295–318.

Milgram, N. W., Siwak-Tapp, C. T., Araujo, J., & Head, E. (2006). Neuroprotective effects of cognitive enrichment. *Ageing Research Reviews*, *5(3),* 354–69.

Monacelli A.M., Cushman L.A., Kavcic V., Duffy C.J. (2003) Spatial disorientation in Alzheimer's disease: the remembrance of things passed. *Neurology 61,* 1491–1497.

Morishima M, Harada N, Hara S et al. (2006). Monoamine oxidase A activity and norepinephrine level in hippocampus determine hyperwheel running in SPORTS rats. *Neuropsychopharmacology. 31(12),* 2627–2638.

Morris, J.C. (2013). Persistent failure of "disease-modifying" drugs to benefit Alzheimer's disease. Now what? *The Dana Foundation: Report on Progress.*

Morris, M. C. (2009). The role of nutrition in Alzheimer's disease : epidemiological evidence, *16*, 1–7.

Neeper S., Gomez-Pinilla F., Choi J., Cotman C. (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth, *Brain Research, 726,* 49-56.

Neuropathology Group. Medical Research Council (2001). Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. *Lancet 357,* 169–175.

Nichol, K.E., Parachikova, A.I., Cotman, C.W., (2007). Three weeks of running wheel exposure improves cognitive performance in the aged Tg2576 mouse. *Behav. Brain Res. 184*, 124–132

Nichol, K., Deeny, S. P., Seif, J., Camaclang, K., & Cotman, C. W. (2009). Exercise improves cognition and hippocampal plasticity in APOE epsilon4 mice. *Alzheimer's & Dementia : The Journal of the Alzheimer's Association*, *5*(4), 287–94.

Nichol, K., Deeny, S. P., Seif, J., Camaclang, K. & Cotman, C. W. (2009). Exercise improves cognition and hippocampal plasticity in APOE epsilon4 mice. *Alzheimer's & dementia : the journal of the Alzheimer's Association*, *5*(4), 287–94.

Nicoll, J. a R., Yamada, M., Frackowiak, J., Mazur-Kolecka, B., & Weller, R. O. (2004). Cerebral amyloid angiopathy plays a direct role in the pathogenesis of Alzheimer's disease. Pro-CAA position statement. *Neurobiology of Aging*, *25*(5), 589–97; discussion 603–4.

Nithianantharajah, J., & Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature reviews. Neuroscience*, *7*(9), 697–709.

Noack, H., Lovden, M., Schmiedek, F. (2014). On the validity and generality of transfer effects in cognitive training research. *Psychological Research, 78,* 773-789.

Norton S., Matthews F.E., Barnes D.E., Yaffe K., Brayne C. (2014). Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *Lancet Neurol 13:* 788–94.

O'Keefe, J.H., Patil, H.R., Lavie, C.J., Magalski, A., Vogel, R.A., McCullough, P.A. (2012). Potential Adverse Cardiovascular Effects From Excessive Endurance Exercise. *Mayo Clin Proc, 87(6),* 587-595.

Oliff H., Berchtold N., Isackson P., Cotman C. (1998). Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. *Mol Brain Res 61,* 147–53.

Owen, A.M., Hampshire, A., Grahn, J.A., Stenton, E., Dajani, S., Burns, A.S., Howard, R.J. & Ballard, C.G. (2010). Putting brain training to the test. *Nature*, *465(7299),* 775-778.

Parachikova, A., Nichol, K. E. & Cotman, C. W. (2008). Short-term exercise in aged Tg2576 mice alters neuroinflammation and improves cognition. *Neurobiology of disease*, *30*(1), 121–9.

Pfeifer LA, White LR, Ross GW, Petrovitch H, Launer LJ (2002) Cerebral amyloid angiopathy and cognitive function. Neurology 58, 1629-1634.

Qaseem, A., Snow, V., Cross, T., Forciea, M.A., Hopkins, R. & Shekelle, P. (2008). Current pharmacologic treatment of dementia: A clinical practice guideline from the American College of Physicians and the American Academy of Family Physicians. *Annals of Internal Medicine, 148(5)*, 370-378.

Radak, Z., Hart, N., Sargam, L., Koltai, E., Atalay, M., Ohno, H., and Boldogh, I. (2010)

Exercise plays a preventive role against Alzheimer's disease. J. Alz. Dis. *20,* 777-783.

Radak, Z., Kumagai, S., Taylor, A.W., Naito, H., Goto, S. (2007). Effects of exercise on brain function: role of free radicals, *Applied Physiology, Nutrition, and Metabolism, 32(5),*942-946.

Redick, T. S., Shipstead, Z., Harrison, T. L., Hicks, K. L., Fried, D. E., Hambrick, D. Z., & Engle, R. W. (2013). No evidence of intelligence improvement after working memory training: A randomized, placebo-controlled study. *Journal of Experimental Psychology, 142,* 359-379.

Rensink, A.A., de Waal, R.M., Kremer, B., and Verbeek, M.M. (2003) Pathogenesis of cerebral amyloid angiopathy. Brain Res. Brain Res. Rev. *43*, 207-223.

Rogers, R.L. et al. (1990). After reaching retirement age physical activity sustains cerebral perfusion and cognition. J. Am. Geriatr. Soc. 38, 123–128 5 Hill, R.D. et al. (1993). The impact of long-term exercise training on psychological function in older adults. *J. Gerontol. 48*, 12-17

Rolland, Y., Pillard, F., Klapouszczak, A., Reynish, E., Thomas, D., Andrieu, S. & Vellas, B. (2007). Exercise program for nursing home residents with Alzheimer's disease: a 1-year randomized, controlled trial. *Journal of the American Geriatrics Society*, *55*(2), 158–65.

Roig, M., Nordbrandt, S., Geertsen, S. S., & Nielsen, J. B. (2013). The effects of cardiovascular exercise on human memory: A review with meta-analysis. *Neuroscience and Biobehavioral Reviews*, *37*, 1645–1666

Rosen, A.C., Sugiura, L., Kramer, J.H., Whitfield-Gabrieli, S., Gabrieli, J.D. (2011). Cognitive training changes hippocampal function in mild cognitive impairment: a pilot study. *Journal of Alzheimer's disease, 26,* 349-357.

Rosenzweig, M.R; Krech, D., Bennett, E.L., Diamond, M.C. (1962). Effects of environmental complexity and training on brain chemistry and anatomy: A replication and extension. *Journal of Comparative and Physiological Psychology, 55,* 429-437.

Rozemuller, A.J.M., Roos, R.A.C., Bots, G.T.A.M., Kamphorst, W., Eikelenboom, P., and Van Nostrand, W.E. (1993) Distribution of ß/A4 and amyloid precursor protein in hereditary cerebral hemorrhage with amyloidosis-Dutch type and Alzheimer's disease. *Am. J. Pathol. 142*, 449-1457

Salam, J.N., Fox, J.H., DeTroy, E.M., Guignon, M.H., Wohl, D.F. & Falls, W.A. (2009). Voluntary exercise in C57 mice is anxiolytic across several measures of anxiety, *Behavioural Brain Research, 197(1),* 31-40.

SantaCruz, K., Lewis, J., Spires, T., Paulson, J., Kotilinek, L., Ingelsson, M., Guimaraes, A., DeTure, M., Ramsden, M., McGowan E., Forster, C., Yie, M., Orne, J., Janus, C., Mariash, A., Kuskowski, M., Hyman, B., Hutton, M., Ashe, K.H., (2005) Tau Suppression in a Neurodegenerative Mouse Model Improves Memory Function, *Science (309)* 5733, 476-481.

Santos-Soto, I.J., Chorna, N., Carballeira, N.M., Velez-Bartolomei, J.G. Mendez-Merced, A.T., Chornyy, A.P., Pena de Ortiz, S. Voluntary running in young adult mice reduces anxiety-like behavior and increases the accumulation of bioactive lipids in the cerebral cortex. *PLoS One, 8(12)* e81459.

Scarmeas, N., Luchsinger, J.A., Schupf, N., Brickman, A.M., Cosentino, S., Tang, M.X., and Stern, Y. (2009) Physical activity, diet, and risk of Alzheimer disease. *JAMA* 302, 627-637.

Scarmeas, N., Albert, S. M., Manly, J. J., & Stern, Y. (2006). Education and rates of cognitive

decline in incident Alzheimer's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *77*(3), 308–16.

Schinder, A.F. & Poo, M.M. (2000). The neurotrophin hypothesis for synaptic plasticity. *Trends in Neuroscience, 23*, 639-645.

Schmand B., Smit J.H., Geerlings M.I. et al. (1997). The effects of intelligence and education on the development of dementia. A test of the brain reserve hypothesis. *Psychol Med. 27,* 1337-1344.

Schultz, R.L., Swallow, J.G., Waters, R.P., Kuzman, J.A., Redetzke, R.A., Said, S., Morreale de Escobar, G., Gerdes, A.M. (2007). Effects of Excessive Long-Term Exercise on Cardiac Function and Myocyte Remodeling in Hypertensive Heart Failure Rats, *Hypertension, 50,* 410-416.

Selkoe, D. (2002). Alzheimer's Disease is a Synaptic Failure. *Science, 298(5594),* 789-791.

Selkoe, D. J. (2001). Alzheimer ' s Disease : Genes , Proteins , and Therapy, *81*(2), 741–767.

Shin, Y., Cho, H.S., Rebeck, G.W., and Greenberg, S.M. (2002) Vascular changes in Iowa-type hereditary cerebral amyloid angiopathy. *Ann. N.Y. Acad. Sci. 977,* 245-251.

Shipstead, Z., Redick, T. S., & Engle, R. W. (2012). Is working memory training effective? *Psychological Bulletin*

Sinoff G, Werner P. (2003). Anxiety disorder and accompanying subjective memory loss in the elderly as a predictor of future cognitive decline. *Int J Geriatr Psychiatry 18*, 951–959.

Snowdon, D.A., Greiner, L.H., Mortimer, J.A., Riley, K.P., Greiner, P.A., Markesbery, W.R. (1997). Brain infarction and the clinical expression of Alzheimer's disease. *JAMA. 227 (10),* 813-817.

Spector, A., Thorgrimsen, L., Woods, B. O. B., Royan, L., Davies, S., Butterworth, M. & Terworth, M. B. U. T. (2003). Efficacy of an evidence-based cognitive stimulation

therapy programme for people with dementia : Randomised controlled trial, *British Journal of Psychiatry, 183,* 248–254.

Stern, Y., Gurland, B., Tatemichi, T.K., Xin Tang, M., Wilder, D., & Mayeux, R. (1994) Influence of Education and Occupation on the Incidence of Alzheimer's Disease. *Journal of the American Medical Association*. *271(13),* 1004-1010.

Stern, Y. (2006). Cognitive reserve and Alzheimer disease. *Alzheimer disease and associated disorders*, *20*, S69–74.

Stern, Y. (2001). What is cognitive reserve? Theory and research application of the reserve concept. *Journal of the International Neuropsychological Society : JINS*, *8*(3), 448–60.

Stern, Y. (2009). Cognitive reserve. *Neuropsychologia*, *47*(10), 2015–28.

Stern, Y., Gurland, B., Tatemichi, T.K., Xin Tang, M., Wilder, D., & Mayeux, R. (1994) Influence of Education and Occupation on the Incidence of Alzheimer's Disease. *Journal of the American Medical Association*. *271(13),* 1004-1010.

Steptoe, A., Edwards, S., Moses, J., & Mathews, A. (1989). The effects of exercise training on mood and perceived coping ability in anxious adults from the general population. *Journal of Psychosomatic Research, 33,* 537-547

Thal, D.R., Ghebremedhin, E., Rub, U., Yamaguchi, H., Del, T.K., and Braak, H. (2002) Two types of sporadic cerebral amyloid angiopathy. *J. Neuropathol. Exp. Neurol. 61*, 282-293.

Thal D.R., Ghebremedhin E., Orantes M., Wiestler O.D. (2003). Vascular pathology in Alzheimer's disease: Correlation of cerebral amyloid angiopathy and arteriosclerosis/lipohyalinosis with cognitive decline. *J Neuropath Exp Neurol 62,* 1287-1301.

Um, H.S., Kang, E.B., Koo, J.H., Kim, H.T., Jin, L., Kim, E.J., Yang, C.H., An, G.Y., Cho, I.H.,

Cho, J.Y., (2011). Treadmill exercise represses neuronal cell death in an aged trans- genic mouse model of Alzheimer's disease. *Neurosci. Res. 69,* 161–173.

van Dellen A, Blakemore C, Deacon R, York D, Hannan A.J.(2000) Delaying the onset of Huntington's in mice. *Nature 404,*721–722.

van Duinen, S.G., Castano, E.M., Prelli, F., Bots, G.T.A.M., Luyendijk, W., and Frangione, B. (1987). Hereditary cerebral hemorrhage with amyloidosis in patients of Dutch origin is related to Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A. 84,* 5991-5994.

van Praag H., Kempermann G., Gage F.H. (2000). Neural consequences of environmental enrichment. *Nat Rev Neurosci 1*, 191–198.

Van Praag H., Shubert, T., Zhao, C. & Gage, F.H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *The Journal of Neuroscience, 25(38),* 8680-8685.

Vinters, H.V. (1987). Cerebral amyloid angiopathy: A critical review. *Stroke 18*, 311-324.

Volkmar F.R. & Greenough W.T. (1972). Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science 176,* 1445–1447.

Wattendorff, A.R., Frangione, B., Luyendijk, W., and Bots, G.T.A.M. (1995). Hereditary cerebral haemorrhage with amyloidosis, Dutch type (HCHWA-D): clinicopathological studies. *J. Neurol. Neurosurg. Psychiatry 59*, 699-705.

Wetherell J.L., Reynolds C.A., Gatz M. et al. (2002). Anxiety, cognitive performance, and cognitive decline in normal aging. *J Gerontol B Psychol Sci Soc Sci 57*, 246–255.

Whalley, L.J., Deary, I.J., Appleton, C.L., Starr, J.M., (2004). Cognitive reserve and the neurobiology of cognitive aging. *Ageing Res. Rev. 3*, 369–382.

Williams, C. L., & Tappen, R. M. (2007). Effect of exercise on mood in nursing home residents

with Alzheimer's disease. *American Journal of Alzheimer's Disease and Other Dementias*, *22(5)* 389-397.

Wilson, R. S., Mendes De Leon, C. F., Barnes, L. L., Schneider, J. a, Bienias, J. L., Evans, D. A. & Bennett, D. A. (2002). Participation in cognitively stimulating activities and risk of incident Alzheimer disease. *JAMA : the journal of the American Medical Association*, *287*(6), 742–8.

Wilson, R. S., Mendes De Leon, C. F., Barnes, L. L., Schneider, J. a, Bienias, J. L., Evans, D. A. & Bennett, D. A. (2002). Participation in cognitively stimulating activities and risk of incident Alzheimer disease. *JAMA : the journal of the American Medical Association*, *287*(6), 742–8.

Xu, W. Xu, F. Anderson, ME. Kotarba, AM., Davis, J. Robinson, JK., VanNostrand, WE (2014) Cerebral Microvascular Rather than Parenchymal Amyloid-β Protein Pathology Promotes Early Cognitive Impairment in Transgenic Mice. *Journal of Alzheimer's Disease, 38*, 621-632

Xu F., Grande AM., Robinson JK., Previti ML., Vasek M., Davis J., Van Nostrand WE. (2007). Early-onset subicular microvascular amyloid and neuroinflammation correlate with behavioral deficits in vasculotropic mutant amyloid -protein precursor transgenic mice. *Neuroscience, 146*: 98-107

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *The European Journal of Neuroscience*, *20*(10), 2580–90.

Yuede, C. M., Zimmerman, S. D., Dong, H., Kling, M. J., Bero, A. W., Holtzman, D. M. & Csernansky, J. G. (2009). Effects of voluntary and forced exercise on plaque deposition,

hippocampal volume, and behavior in the Tg2576 mouse model of Alzheimer's disease. *Neurobiology of Disease*, *35*(3), 426–32.