EARLY CHANGES IN MEMORY, MOTOR COORDINATION AND STRENGTH IN THE TRANSGENIC RAT MODEL OF ALZHEIMER'S DISEASE, TgF344-AD

by

Mark Andrew Winslow

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Kinesiology

Boise State University

August 2019

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Mark Andrew Winslow

Thesis Title: Early Changes in Memory, Motor Coordination and Strength in the Transgenic Rat Model of Alzheimer's Disease, TgF344-AD

Date of Final Oral Examination: 25 April 2019

The following individuals read and discussed the thesis submitted by student Mark Andrew Winslow, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Stephanie Greufe-Hall, Ph.D.	Chair, Supervisory Committee
Shawn R. Simonson, Ed.D.	Member, Supervisory Committee
Troy Rohn, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by Stephanie Greufe-Hall, Ph.D., Chair of the Supervisory Committee. The thesis was approved by the Graduate College.

DEDICATION

This is dedicated to my parents who always gave me support when I needed it most. To the “Blue Tank Warriors,” there’s always one more tank and one more mountain climb. To my comrades in Chestnut Ridge Forestry, it’s always easier going through hell when you’re laughing. To you, Life’s not always going to be lollipops and cupcakes, bud.

ACKNOWLEDGEMENTS

I would like to thank Dr. Troy Rohn for agreeing to be on my committee because reading about neurology written by someone who has no background in the field is not easy. Thanks to Dr. Shawn Simonson, for fielding all my questions I had about physiology and dealing with my shenanigans in class. Finally, I would like to thank Dr. Stephanie Hall for providing me with this research opportunity; I honestly do not know what I would be doing if I were not at BSU. Dr. Hall do not stop “suggesting” projects for me to work on.

ABSTRACT

Introduction: Alzheimer's disease (AD) is the most common form of dementia and an estimated 5.7 million American's are currently suffering from this disease resulting in $277 billion in health care costs as of 2018. As the number of cases and cost are expected to double by 2050, new approaches are needed to properly identify at-risk individuals prior to irreversible changes to the brain caused by this disease. **Purpose:** Through the use of a novel rodent model, this study assessed early changes in muscular coordination and strength relative to cognitive decline due to AD, as well as providing insight into the progression of these changes and whether sex differences exist within AD development. **Hypothesis:** Declines in muscular coordination and strength will precede cognitive decline. Males will exhibit significantly worse declines among all measures earlier than females. **Methods:** Thirty-eight TgF-344AD rats were compared to 38 wild type (WT) TgF-344 rats for muscle atrophy (grip strength test), coordination (rotarod), and spatial memory (Morris Water Maze test). Testing periods occurred at 3, 6, and 9 m and body weight was measured prior to assessments for these time periods. **Statistical Analysis:** Repeated measures ANOVA was used to identify within group differences. Mixed model 2-way ANOVA (group x age) with a Bonferroni post hoc test was used to identify any significant effects. ANOVA with a Tukey post hoc was used to determine between group differences. Unpaired independent T-tests were used to determine the degree of significance based on significant P-values ($p \leq 0.05$) determined by the Tukey post hoc test. **Results:** AD males displayed significantly heavier body weight at 3 m, 6 m,

and 9 m and AD females displayed significantly heavier body weight at 6 m and 9 m compared to the WT cohort. No significant difference was seen in grip strength between any groups. AD females and AD males expressed a significant decline in motor coordination at 6 m but by 9 m all groups expressed a significant decline in motor coordination. No significant difference was seen in motor coordination between groups. The AD group as a whole displayed significantly worse memory function at 9 m compared to WT group. **Conclusion:** Body weight may be a risk factor for developing AD. AD may cause an accelerated rate of decline in motor coordination. Memory dysfunction precedes deficits in strength and coordination. No AD-related sex differences were identified but 2-way ANOVA identified a significant effect for AD females on motor coordination decline seen at 6 m but not males.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
Aβ	amyloid *beta*
NFTs	neurofibrillary tangles
MCI	Mild Cognitive Impairment
MMSE	Mini-Mental State Examination
APP	amyloid precursor protein
PS1	presenilin 1
α7nAChRs	α7 nicotinic acetylcholine receptors
eNMDARs	extrasynaptic N-methyl-D-aspartate receptors
NO	nitric oxide
GABAA	γ-aminobutyric acid type A
MAPK	mitogen-activated protein kinase
CAA	cerebral amyloid angiopathy
MWM	Morris Water Maze
BMT	Barnes Maze Task
DNMS	delayed nonmatch-to-sample
BDNF	brain derived neurotrophic factor
OXPHOS	oxidative phosphorylation
ANOVA	analysis of variance

CHAPTER ONE: INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia accounting for 60-80% of all cases. An estimated 5.7 million Americans are suffering from AD and that number is expected to rise to 14 million by 2050 (Alzheimer's Association, 2018). The estimated total cost for these individuals and their families is $277 billion for 2018. The number of cases and cost is estimated to double over the next 50 years as there is no current treatment to cure or slow the progression of this disease. Although AD is typically associated with aging, an estimated 200,000 of the 5.7 million cases develop AD before reaching age 65.

Currently there is no cure or treatment to slow AD disease progression. However, in addition to finding treatments, research is needed to improve the diagnosis of AD. AD is commonly identified in patients by memory loss, cognition and behavior alterations. Patient's suffering from previously mentioned symptoms will undergo a functional magnetic resonance imaging (fMRI) of their brain. If these tests reveal Amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs) with brain atrophy they will be diagnosed with AD. Prior to diagnosis, elderly subjects who develop AD present significantly lower cognition and memory capabilities when compared to elderly subjects who do not develop AD (Baker et al., 2010; Verghese et al., 2003). Recently, AD has also been strongly correlated with declines in muscular strength and motor control (Roberts et al., 2013; Taekama et al., 2012). These strong and well documented correlations could provide an avenue for diagnostic tools as well as elevating our understanding of AD progression.

Natural aging induces muscle and brain atrophy (Pollack, Phaneuf, Dirks, & Leeuwenburgh, 2002); however, there are subtle yet key differences between natural aging and AD development. While regions of the brain impacted with age and AD are the same, the rates and patterns of muscle atrophy can identify individuals who have AD or are at risk of developing AD. This, with more research, will likely lead to better diagnostic practices. Biomarkers Aβ and modified tau in the form of NFTs also distinguish AD from natural aging. In AD, Aβ and modified tau accumulate in the centers of the brain responsible for learning and memory leading to synapse loss and death, which ultimately leads to impaired cognition and memory. This study will aim to identify physiological changes (i.e., memory, motor coordination, and skeletal muscular strength) pre-onset of the development of the pathological cascades.

Current rodent models genetically modified to exhibit AD are problematic in that they only exhibit a part of the AD pathogenesis and do not fully recapitulate human AD pathology. For example, transgenic rat models modified to overexpress Aβ may only exhibit this side of AD development without the presence of NFTs, which are a key feature of human AD. However, a new transgenic model is available, TgF344-AD, which displays the complete repertoire of AD pathological features, including, age-dependent accumulation of cerebral amyloid-β that preempts tauopathy, cognitive disturbance, apoptosis, and neuronal loss. (Cohen et al., 2013; Joo et al., 2016; Pentkowski et al., 2018; Munoz-Moreno et al., 2018; Rorabaugh et al., 2017; Smith, & McMahon, 2018; Tsai et al., 2013; Vorhees et al., 2018). Nine studies have been conducted to date with this model; however, it promises to be a viable model to use for AD research. This study will be the first to analyze skeletal muscle strength and motor control employing this rat model of AD.

Research has identified gender differences in AD progression (Burke et al., 2018; Nead et al., 2015; Phung et al., 2010), which has been confirmed using rodent models (Carroll e. al., 2010; Munetumo et al., 2015; Woolley, & McEwen, 1992). The dichotomy of gender-differentiated AD progression is due to sex hormone interaction in neuronal growth and repair (Cacciottolo et al. 2016; Rosario, Chang, Head, Stanczyk, & Pike, 2011). The primary hormones used for neuronal growth for females is estradiol and testosterone for males. The difference in hormonal decline between genders due to aging and the use of estradiol versus testosterone relates to differences in Aβ load, brain region affected, and rate of cognitive decline in AD progression (Cacciottolo et al., 2016; Carroll et al., 2010). These differences suggest that males exhibit more abrupt cognitive impairment earlier in life than females but it eventually plateaus. Females; however, exhibit a gradual decline and show worse performances on cognitive tests at later time points in life than males (Burke et al., 2018; Carroll et al., 2010). This study, accordingly, will analyze these gender differences in the development of AD. Understanding this dichotomy will aid in the effectiveness of diagnosing and treating individuals.

The primary hypothesis of this study is muscular coordination and strength declines in Alzheimer's disease and these muscular changes will precede memory declines. The secondary hypothesis is males will exhibit significantly worse performances on all parameters being tested at the 9m test period. Research indicates that males express more drastic declines in cognition earlier than females due to differences in hippocampal volume and Aβ load, which may be the result of differences in sex hormones (Burke et al., 2018; Carroll et al., 2010; Irvine, Laws, Gale, & Kondel, 2012; Leranth, Pentnehazy, & Maclusky, 2003; Nead et al., 2015). Muscle strength and coordination will be assessed by

a grip strength test and rotarod test (Bioseb, Panlab), respectively. Spatial memory will be assessed using a Morris Water Maze test (Voorhees, & Williams, 2006). The first aim of this study is to enhance the protocol for identifying AD or risk of developing AD among humans using physiological tests such as strength and motor control tests. The second aim is to validate the rotarod test in an AD rat model. The third and final aim is to identify sex differences among the development of AD: previous studies have indicated males tend to exhibit a steeper decline in cognition earlier, whereas females exhibit a steady decline and exhibit a steeper decline as AD progresses overall (Carroll et al., 2010).

CHAPTER TWO: LITERATURE REVIEW

The Effect of Exercise on the Progression of Alzheimer's Disease

Exercise can slow the rate of cognitive decline among individuals suffering from AD; even general physical activity and higher fitness levels decrease risk of AD (Best, Chiu, Hsu, Nagumatsu, & Liu-Ambrose, 2015; Maass et. al, 2015; Matura et al., 2017; Nagumatsu et al., 2013; Voss et al., 2013). Nouchi et al. (2014) studied the effects of short-term exercise on cognitive functions among 64 healthy subjects over the age of 62. The exercise intervention consisted of interval training and strength training at 60-80% of their maximum heart rate for 24 minutes 3 days a week for 4 weeks (Nouchi et al., 2014), while the control group was asked to refrain from exercise during this 4-week period. Cognitive function was measured across six categories: executive function, episodic memory, working memory, reading ability, attention, and processing speed. The results indicated that subjects receiving the intervention improved on all measures of executive function, processing speed, and one measure of episodic memory (logical memory), which suggests exercise can improve cognitive function. Although this study did not include outcome measures of muscular strength or cardiovascular output other studies have identified improvements between these measures and improved cognitive function and brain plasticity (Baker et al., 2010; Chan et al., 2005; Chapman et al., 2016; Nagumatsu et al., 2016; Voelcker-Rehage, Godde, & Staudinger, 2011). For example, Baker et al. demonstrates that aerobic exercise intervention improves $\dot{V}O_{2peak}$ and cognitive function among 33 subjects suffering from mild cognitive impairment (MCI). MCI is a termed used

to identify individuals who are experiencing cognitive decline greater than expected for their age but does not interfere with daily activities (Gauthier et al., 2006). $\dot{V}O_{2peak}$ is a measure of a person's capacity for aerobic ATP synthesis (McArdle, Katch, & Katch, 2010). This study used the modified Balke maximal-graded exercise treadmill test, which maintains a constant speed but increases 1% grade every minute (McArdle, Katch, & Katch, 2010). Higher $\dot{V}O_{2peak}$ values indicate higher fitness levels and vice versa for lower $\dot{V}O_{2peak}$ values. Improvements in cognitive function due to the aerobic training was greatest among women. Furthermore, changes in $\dot{V}O_{2peak}$ among women was correlated with improved glucoregulation and insulin sensitivity and changes in insulin sensitivity predicted $\dot{V}O_{2peak}$ and executive function. Exercise is commonly used to treat diabetic (type II) patients but can be extended as a prevention measure among individuals at risk for AD (Umpierre et al., 2011). Being that glucose is the primary source of energy for the brain, these results suggest that MCI and AD may cause a dysfunction among glucose uptake, which exercise can remedy by changes made centrally (brain) or peripherally (muscle).

Exercise Increases Brain Volume and Function

Exercise has also been shown to increase brain volume with aging and stabilize brain atrophy associated with AD (Erickson et al., 2009; 2010; 2011; Burns et al., 2008), which correlates to an enhancement of brain function, as measured with MRI and a spatial memory task (Voss et al., 2010; 2013). A study involving healthy and AD subjects between the ages of 59-81 years old identified a significant correlation between fitness level measured by VO_{2peak} and hippocampus size. The individuals with higher cardiorespiratory fitness express larger hippocampus size, which correlated with better scores on the spatial memory assessment (Erickson et al., 2009). To understand whether AD impacts fitness

level, the next logical step was to analyze the impact of an exercise regimen on brain volume among individuals with AD. Erickson et al. implemented a 12-month walking program among a similar population and found that aerobic exercise increases hippocampus size, which correlates with significant improvements in spatial memory (Erickson et al., 2011). As these improvements are a result of exercise, Voss et al. (2010) indicates that connectivity between brain regions improves with exercise. Improved connectivity was indicated by functional MRI scans showing an increase in activity between the bilateral parahippocampus and the bilateral middle temporal gyrus post exercise intervention. Altogether these studies indicate that exercise increases brain volume which produces better connectivity between regions resulting in improvements in cognition among patients suffering from AD. Furthermore, exercise is used to treat people suffering from obesity, hypertension, and type II diabetes which are common risk factors for dementia (Baumgart et al., 2015; Chatterjee et al., 2016). This extends the notion that exercise may play an important role in maintaining one's brain health.

Exercise-Induced Changes in Aβ/tau are Unknown

Aβ plaque and NFTs are important hallmarks to AD and the pathogenic development of these proteins are described in a latter section but again there is evidence suggesting exercise can be used to alleviate symptoms caused by these brain lesions. Using an AD transgenic mouse model, Ke et al. (2011) implemented an aerobic exercise protocol, which consisted of treadmill running 5 days a week for 1 month. For the first week, the mice ran for 10 min/day; by the second week training time increased 10 min every day until 60 min of continuous running was achieved and was maintained for the remainder of the intervention. Results of this study indicated that Aβ40 and Aβ42 levels were

significantly reduced by exercise but had no effect on plaque formation (Ke, Huang, Liang, & Hsieh-Li, 2011). These results are potentially confounding because Aβ40 and Aβ42 are typically precursors to plaque formation; however, as mentioned below there are other Aβ substituents that can cause plaque formation as well. These results differ from other studies involving different AD transgenic mice (Adlard, Perreau, Pop, Cotman, 2005; Yuede et al., 2009). Therefore, when examining the impact of exercise, it may be dependent upon which model is employed. Overall, there does appear to be a correlation between exercise and improving brain health. For example, one study indicates a reduction in plaque formation as well as a reduction Aβ40 and Aβ42 formation induced by exercise. This study used the TgCRND8 mouse line (Adlard et al., 2005), whereas the previous study used an amyloid precursor protein/presenilin 1 (APP/PS1) mouse model. In the Adlard et al. (2005) study, the authors overexpressed a double mutant form of the APP gene, which only incorporated the Aβ arm of AD pathogenesis, whereas the Ke et al. (2011) model expressed both Aβ and tau formation. The importance of the model employed is described below but mouse models do not express the same similarities in genome in comparison to rats as they relate to the human genome with rats being a better match to the human genome and development and progression of AD. Furthermore, the few studies that have addressed the impact exercise has on NFTs are promising as these reports show a suppression of NFTs caused by exercise (Belrabi et al., 2011; Leem et al., 2009; Um et al., 2011). These reports used models expressing NFTs without the expression of Aβ plaques, which based on the Aβ hypothesis, Aβ plaques are a necessary component in the development of neurotoxic tau during human pathogenesis of AD. Regardless of the differences among these models and the human development of AD, muscle plasticity seems to play an important role in the

development of AD and future research should be directed towards identifying the mechanisms involved that lead to improved brain health.

Development of Alzheimer's Disease

The development of AD progresses with age and is typically identified by cognitive decline and memory loss. The progressive loss of cognitive function is usually identified behaviorally using tests like the Mini-Mental State Examination (MMSE). However, scientists can detect changes in cognition and brain atrophy by testing the motor control of elderly individuals because motor function is a measurable output of central and peripheral nervous systems (Allali, Annweiler, Pedrovan, Bherer, & Beauchet, 2016; Beauchet, Launay, Annweiler, & Allali, 2015; Buchman, Wilson, Boyle, Bienias, & Bennett, 2007b; Callisaya et al., 2013; Macdonald et al., 2017). As a result of declining motor control, elderly individuals have less control over their movements resulting in falls and the inability to complete normal household chores (activities of daily living, ADLs), which leads them to perform fewer physical activities further progressing the loss of muscular strength, which further exacerbates declines in motor control, falls, and ADLs. This connection provides the basis for examining the muscular strength and correlating strength to declines in cognition.

Alzheimer's Disease Leads to Declines in Muscular Strength and Motor Control

Grip strength tests are easily employed and widely used to assess muscular strength among the elderly. Recent studies have correlated muscular strength assessed by the grip strength tests with age, height, weight, cognitive ability, MCI to AD development, motor control, and mortality (Boyle et al., 2010; Buchman, Wilson, Boyle, Bienias, & Bennett, 2007a; Roberts et al., 2013; Sternäng et al., 2015; Taekema et al., 2012). The progression

from MCI to dementia is unclear; however, research indicates 88% of people with MCI and reduced memory function will develop dementia within 3 years (Olichney et al., 2008). Another study suggests that 50-70% of individuals with MCI and high Aβ loads will develop dementia within 5-7 years (Drago et al., 2011) The majority of these studies assessed grip strength and its correlation with cognitive decline and found that muscular strength is a controllable factor that can slow cognitive decline correlated to AD. That is, strength and motor function starts to decline before the onset of cognitive impairment, which may contribute to the onset of dementia. One such study performed by Boyle et al. (2010) looked at the correlation between physical frailty, MCI, and progression to AD. This study included 750 community based elderly people with no dementia or MCI from the Rush Memory and Aging project. After analyzing the data, Boyle et al. (2010) reported physical frailty, as defined by grip strength, timed walk, body composition, and fatigue showed positive correlation to age and negative correlation to education and global cognitive function (Boyle et al., 2010). Increasing frailty correlated with normal aging; however, 40% of the population who developed MCI expressed higher levels of frailty at baseline (Boyle et al., 2010). In this context, dementia enhances the rate of frailty more so than normal aging but increased frailty at baseline could be an early indicator of dementia, which is why people who developed MCI had higher baseline frailty scores than those who did not develop MCI. By eliminating the possible confounding variables, such as age, sex, and education, Boyle, et al., found that baseline frailty is correlated with an even greater risk of developing MCI, further enhancing the relationship between these two variables. This study also indicated that demographic characteristics do not impact the correlation between frailty and MCI. Development of MCI is reversible but individuals who express

persistent MCI correlated with weaker grip strength, whereas as frailty as a whole correlated with first occurrence of MCI (Boyle et al., 2010). This study also indicates higher levels of physical frailty correlated to lower global cognition and more rapid decline in global cognition as measured by MMSE (Boyle et al., 2010). There is a clear correlation between cognitive function and physical parameters such as muscle strength and motor control. However, identifying what happens first in the pathogenesis of AD is highly debated but the prevailing view is that a decline in physical ability may precede the cognitive decline observed in AD ((Alfaro-Acha et al., 2006; Buchman et al., 2007b; Gray et al., 2013).

Sternäng et al. (2015) analyzed grip strength and multiple parameters of cognitive function such as verbal ability, spatial ability, processing speed, and memory. The results of the study indicated significant unidirectional correlations between grip strength and verbal/spatial ability and memory. Whereas, the relationship between grip strength and processing speed shows a bidirectional correlation. This suggests that the decline in muscular strength impacts certain parameters of cognitive function and vice versa. Other investigations indicate that muscular strength relates to the rate of cognitive decline and motor performance (Buchman et. al, 2007a; Roberts et. al, 2013). However, Taekema et al's. (2012) findings suggest the opposite, cognitive decline impacts the decline in muscular strength. In the Taekema et al. (2012) study, the authors only used elderly individuals between 85-89 years of age, whereas Sternäng et al. (2015) investigated these parameters among individuals ranging from age 40-86 years. Sternäng et al. (2015) found that decline in strength and cognition do not significantly change until 65 years of age. If this is true, Taekema et al. (2012) subjects had 20+ years of muscular and cognitive decline,

so these findings might be only generalizable to individuals who are among the oldest of old in a population.

While it is well documented that strength and coordination decline with age, the question remains whether declines seen with AD are independent of age. The brain regions affected by aging and AD directly affect motor control with AD causing the greatest reductions in brain volume and motor control. The specific regions of the brain most impacted by AD are hippocampus, amygdala, entorhinal cortex, parietal lobe, and the basal ganglia (Beheshti, Demirel, & Alzheimer's Disease Neuroimaging Iniative 2016; Cai et. al, 2018; Davatzikos, Bhatt, Shaw, Batmanghelich, & Trojanowski, 2011; Misra, Fan, & Davatzikos, 2009; Nagumatsu et al., 2016). As information is received by a person's senses, the sensory input is distorted by alterations in these brain regions and this further distorts one's response, which is reflected by the coordination of the movement. Determining the region of the brain that is the base of all the AD-related problems is extremely difficult. However, if the first AD-associated decline is in one's motor control that would suggest the formation of AD begins in the basal ganglia. Nagamatsu et al. (2016) identified that increasing mobility correlated with little to no change in putamen volume (structure of basal ganglia) and the decline in mobility significantly correlated with this part of the basal ganglia. This study only analyzed healthy elderly individuals but Cai et al. (2018) confirmed that feeder and local connection strength in the basal ganglia is significantly lower among subjects suffering from AD compared to healthy elderly. This disruption will be further analyzed in the following sections addressing the proteins associated with neuronal connectivity and volume decline.

Both Amyloid Beta Plaque and Tau Protein Tangles Play a Significant Role in AD

There are many hypotheses to explain the pathogenesis of AD: cholinergic hypothesis, tau hypothesis, inflammation hypothesis, and amyloid (Aβ) hypothesis. The most prominent hallmark to AD is Aβ plaque formation and the progression of this pathogenesis regardless of the hypothesis relies on the APP. Furthermore, more and more studies have contributed evidence towards the Aβ hypothesis (Andrews-Zwilling et al., 2010; Hansson et al., 2007; Jin et al., 2011; Kretner et al., 2016; Roberson et al., 2007; Rapoport et al., 2002; Talantova et al., 2013; Yasojima, McGreer, & McGreer, 2001). This section will focus on explaining the mechanisms involved in the Aβ hypothesis and the progression of the AD pathogenic cascade.

The Aβ hypothesis was first described by Glenner and Wong (1984) and refined by Hardy and Selkoe (2002) in their review and is depicted in Figure 1 in the appendix. The Aβ hypothesis progresses in the following manner: missense mutations in APP and PS1 induces Aβ42 production and accumulation leading to Aβ42 oligomerization and deposition as diffuse plaques. Aβ oligomer effects synapses leading to microglial and astrocytic activation inducing synaptic and neuritic injury causing altered neuronal ionic homeostasis and oxidative injury. These changes alter kinase/phosphatase activities resulting in tau tangles and neuronal/neuritic dysfunction and cell death with transmitter deficits ultimately leading to dementia. Below will describe how each step occurs at the molecular level.

Research supports the aberrant cleavage of APP results in the production and accumulation of Aβ42 and Aβ43. Although a mutation in PS2 can cause the production of these amyloid peptides PS1 tends to have a dominant influence on the pathological cascade

(Wolfe et al., 1999). Following the PS1 pathway, as APP produces Aβ peptides the mutation in the PS1 causes an increased production of longer amyloid peptide Aβ42. However, a recent study has indicated that the loss of PS1 function increases the production of Aβ43 and is preferentially deposited in the frontal cortex and hippocampus (Kretner et al., 2016). The APP mutant pathway is caused by microduplications of the APP gene on chromosome 21, which ultimately causes overexpression of APP leading to higher Aβ load. Oligomers are created by the misfolding of Aβ42 and Aβ43 and are deposited as plaques in the brain (Bernstein et al., 2009; Klein, Krafft, & Finch, 2001; Kirkitadze, Bitan, & Teplow, 2002). Jin et al. also identified Aβ dimers having a role in the pathogenesis of AD but the relationship between dimers and plaques are unclear but show a correlation (Jin et al., 2011; McDonald, Craig, & Hong, 2010). The formation of these plaques has been identified in a variety of ways in the damage and reduction of brain tissue.

An extensive amount of research has implicated oligomer formation in the continuous astrocytic activation. Talantova et al's. (2013) study provided a clear picture for this portion of the pathological cascade. This study indicated Aβ oligomers activate α7 nicotinic acetylcholine receptors (α7nAChRs) on astrocytes, which causes a release of Ca^{2+} inducing glutamate release. Using α7nAChR null mice and α-Bgtx (α7nAChR antagonist) almost completely inhibits the glutamate release (Talantova et al., 2013). The increase in glutamate is an iconic characteristic of synaptic damage, which this study indicated is dependent on Ca^{2+} availability. Once glutamate is released, it activates extrasynaptic N-methyl-D-aspartate receptors (eNMDARs). eNMDAR mediates the increase in nitric oxide (NO), tau, hyperphosphorylated tau, and caspase-3 activity leading to synaptic damage. The region primarily impacted was the hippocampus (Talantova et al., 2013). This study

provided crucial information in the pathological process of AD by identifying the hippocampus as the primary location for these events. Additionally, by identifying the increase in NO, tau and cholinergic receptor activity as a product of Aβ oligomer stimulation implies that inflammation, tau, and cholinergic receptors have a role in AD but is a product encapsulated by the Aβ hypothesis. Additionally an increase in caspase-3 was identified, which is a key protein responsible for apoptosis indicating a pathway causing synaptic loss resulting in a decline in brain volume commonly seen in the pathogenesis of AD. Studies such as this one implicates APP dependent hyperphosphorylation of tau (Jin, et al., 2011; Talantova, et al., 2013; Puzzo, et al., 2017; Takahashi, et al., 2015) but other findings suggest the neurotoxicity of Aβ oligomers are dependent on NFT expression (Andrews-Zwilling, et al., 2010; Rapoport, et al., 2002; Roberson, et al., 2007). As some research has shown Aβ plaque formation but no AD-like symptoms and AD pathologies without plaque formation (Erten-Lyons et al., 2009).

To address the cognitive effects of Aβ plaque and tau expression Roberson et al. (2007) crossed human APP (hAPP) mice with mice expressing two, one or no endogenous tau alleles and compared these models to mice only expressing one of the three tau allele genotypes. The results of this study indicate that mice expressing hAPP/Tau+/+ perform significantly worse on all cognitive tests. Interestingly, the mice that were not crossed hAPP mice perform better on these tests indicating tau expression alone did not cause significant cognitive decline – further suggesting the pathogenesis of AD must have the expression of both Aβ and tau. The most puzzling characteristic identified in all three hAPP models was they all express similar neuritic dystrophy, yet mice expressing hAPP/Tau+/+ exhibit significantly more premature deaths. Denoting tau is downstream of plaque

formation and a decline in brain tissue but cognitive decline may be closely correlated with the expression of tau. This study did indicate tau expression causes over excitation of γ-aminobutyric acid type A (GABAA), which causes seizures and death common among AD patients. The relationship between tau and GABAA has been verified in other studies (Andrews-Zwilling et al., 2010) with the removal of tau protecting against GABAA impairment. Rapoport et al. (2002) also indicated tau is downstream of Aβ and is essential to neurite degeneration. Furthermore, tau was identified to initiate continuous activity of mitogen-activated protein kinase (MAPK) (Rapoport et al., 2002), which could provide another link between increased levels of inflammation, apoptosis by caspase-3 and oxidative stress.

Some of the aforementioned findings may not be contiguous with the human development of AD as most use rodent models, which may express parts of the pathogenesis. The following section will identify most commonly used rat models and their specificity to AD development in comparison to human AD development. As well as address the rodent model being used for this study and its specificity to the development of AD.

The Rat Model of AD, TgF344-AD Closely Mimics Human Pathology

Most of the current transgenic rat models in use develop only portions of the AD pathological cascade. The few models that express both an increased Aβ load and p-Tau only form plaques without NFTs or NFTs without plaques (Kumar, & Singh, 2015). However, the model we chose to address my hypotheses is the only model that expresses both Aβ plaque formation and NFTs.

Prior to the development of TgF344-AD, three rat models were commonly used: UKUR25, Tg6590, and PSAPPTg478/Tg1116/Tg11587 (Do Carmo & Cuello, 2013). The UKUR25 only expresses insoluble Aβ and p-tau with onset between 6 and 9m of age (Echeverria et al., 2004; Vercauteren et al., 2004). Furthermore, insoluble Aβ has been linked to neurotoxicity via hyperphosphorylated tau but does not form plaques (Echeverria et al., 2004). This suggests that the AD like pathology does not develop in an age-dependent manner because the development of the disease happens abruptly at a very young age. The problem of early pathology development is due to cellular plasticity; cells are more viable in younger models posing the possibility that cellular interactions are different among youthful models compared to aged models. This may also eliminate the age-related decline in physiological function and would not relate to the human model of AD development and progression. Not that the findings from this model are irrelevant but the scope of the findings from research using this model is very limited. By using this model, one may only speculate on the actions of p-tau and not the full progression of the AD pathogenesis involving both NFTs and plaques. The Tg6590 is another model for studying cerebrovascular dysfunction and p-tau in an age dependent manner as these pathologies develop around 15m and studies have cited some plaque formation (Folkesson et al., 2007; Kloskowska et al., 2010). Cerebrovascular dysfunction is exhibited by Aβ cerebrovascular deposits which produces toxic levels of NO and increases in p-tau leading to damaged arteries resulting in loss in brain volume (Talantova et al., 2013). In this model phosphorylated tau levels increase as age increases, whereas the previous model exhibits abrupt induction of phosphorylated tau that does not increase with age. The TgF344-AD

model captures all effects of the previously mentioned models and more in an age dependent manner.

The TgF344-AD rat model has been demonstrated to develop plaques and NFTs around 16m of age (Cohen et al., 2013) and the progression of this disease is age-dependent and most similarly mirrors human development of AD (Cohen et al., 2013; Rorabaugh et al., 2017). Very few studies have been published using this model to study AD development but the research indicates trends in memory deficits and synapse dysfunction between 6 and 16m of age (Cohen et al., 2013; Joo et al., 2017; Muñoz-Moreno et al., 2018; Smith & McMahon, 2018; Pentkowski et al., 2018; Rorabaugh et al., 2017; Tsai et al., 2014; Vorhees et al., 2018). Currently, none of the previous studies on this model have used physiological tests like grip strength or rotarod (Panlab) to determine how muscle strength and coordination are affected as AD develops in this model. Cohen et al. with the use of the Barnes Maze Task (BMT), which is a learning and memory task used to measure hippocampal function, saw a deficient trend in 6 month TgF344-AD rats. This study also showed cerebral amyloid angiopathy (CAA)-like pathology progression at 6m but no significant findings until the rat progressed to 15m of age (Cohen et al., 2013). If decrements in muscle strength and function do occur prior to significant disease development, these rats may exhibit significant losses in muscle strength and coordination between 6 and 16m of age. A few studies have used the Morris Water Maze (MWM), which measures memory to address hippocampal dysfunction much like the BMT. These studies have mixed findings, which may in part be due to the differences in sample size. Pentkowski et al. (2018) used 24 Tg rats vs 20 WT rats and found no significant findings between the groups at 4-6m of age. Whereas, Rorabaugh et al. (2017) observed significant

differences between the 6m old groups using a sample size of 6 WT and 6 Tg rats. As reported by Cohen's BMT in 6m old rats, there was a trend towards significance between groups, 21 WT and 21 Tg rats. Muñoz-Moreno et al. (2018) also found a trend towards significance using the delayed non-match-to-sample (DNMS) task at 6m with 9 WT and 9 Tg rats. Additionally, the few studies that have specifically examined the progression of Aβ plaques, NFTs, and synapse dysfunction within this model do not identify significant development of AD related symptoms until 9-16m old (Cohen et al., 2013; Joo et al., 2017; Rorabaugh et al., 2017). Both Joo et al. (2017) and Smith and McMahon (2018) have identified postsynaptic dysfunction starting at 9m but remain unchanged at 6m within this model. These findings suggest significant changes to the brain start to appear within this model at 9m yet the hallmarks (Aβ plaque and NFT) appear much later. Whether cognitive impairment appears between 6-16m is still inconclusive, which makes this time point a crucial age to determine whether cognitive function and muscular strength and function are significantly different between the cohorts.

Significant Sex Differences are Present in the Development of AD

The divergence between sex and prevalence of AD is overwhelming, 78% of AD cases are women (Alzheimer's Association, 2018). Research has identified possible sex differences across a wide array of variables from brain volume/composition differences, hormonal response, epigenetic modifications and Aβ load. The difference in the development of AD may stem from sexual differentiation and subsequent hormone development in utero.

Carroll et al. (2010) analyzed sex differences in AD development among transgenic AD adult mice and adult mice that were hormonally manipulated at the neonatal phase.

Hormonal manipulation was carried out by demasculinizing males with androgen receptor antagonist (flutamide) and defeminizing females with testosterone proprionate. The initial findings indicated females expressed significantly higher Aβ loads in the frontal cortex at both time points, whereas the males expressed no Aβ formation in this region. Furthermore, both expressed significant increases in Aβ load in the hippocampus and subiculum but at 12m females expressed significantly higher expression in these regions. Other studies using human models have suggested that sex hormones may influence circulating Aβ (Gandy et al., 2001; Rosario et al., 2011). What's more interesting about the Carroll et al. (2010) study is that males performed significantly worse than the females on a hippocampal dependent task at 6m but at 12m the females performed significantly worse than the males on the same task. Irvine et al. (2012) identified a similar interaction among humans; males performed significantly better on cognitive tests and that age and severity of dementia could not explain the differences in performance between the sexes. To identify the role of hormones on brain development Carroll et al. (2010) manipulated the hormonal state of these neonatal transgenic mice by administering an androgen receptor antagonist to males and testosterone propionate to females. At 7m of age, males showed significantly higher Aβ loads in the hippocampus and subiculum with no effect on the frontal cortex. Females expressed a significant decrease in Aβ load within the frontal cortex and increase in the hippocampus with no effect in the subiculum. The results identify a modulating effect of hormones on Aβ and brain development; furthermore, these hormones did not completely oppose the results of natural transgenic adult model suggesting that brain development due to sexual differentiation due to hormonal production begins in the embryonic stage. Based

on these results and the positive correlation between aging and AD and negative correlation between aging and hormone production suggests a hormonal impact on AD.

Nead et al. (2016) analyzed males with prostate cancer and their probability of developing AD. This report revealed individuals using androgen deprivation therapy significantly increased their risk in developing AD. Brain atrophy due to reduced sex-hormones identified in this report was also confirmed in other studies (Leranth, Petnehazy, & MacLusky, 2003; Woolley, & McEwen, 1992); one of which saw an association between reduced dihydrotestosterone and brain derived neurotrophic factor (BDNF) due to aging (Munetumo et al., 2015). BDNF has been cited to improve neuronal plasticity (Caldeira et al., 2007; Garcia-Mesa et al., 2014). The same results are seen among females who underwent hysterectomies, Phung et al. (2010) identified women were at greater risk of developing AD the younger they were when the procedure was performed. The risk of AD due to hysterectomies may be prevented by hormone treatment, as Henderson et al. (2005) saw a significant decrease in AD risk among post-menopausal females between the ages of 50-63 years old who used hormone treatment. However, the hormone treatment did not reduce the risk of AD development among women older than 64, suggesting a window of opportunity within hormone use. These reports identify that sex specific hormones can promote the preservation of brain health and Rosario et al. (2011) confirmed the difference in treatment is gender specific by analyzing the hormonal fluxes among men and women. These reports identify the impact of sex hormones on brain health although how these hormones specifically impact the rate and development of AD is still unclear. Recently Burke et al. (2018) identified that women had greater percentage of hippocampus volume relative to total brain volume compared to men. Additionally, the hippocampal volume

ratio showed a significant relationship to the risk of developing MCI and progressing to AD among women but not men (Burke et al., 2018). Among men, increases in white matter hyperintensities were associated with increased rate of progression to MCI but not AD. These studies explain why women may be more susceptible to AD and why their progression is slower. Having higher Aβ loads that may be due to hormonal differences can explain increased incidence of AD but by having more hippocampal volume provides a buffer and allows for slower progression of AD. To help further explain how hormones may impact the dichotomy of AD development researchers are narrowing the scope.

Bove et al. (2018) indicated that there is a relationship between the reproductive period and epigenetic modifications at genes in the oxidative phosphorylation (OXPHOS) pathway. Epigenetic modifications are heritable changes in gene expression absent of changes to the genomic sequence (Verhoeven, Jansen, van Dijk and Biere, 2010). Among the study participants, reproductive period was associated with epigenetic modifications in both positive and negative directions depending on the gene region to OXPHOS-related genes. Although the study did not link reproductive period with RNA expression or disease outcome, it does point to a potential relationship between hormone exposure and DNA methylation in the context of neurological morbidity and mortality. More work should be done to understand if hormonal exposures throughout the reproductive period inhibit or promote vulnerability to AD.

Purpose of the Research Study

As presented above, there is a clear relationship between exercise and brain health, in fact, numerous studies have identified exercise as an activity that can enhance cognitive function and attenuate brain atrophy. However, little is understood regarding the

mechanisms behind the protection. This is in part due to the lack of an appropriate model of AD, which may overcome with the newly developed TgF344-AD strain. This project will provide the necessary first step in understanding the timeline of dysfunction present in this strain by attempting to identify early changes in memory, motor coordination and muscular strength. This is an essential first step in the discovery of the mechanisms of exercise-induced protection against AD.

CHAPTER THREE: MATERIALS AND METHODS

Animals

One male TgF344-AD rat and two female Fischer 344 rats were purchased from the University of Southern California. Husbandry was carried out by Boise State University (BSU) vivarium. At 3 weeks of age, animals were weaned and housed two per cage on a 12-h light/12-h dark cycle (light between 11pm and 11am). Food and water were available *ad libitum* except during training and testing when they were outside of their cage. AD genotyping was identified with ear tissue sampling. The samples were processed by TransnetYX (Cordova, TN) and the results were withheld from the researchers until experiment completion, 38 total animals (AD group: 12 males, 11 females, WT group: 8 males, 7 females). Animals were weighed and assessed every 3 m for 9 m; all procedures were performed under the accordance of IACUC.

Skeletal Muscle Strength

Grip strength was assessed using the grip strength meter (Model GS3, Bioseb) every 3 m to quantify the muscular strength of the animals. The device has a sampling rate of 1000Hz allowing for the capture of short and low force peaks between 0 and 2kg. Animals were held by the base of the tail and guided towards the device until the forelimbs grasped the T-bar. The animals were gently pulled along the sensor axle until their grip was released. Each animal completed two trials and were given a break between each trial while their littermate was tested. Peak force was recorded and divided by bodyweight for statistical analysis.

Motor Coordination

Motor coordination was assessed using a Rota Rod (Model LE8305, Panlab). This model contains 4 lanes with levers under each lane, time for each lane begins upon the lever being raised. When the lever is depressed upon animal failure, the timer shows the total rotating time as the end point for that animal. The motor ramp increases from 4 to 40 rpm, in this experiment the acceleration time was adjusted to 40 rpm/120 s. The animal began the trial at 4 rpm with the rod reaching 40 rpm at 120 s. For this experiment, the speed increased by 1 rpm every 3 s starting at 4 rpm. Each animal was given three trials with a 2 min recovery between each trial; total time and maximum rpm was recorded for each trial. Coordination was assessed based on how long (s) and at what speed (rpm) the rat can stay on the Rota Rod. The best trial for each animal was used for statistical analyses.

Spatial Memory

The Morris Water Maze (MWM) procedure was used to assess the animal's spatial memory. A clear platform was placed in a cylinder pool with the water temperature at 26^{o}C (water temperature was maintained within 1 degree of this basal temperature). The platform was placed in a fixed location close to the center of the pool; animals were placed in the pool in four different quadrants. During the acquisition period, the platform was one inch above the water surface and the animals were placed 3 times in each quadrant and given 60 s to find the platform. The animal was guided to the platform if it cannot locate the platform in the allotted time. After each trial, the animals were given 10s to rest and to become familiar with the platform location. The testing period used the same placement strategy as the acquisition, however the platform was hidden. The water level was elevated one inch above the platform and condensed milk was mixed into the water. The order of

animal placement for both testing and acquisition was the same: starting at quadrant I followed by II then III (which was opposite of II) then IV. Three trials for each quadrant was recorded, if the animal did not reach the platform a score of 60s was given for that trial and the animal was guided to the platform. Twelve trials total for each animal was recorded for each time point (3, 6, and 9 m). Total time taken to complete MWM was averaged for each animal and used for statistical analysis.

Data Analysis

A repeated measures ANOVA was used to identify change over time and a mixed model 2-way ANOVA with a Bonferroni post hoc test was used to assess the effect of group x age has on the outcome measures. A one-way analysis of variance (ANOVA) was used to identify which variables contained significant findings. A Tukey post hoc test will then be performed to identify significant group differences within the groups. Linear regression and Pearson correlation was used to identify any correlations between the variables. P values less than or equal to 0.05 will be considered statistically significant.

CHAPTER FOUR: RESULTS

Results

Animals were housed with their same-gendered littermate for the whole study. All animals tolerated the testing with no noticeable adverse effects to testing. One animal developed an eye tumor at 3 m of age and another developed glaucoma at 6 m of age; however, these animals were not removed from the study following veterinarian examination. Post-acquisition period of the MWM, all animals learned the location of the platform based on their ability to recall the platforms location during the first test period.

At 3 m, 6 m and 9 m, males were significantly heavier than females as illustrated in Figure 1A ($p<.05$). Alzheimer's disease (AD) males were significantly heavier than wild type control (WT) males at 3 m (AD males: 360.3±26.3, WT males: 335.6±10.6, $p<0.05$) but no significant difference in body weight was seen between AD females and WT females (AD females: 211±12.3, WT females: 196.4±6.079, $p > 0.05$). AD males remained significantly heavier weight than WT males at 6 m (AD males: 428.6±17.8, WT males: 408.4±12.1, $p<0.05$) and at 9 m (AD males: 462.6±22.7, WT males: 441.6±14.4, $p<0.05$). AD females are significantly heavier than WT females at 6 m (AD females: 244.9±10.3, WT females: 223.4±5.2, $p<0.0001$) and 9 m (AD females: 255.4±9.3, WT females: 233±5.8, $p<0.0001$). Differences between female groups are illustrated in Figure 1B and males are illustrated in Figure 1C. There was no significant difference among body weight at any time point between the AD or WT group as a whole. Repeated measures ANOVA revealed a significant change in body weight at between 6 and 9 m among all groups

($p<0.05$). Mixed model 2-way ANOVA (group x age) identified groups WT, AD, WT male and AD male and age show significant effect for body weight at 9 m but no interaction was seen between group and age. Changes in WT and AD body weight are displayed in Table 1.

Table 1. Body Weight (g)

	3 m	6 m	9 m	Δ
WT	271±72	322±96$^{\beta}$	344±108$^{\beta}$	73
AD	289±79	341±95$^{\beta}$	364±107$^{\beta}$	75

	3 m	6 m	9 m	Δ
WT Fem	196±6	223±5$^{\beta}$	233±6$^{\beta}$	37
WT Male	336±11	408±12$^{\beta}$	442±14$^{\beta}$	106
AD Fem	211±12	245±10$^{\beta}$	255±9$^{\beta}$	44
AD Male	360±26	429±18$^{\beta}$	463±23$^{\beta}$	103

Note. β significantly different than 3 m ($p<0.05$)

Figure 1. Body Weight (g)

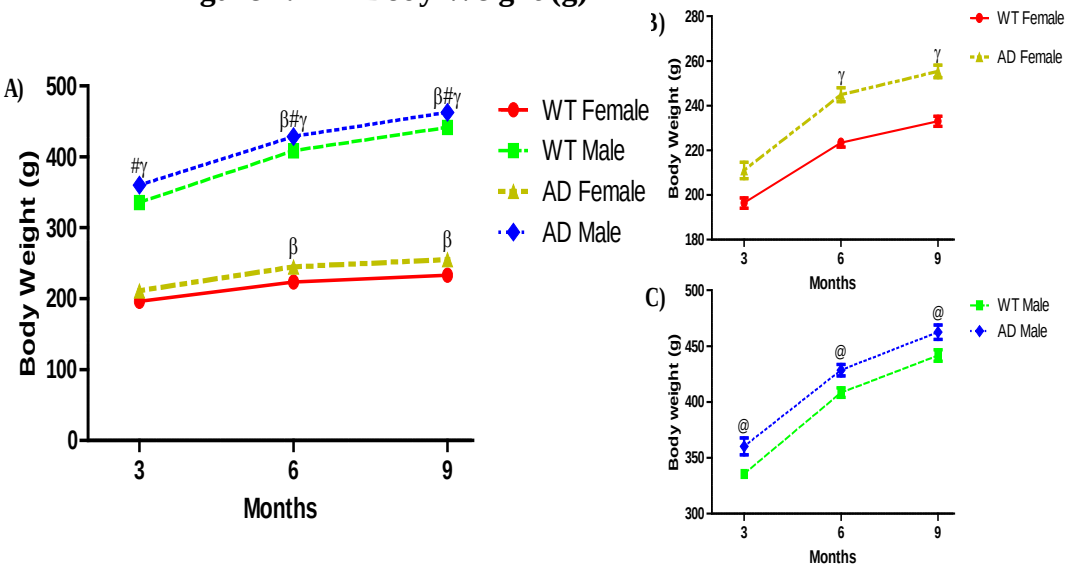

Figure 1. A) Males are significantly heavier than females at each time point and all groups begin to display significant heavier body weight at 6 m, which continues through 9 m. **B)** AD females exhibit significantly heavier body weight at 6 m and 9 m compared to WT females. **C)** AD males exhibit significantly heavier body weight at 3 m, 6 m, and 9 m compared to WT males.
β significantly different than 3 m ($p<0.05$)
significantly different than WT female ($p<0.05$)
γ significantly different AD females ($p<0.05$)
@ significantly different than WT male ($p<0.05$)

Among grip strength, WT females recorded higher peak force relative to body weight than WT males at 3 m (WT female: 3.94±0.552, WT male: 2.86±0.525, p=0.0019) and 9m (WT female: 4.71±0.59, WT male: 3.33±0.58, p=0.0005). As depicted in Figures 2A and 2B no difference was seen between these two groups at 6 m (WT female: 4.27±0.92, WT male: 3.04±1.03, p > 0.05). AD females also express significantly higher peak force relative to body weight AD males at all three time points; 3 m (AD female: 4.61±0.88, AD male: 3.11±0.77, p<0.001), 6 m (AD female: 4.28±1.03, AD male:

2.58±0.83, p<0.001), and 9 m (AD female: 4.83±0.66, AD male: 3.08±0.7, p<0.0001). The differences between AD groups are displayed in Figures 2A and 2C. There is no significant difference in grip strength between AD females and WT females or AD males and WT males at any time point. No significant differences were seen between AD and WT at any time point. The change in grip strength over time was not significant for any group, and there was no interaction between genotype or age and grip strength. Table 2 illustrates changes in grip strength over time for the WT and AD groups.

Table 2. Grip Strength

	3 m	6 m	9 m	Δ
WT	3.4±0.8	3.7±1	4±0.9	0.6
AD	3.8±1.1	3.5±1.2	3.9±1.1	0.1

	3 m	6 m	9 m	Δ
WT Fem	3.9±0.6	4.3±0.8	4.7±0.6	0.8
WT Male	2.9±0.5	3.1±0.9	3.3±0.6	0.4
AD Fem	4.6±0.9	4.3±1	4.8±0.7	0.2
AD Male	3.1±0.8	2.7±0.7	3.1±0.7	0

Note. No significant changes over time were seen among any groups

Figure 2. Grip Strength

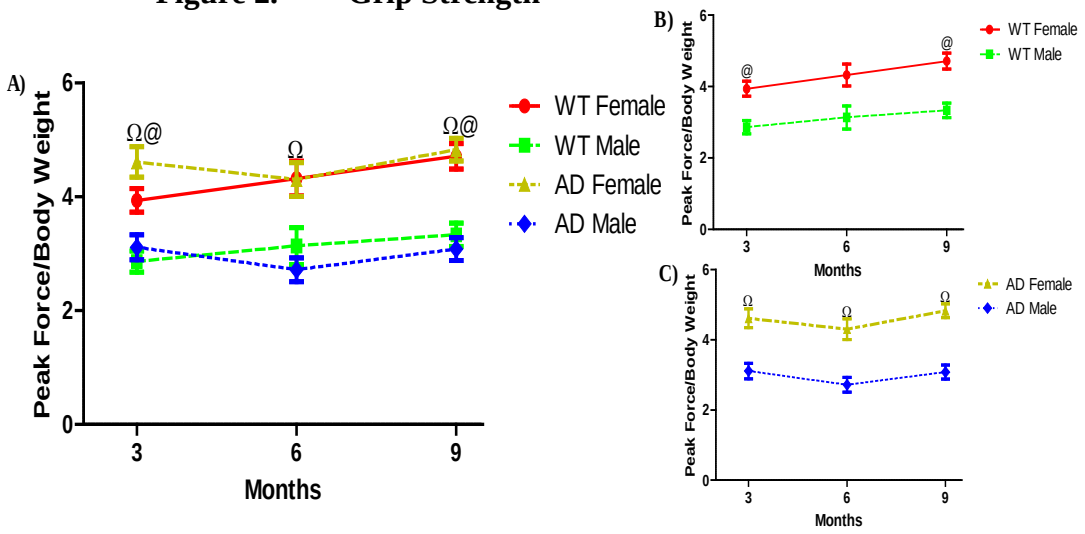

Figure 2. A) AD females express significantly higher grip strength than AD males at each time point. WT females express significantly higher grip strength than WT males at 3 m and 9 m. **B)** WT females express significantly higher grip strength than WT males at 3 m and 9m. **C)** AD females express significantly higher grip strength than AD males at each time point.
@ significantly different than WT male ($p<0.05$)
Ω significantly different than AD male ($p<0.05$)

Similar to grip strength both female groups exhibited significantly better performances on the rotarod than their male cohort, which is illustrated in Figure 3B. At 3 m WT females indicated significantly higher motor coordination than WT males (WT female: 71.7±23.5, WT male: 47.6±12.2, $p<0.05$) and remained significant at 6 m (WT female: 58.6±20.6, WT male: 33.5±9.3, $p<0.01$) and 9 m (WT female: 45±13.4, WT male: 23.6, $p<0.01$) as seen in Figure 3C. AD Females display significantly better motor coordination than AD males at 3 m (AD female: 71.2±17, AD male: 51.17±15.09, $p<0.01$) and 9 m (AD female: 35.36±12.76, AD male: 26±5.22, $p<0.01$), but at 6 m there was no significant difference in performance (AD female: 48.4±13.6, AD male: 41±8.5, $p > 0.05$). The differences between the AD genders and rotarod task is shown in Figure 3D. Repeated

measures identified significant change among AD females, AD males, WT and AD group in rotarod performance from 3 to 6 m ($p<0.01$) and all groups displayed a significant decline from 3 m to 9 m ($p<0.05$). No significant differences were found between AD vs. WT. 2-way ANOVA revealed AD females, AD group and age had a significant effect of at 6m ($p<0.01$) and all groups by 9 m ($p<0.05$). The significant decline in rotarod performance among the WT and AD groups are illustrated in Figures 3A, 3B and Table 3.

Table 3. Rotarod (s)

	3 m	6 m	9 m	Δ
WT	59±22	45±20	$34±16^{\beta}$	-25
AD	61±19	$45±12^{\beta}$	$31±11^{\beta}$	-30

	3 m	6 m	9 m	Δ
WT Fem	72±24	59±21	$45±13^{\beta}$	-27
WT Male	48±12	34±9	$24±10^{\beta}$	-24
AD Fem	71±17	$48±14^{\beta}$	$35±13^{\beta}$	-36
AD Male	51±15	$41±9^{\beta}$	$26±5^{\beta}$	-25

Note. β significantly different than 3 m ($p<0.05$)

Figure 3. RotaRod

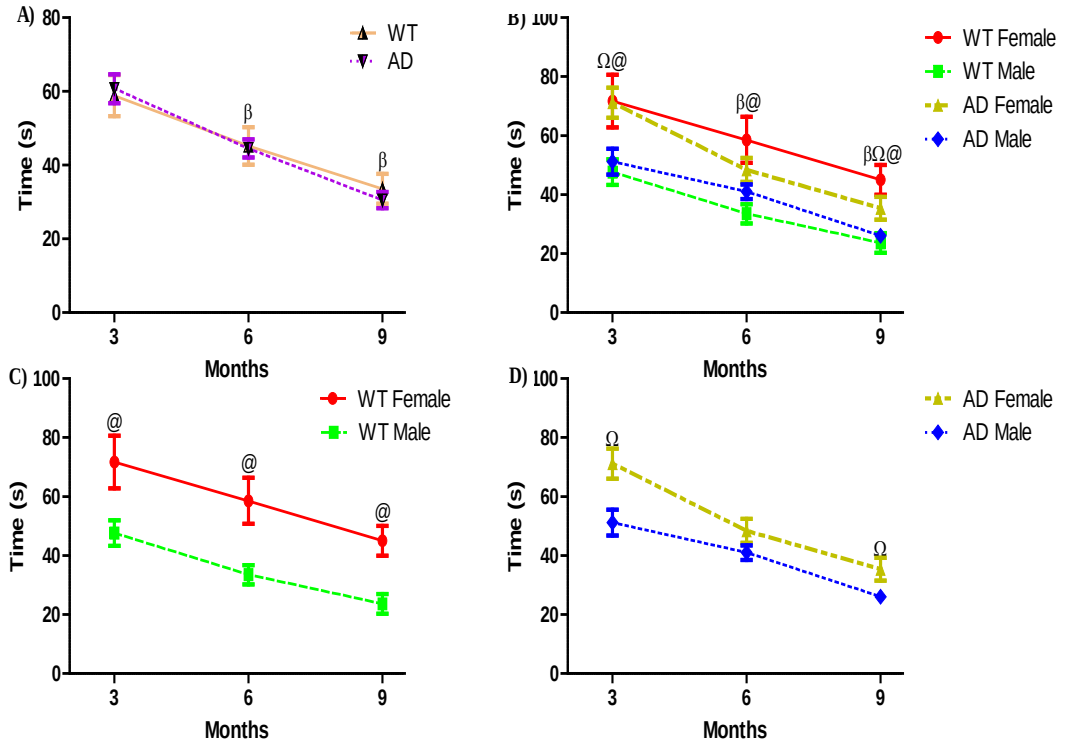

Figure 3. A) Both AD and WT group's significantly declined in rotarod performance at 6 m and 9 m. **B)** WT females display significantly better rotarod performance than WT males at each time point. AD females display significantly better rotarod performance than AD males at 3 m and 9 m. Both AD females and AD males significantly declined in rotarod performance at 6 m and all groups significantly declined in performance at 9 m. **C)** WT females display significantly better rotarod performance than WT males at each time point. **D)** AD females display significantly better rotarod performance than AD males at 3 m and 9m.
@ significantly different than WT male ($p<0.05$)
Ω significantly different than AD male ($p<0.05$)
β significantly different than 3 m ($p<0.05$)

Using the Morris Water Maze to assess memory, no group displayed significantly different performances from each other at 3 m (WT female: 17.9±12.3, AD female: 25.5±13.4, WT male: 22.5±19.5, AD male: 25.6±13.7, $p > 0.05$). At 6 m males did not display statistically different means (WT male: 22.75±14.43, AD male: 22.27±14.03, $p > 0.05$). However, Figure 4C shows WT females displayed significantly better memory function than AD females at 6 m (WT female: 15.3±10.7, AD female: 24.5±16.1, $p<0.01$.

At 9m significance between females was lost but the WT females performed significantly better than AD males (WT female: 12.2±6.4, AD male: 23.16±14.46, $p<0.0001$), whereas AD females (20.9±15) and WT males (19.2±15.8) were not significantly different. The differences between genders are illustrated in Figure 4B. There was no significant difference between WT and AD at 3 m or 6 m but by 9 m the AD group displayed significantly longer time to reach the platform than WT (AD: 22.1±14.7, WT: 15.9±12.7, $p<0.01$). The significant difference between WT and AD groups at 9 m are shown in Figure 4A. No group expressed a statistically significant change in performance from 3 m to 6 m or 9 m but these groups express negative trend over time on this task, which is displayed in Table 4.

Table 4. Morris Water Maze (s)

	3 m	6 m	9 m	Δ
WT	20±16	19±13	16±13	-4
AD	26±13	23±15	22±15	-4

	3 m	6 m	9 m	Δ
WT Fem	18±12	15±11	12±6	-6
WT Male	23±20	23±14	19±16	-4
AD Fem	26±13	25±16	21±15	-5
AD Male	26±14	22±14	23±16	-3

Note. No significant change over time were seen among any groups

Figure 4. Morris Water Maze

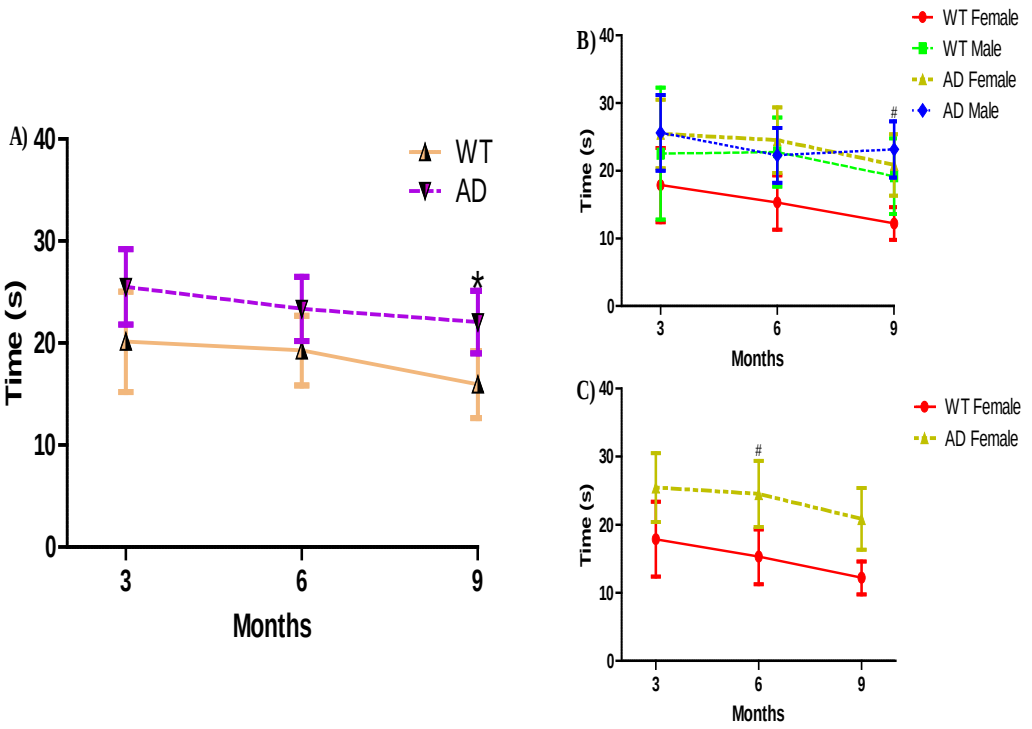

Figure 4. A) AD group displayed significantly worse performance at 9 m compared to WT group. **B)** AD males took significantly longer than WT females to find the platform at 9m. **C)** AD females took significantly longer than WT females to find the platform at 6 m.
significantly different than WT female ($p<0.05$)

After running a linear regression and Pearson correlation age and body weight exhibited strong correlation to one another and the rotarod task, which measures motor coordination. Age and body weight displayed a strong positive correlation to each other among all groups (WT female: $r = 0.9126$, $p < 0.0001$; WT male: $r = 0.9455$, $p < 0.0001$; AD female: $r = 0.8421$, $p < 0.0001$; AD male: $r = 0.8753$, $p < 0.0001$). The correlation between body weight and rotarod performance indicated a moderate but significantly negative correlation among WT females ($r = -05924$, $p<0.005$), WT males ($r = -0.7443$, $p<0.0001$), AD females ($r = -0.6865$, $p<0.0001$), and AD males ($r = -0.6375$, $p<0.0001$).

WT females were the only group to have a significant correlation between body weight and MWM ($r = -0.4631$, $p = 0.0459$), as well as, age and grip strength ($r = 0.4345$, $p<0.05$). Age and rotarod performance also displayed a moderate to high negative correlation among all groups: WT female ($r = -0.5145$, $p<0.05$), WT males ($r = -0.7068$, $p<0.0001$), AD females ($r = -0.72$, $p<0.0001$) and AD males ($r = -0.7144$, $p<0.0001$).

The AD group and WT group expressed similar correlations, body weight among both groups showed a negative correlation to grip strength (WT: $r = -0.5271$, $p<0.001$, AD: $r = -0.6769$, $p<0.0001$) and rotarod performance (WT: $r = -0.6859$, $p<0.0001$, AD: $r = -0.5110$, $p<0.0001$). Age and rotarod performance expressed a negative correlation between these two groups as well (WT: $r = -0.4864$, $p<0.001$, AD: $r = -0.6674$, $p<0.0001$) but no significant correlations were found between age grip strength.

CHAPTER FIVE: DISCUSSION

This is the first study to characterize early changes in body weight, muscular strength, motor coordination and memory within the TgF344-AD model. The major finding of this study was TgF344-AD rats displayed significantly impaired memory at 9 m compared to WT control. This indicates that memory dysfunction occurs prior to significant plaque and NFT accumulation, as previous work with this model showed no significant accumulation at 9 m and significant accumulation by 15 m (Cohen et al., 2013; Rorabaugh et al., 2017). However, because this study did not analyze AD pathology at any time, changes seen in memory, body weight, and coordination cannot be attributed to AD pathology. In addition, at 12 m animals were not yet showing signs of decreased muscular strength and coordination. These findings confirm that memory decline occurs prior to muscular strength and coordination decline, but motor coordination declines at a faster rate among rodents with AD in this study. Finally, AD may cause early weight gain among both males and females, which was an unexpected result, requiring further research.

TgF344-AD rats display memory dysfunction at 9 m. The Morris water maze was used to assess memory and the results suggest that memory dysfunction occurs before strength and motor decline among the rodents expressing AD. According to previous studies on the TgF344-AD model, memory dysfunction reported here occurs months before appreciable plaque and NFT development, which is expressed between 15-19 m (Cohen et al., 2013; Rorabaugh et al., 2017; Tsai et al., 2013). Previous literature using this task to measure memory indicates that swim speed does not differ between groups at 6 or 16 m (Rorabaugh et al., 2017), which is why swim speed was not accounted for in this report. Memory dysfunction prior to the onset of AD seen in this study is supported by literature

investigating this TgF344-AD and the human model (Muñoz-Moreno et al., 2018; Rorabaugh et al., 2017). The timeline for decline in strength and motor coordination is still unknown for this model.

Early synapse dysfunction in the locus coeruleus and hippocampus not Aβ plaques or NFTs caused the early memory dysfunction. As stated previously there is still a debate between whether physiological declines precedes AD pathology but this report indicates that memory dysfunction occurs prior to any significant physiological decline or plaque and NFT build-up (Cohen et al. 2013). Other investigations on the TgF344-AD model of AD progression have revealed significant synapse dysfunction and hyperphosphorylated tau in the locus coeruleus by 6 m and a decrease in connectivity in the hippocampus by 9 m (Rorabaugh et al., 2017; Smith & McMahon, 2018), which may cause the memory dysfunction seen at 9 m in the AD group in this study. Smith and McMahon indicated that altered synaptic activity becomes significantly present in the hippocampus of males at 9m and the females by 12 m. Although this study did not find a significant difference in memory between AD females and males at 9 m, AD males show a positive trend by 9 m, whereas the females remain negatively trending. Sex differences in memory decline are still unknown to exist in this model but may occur prior to 15 m age in the current model. Future research should analyze synapse dysfunction and other AD related pathology to elucidate the cause of memory dysfunction occurring at this early time point.

No significant sex differences were seen in memory dysfunction. Previous studies support a sex difference in memory decline, suggesting males exhibit an earlier and more drastic decline in memory and females experience a slow but steady decline (Carroll et al., 2010). This may be due to females having higher percent hippocampus volume compared

to males, which may be the result of slower progression of AD symptoms in females (Burke et al., 2018). Yet no sex differences have been revealed at these time points in memory dysfunction, which may be more present later. To elucidate sex differences that are relatable to the human model, future research should identify brain volume differences and memory decline between 6 m and 15 m among the TgF344-AD model. WT females were the only group to express a negative correlation between body weight and MWM performance. However, studies have yet to be carried out to assess the correlation between body weight and memory. Furthermore, body composition was not accounted for so inferences on lean mass or adipose tissue impacting memory cannot be made. More research is needed to assess the correlation between body weight/body composition and memory to better understand why this relationship may exist.

AD does not significantly impact early motor coordination because all AD and WT groups expressed a decline in performance on this task, which was not significantly different at any time. Motor coordination and strength share a close relationship (Sheppard, & Young, 2006), for example, if motor coordination is declining strength should be declining as well but the results indicate the opposite has occurred. The early decline in motor coordination is a result of a Fischer 344 background and using the rotarod task to assess motor coordination. Previous studies have indicated that Fischer 344 rats typically express early declines in the rotarod task prior to declines in strength (Shukkit-Hale, Mouzakis, & Joseph, 1998). This indicates that the assessment of motor coordination using the rotarod may not give a true account of Fischer 344 motor coordination abilities. Other studies have used alternative motor coordination tasks along with the rotarod task on Fischer 344 rats and found motor coordination on the alternative tasks decline later at a

slower rate and follow declines in strength (Shukkit-Hale et al., 1998). An alternate motor task or an alteration to the rotarod task should be considered when measuring motor coordination on the rodent model TgF344-AD.

Body weight may have caused the decline in motor coordination. The decline in rotarod performance shows a moderate to high negative correlation to age and body weight among all groups. Salvatore et al. (2016), study suggests that body weight may have a significant impact on rotarod performance for Fischer 344 rats as caloric restriction prevents early decline in motor coordination as measured by the rotarod (Salvatore et al. 2016). However, AD females and the AD group as a whole showed a significant decline in motor coordination by 6 m, whereas the other groups began expressing a significant decline from their baseline at 9 m. AD females showed significantly higher body weight at 6 m compared to WT females which may have resulted in the significant decline in motor coordination at 6 m. Since the AD group also indicated a significant decline at 6 m body weight differences cannot fully explain this decline.

AD may accelerate age related motor neuron cycling. The early decline in performance may be a result of AD, previous studies have seen synapse dysfunction, increases in microglia activity and hyperphosphorylated tau by 6 m, but brain atrophy caused by plaques and NFTs do not become significantly present until 15 m at in the TgF344-AD model (Cohen et al., 2013; Muñoz-Moreno et al.,2018; Rorabaugh et al., 2017; Smith, & McMahon, 2018). If this enhanced rate of motor decline is caused by AD, this may be a result of motor neuron alterations. As stated previously, AD progresses with age and age showed a negative correlation with the rotarod task. Research has shown that motor neurons cycle through denervating and reinnervating motor units causing a

restructuring of that motor unit each time which may enhance muscular atrophy (Baloh, Rakowicz, Gardner, & Pestronk, 2007; Butikofer, Zurlinden, Bollinger, Kunz, & Sonderegger, 2011; Holloszy, & Larson, 1995). Since AD is a CNS disorder and progresses with age, it is likely AD may impact motor units by enhancing motor neuron innervation cycling or by disrupting neural output to the periphery. Currently no research has investigated AD impact on motor neurons but AD does share commonalities between other brain diseases that impact motor control like Parkinson's disease (Forloni, Artuso, La Vitola, & Balducci, 2016; Maple-Grødem et al., 2018). Since AD has been implicated in increased muscle loss and early strength and motor coordination deficits (Boyle et al., 2010; Davatzikos et al., 2011), AD may impact these symptoms by altering motor neuron activity; however, no research has analyzed whether AD has an impact on motor neurons. If AD impacts motor neurons it is likely to see motor coordination deficits prior to strength deficits because muscles used for coordinated movements require more motor units per muscle volume, which means more motor neurons per fiber. Future research should investigate how AD may impact the peripheral nervous system during preclinical and clinical stages of AD, specifically in muscles used for fine motor movements.

AD did not affect muscular strength in early and mid-life animals. There was no significant difference in grip strength or change in grip strength other than both female groups displayed greater grip strength relative to body weight compared to their male cohorts. Suggesting that AD does not impact grip strength prior to memory loss among familial AD cases. These results contradict some of the literature investigating baseline strength and risk of developing of AD, which indicate lower baseline strength may be a pre-onset symptom of AD (Buchman et al., 2007a,b). However, some sources do indicate

memory loss precedes muscular based declines (Taekama et al., 2012), which is supported by the present findings. Although the literature is mixed on this topic as early declines in muscular strength may be more relatable to sporadic AD compared to familial AD because familial AD comprises only 5% of all AD cases (Alzheimer's Association, 2018) and TgF344-AD represents familial AD development and progression. Nonetheless, future research should investigate whether grip strength declines prior to significant plaque and NFT formation using this model. Identifying possible physiological changes prior to irreversible damage caused by AD may provide a window of opportunity for preventing or moderating AD.

Weight gain was similar between WT and AD groups as there was no significant difference expressed between these two groups at any time. All groups displayed significant weight gain by 6 and 9 m but this significant increase in weight may be due to sex differences because only WT males and AD males expressed a significant effect for body weight by 9 m. The significant gain in weight exhibited by males from baseline is a sex difference due to physiological differences in aging and not due to the sex differences that may exist in the early development of AD, as aging and body weight showed a strong positive correlation in the present study.

AD effects weight gain among females and males. Both AD males and females expressed significantly heavier body weight than their cohort indicating that AD may have an impact on weight gain. Midlife obesity is correlated to the risk of developing dementia and AD (Kivipelto et al., 2005). However, the present study cannot indicate whether the weight gain was due to an increase in lean mass but the results support the role AD has on weight gain when analyzing the groups separated by sex. Other studies have indicated

humans suffering from AD experience significant body weight fluctuations compared with normal aging (White, Pieper, Schader, & Fillenbaum 1996; Beydoun et al., 2008). Furthermore, research suggests that underweight males and overweight females between 30 and 50 years old are at higher risk of developing AD (Beydoun et al., 2008).The current study does not show that these weight fluctuations between the sexes occur but these this model is still in the preclinical AD stage, whereas the previous studies analyzed data from individuals during AD. Weight change due to sex may not occur in this model of AD but the results do support previous work indicating AD may cause early weight gain. Future research should analyze body composition and food intake to understand why heavier weights were seen among AD animals.

CONCLUSION

Memory dysfunction in TgF344-AD occurs at 9 m, prior to significant loss in strength and coordination compared to the WT cohort. There was no significant differences in strength or motor coordination between WT or AD groups indicating AD does not significantly impact physiological parameters prior to memory deficits. No clear sex-differences were seen in the early development of AD. However, AD males begin to show a positive trend in the memory task by 9 m, whereas the AD females and WT groups continue to show a negative trend. Additionally, females at risk for AD may express a higher rate motor coordination decline prior to clinical diagnoses. Taken together, the results confirm memory deficits occur during the preclinical phase of AD and further suggest that memory tests may be more sensitive to males, whereas motor coordination tests may be more sensitive for females. Additionally, higher body weights may be an early sign for at risk individuals.

REFERENCES

Adlard, P. A., Perreau, V. M., Pop, V., & Cotman, C. W. (2005). Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *Journal of Neuroscience*, *25*(17), 4217-4221. doi:10.1523/JNEUROSCI.0496-05.2005

Alfaro-Acha, A., Snih, S. A., Raji, M. A., Kuo, Y. F., Markides, K. S., & Ottenbacher, K. J. (2006). Handgrip strength and cognitive decline in older Mexican Americans. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *61*(8), 859-865. doi:10.1093/Gerona/61.8.859

Allali, G., Annweiler, C., Predovan, D., Bherer, L., & Beauchet, O. (2016). Brain volume changes in gait control in patients with mild cognitive impairment compared to cognitively healthy individuals; GAIT study results. *Experimental Gerontology*, *76*, 72-79. doi:10.1016/j.exger.2015.12.007

Alzheimer's Association. 2018 Alzheimer's Disease Facts and Figures. Alzheimers Dement 2018;14(3):367-429.Retrieved from: https://www.alz.org/documents_custom/2018-facts-and-figures.pdf

Andrews-Zwilling, Y., Bien-Ly, N., Xu, Q., Li, G., Bernardo, A., Yoon, S. Y., ... & Huang, Y. (2010). Apolipoprotein E4 causes age-and Tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. *Journal of Neuroscience*, *30*(41), 13707-13717. doi:10.1523/JNEUROSCI.4040-10.2010

Baker, L. D., Frank, L. L., Foster-Schubert, K., Green, P. S., Wilkinson, C. W., McTiernan, A., ... & Duncan, G. E. (2010). Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *Archives of Neurology*, *67*(1), 71-79. doi:10.1001/archneurol.2009.307

Baloh, R. H., Rakowicz, W., Gardner, R., & Pestronk, A. (2007). Frequent atrophic groups with mixed-type myofibers is distinctive to motor neuron syndromes. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 36*(1), 107-110. doi: 10.1002/mus.20755

Baumgart, M., Snyder, H. M., Carrillo, M. C., Fazio, S., Kim, H., & Johns, H. (2015). Summary of the evidence on modifiable risk factors for cognitive decline and dementia: a population-based perspective. *Alzheimer's & Dementia*, *11*(6), 718-726. doi:10.1016/j.jalz.2015.05.016

Beheshti, I., Demirel, H., & Alzheimer's Disease Neuroimaging Initiative. (2016). Feature-ranking-based Alzheimer's disease classification from structural MRI. *Magnetic Resonance Imaging*, *34*(3), 252-263. doi:10.1016/j.mri.2015.11.009

Belarbi, K., Burnouf, S., Fernandez-Gomez, F. J., Laurent, C., Lestavel, S., Figeac, M., ... & Grosjean, M. E. (2011). Beneficial effects of exercise in a transgenic mouse model of Alzheimer's disease-like Tau pathology. *Neurobiology of Disease, 43*(2), 486-494. doi:10.1016/j.nbd.2011.04.022

Bernstein, S. L., Dupuis, N. F., Lazo, N. D., Wyttenbach, T., Condron, M. M., Bitan, G., ... & Bowers, M. T. (2009). Amyloid-β protein oligomerization and the importance of tetramers and dodecamers in the aetiology of Alzheimer's disease. *Nature Chemistry, 1*(4), 326-331. doi:10.1038/nchem.247

Best, J. R., Chiu, B. K., Hsu, C. L., Nagamatsu, L. S., & Liu-Ambrose, T. (2015). Long-term effects of resistance exercise training on cognition and brain volume in older women: results from a randomized controlled trial. *Journal of the International Neuropsychological Society*, *21*(10), 745-756. doi:10.1017/S1355617715000673

Beauchet, O., Launay, C. P., Annweiler, C., & Allali, G. (2015). Hippocampal volume, early cognitive decline and gait variability: which association?. *Experimental Gerontology, 61*, 98-104. doi:10.1016/j.exger.2014.11.002

Beydoun, M. A., Lhotsky, A., Wang, Y., Forno, G. D., An, Y., Metter, E. J., ... & Zonderman, A. B. (2008). Association of adiposity status and changes in early to

mid-adulthood with incidence of Alzheimer's disease. *American Journal of Epidemiology, 168*(10), 1179-1189. doi:10.1093/aje/kwn229

Bove, R. M., Patrick, E., Aubin, C. M., Srivastava, G., Schneider, J. A., Bennett, D. A., ... & Chibnik, L. B. (2018). Reproductive period and epigenetic modifications of the oxidative phosphorylation pathway in the human prefrontal cortex. *PloS one, 13*(7), e0199073. doi:10.1371/journal.pone.0199073

Boyle, P. A., Buchman, A. S., Wilson, R. S., Leurgans, S. E., & Bennett, D. A. (2010). Physical frailty is associated with incident mild cognitive impairment in community-based older persons. *Journal of the American Geriatrics Society, 58*(2), 248-255. doi:10.1111/j.1532-5415.2009.02671.x

Buchman, A. S., Wilson, R. S., Boyle, P. A., Bienias, J. L., & Bennett, D. A. (2007a). Change in motor function and risk of mortality in older persons. *Journal of the American Geriatrics Society, 55*(1), 11-19. doi:10.1111/j.1532-5415.2006.01032.x

Buchman, A. S., Wilson, R. S., Boyle, P. A., Bienias, J. L., & Bennett, D. A. (2007b). Grip strength and the risk of incident Alzheimer's disease. *Neuroepidemiology, 29*(1-2), 66-73. doi:10.1159/000109498

Burke, S. L., Hu, T., Fava, N. M., Li, T., Rodriguez, M. J., Schuldiner, K. L., ... & Laird, A. (2018). Sex differences in the development of mild cognitive impairment and probable Alzheimer's disease as predicted by hippocampal volume or white matter hyperintensities. *Journal of Women & Aging*, 1-25. doi:10.1080/08952841.2018.1419476

Burns, J. M., Cronk, B. B., Anderson, H. S., Donnelly, J. E., Thomas, G. P., Harsha, A., ... & Swerdlow, R. H. (2008). Cardiorespiratory fitness and brain atrophy in early Alzheimer disease. *Neurology, 71*(3), 210-216. doi:10.1212/01.wnl.0000317094.86209.cb

Bütikofer, L., Zurlinden, A., Bolliger, M. F., Kunz, B., & Sonderegger, P. (2011). Destabilization of the neuromuscular junction by proteolytic cleavage of agrin

results in precocious sarcopenia. *The FASEB Journal*, *25*(12), 4378-4393. doi:10.1096/fj.11-191262

Cacciottolo, M., Christensen, A., Moser, A., Liu, J., Pike, C. J., Smith, C., ... & Charidimou, A. (2016). The APOE4 allele shows opposite sex bias in microbleeds and Alzheimer's disease of humans and mice. *Neurobiology of Aging*, *37*, 47-57. doi:10.1016/j.neurobiolaging.2015.10.010

Cai, S., Huang, K., Kang, Y., Jiang, Y., von Deneen, K. M., & Huang, L. (2018). Potential biomarkers for distinguishing people with Alzheimer's disease from cognitively intact elderly based on the rich-club hierarchical structure of white matter networks. *Neuroscience Research*. Advance online publication. doi:10.1016/j.neures.2018.07.005

Caldeira, M. V., Melo, C. V., Pereira, D. B., Carvalho, R. F., Carvalho, A. L., & Duarte, C. B. (2007). BDNF regulates the expression and traffic of NMDA receptors in cultured hippocampal neurons. *Molecular and Cellular Neuroscience*, *35*(2), 208-219. doi:10.1016/j.mcn.2007.02.019

Callisaya, M. L., Beare, R., Phan, T. G., Blizzard, L., Thrift, A. G., Chen, J., & Srikanth, V. K. (2013). Brain structural change and gait decline: a longitudinal population-based study. *Journal of the American Geriatrics Society*, *61*(7), 1074-1079. doi:10.1111/jgs.12331

Carroll, J. C., Rosario, E. R., Kreimer, S., Villamagna, A., Gentzschein, E., Stanczyk, F. Z., & Pike, C. J. (2010). Sex differences in β-amyloid accumulation in 3xTg-AD mice: role of neonatal sex steroid hormone exposure. *Brain Research*, *1366*, 233-245. doi:10.1016/j.brainres.2010.10.009

Chan, A. S., Ho, Y. C., Cheung, M. C., Albert, M. S., Chiu, H. F., & Lam, L. C. (2005). Association between mind-body and cardiovascular exercises and memory in older adults. *Journal of the American Geriatrics Society*, *53*(10), 1754-1760. doi:10.1111/j.1532-5415.2005.53513.x

Chapman, S. B., Aslan, S., Spence, J. S., Keebler, M. W., DeFina, L. F., Didehbani, N., ... & D'Esposito, M. (2016). Distinct brain and behavioral benefits from cognitive

vs. physical training: a randomized trial in aging adults. *Frontiers in Human Neuroscience*, *10*(338), 1-15. doi:10.3389/fnhum.2016.00338

Chatterjee, S., Peters, S. A., Woodward, M., Arango, S. M., Batty, G. D., Beckett, N., ... & Hassing, L. B. (2016). Type 2 diabetes as a risk factor for dementia in women compared with men: a pooled analysis of 2.3 million people comprising more than 100,000 cases of dementia. *Diabetes Care*, *39*(2), 300-307. doi:10.2337/dc15-1588

Cohen, R. M., Rezai-Zadeh, K., Weitz, T. M., Rentsendorj, A., Gate, D., Spivak, I., ... & Rakic, P. (2013). A transgenic Alzheimer rat with plaques, tau pathology, behavioral impairment, oligomeric aβ, and frank neuronal loss. *Journal of Neuroscience*, *33*(15), 6245-6256. doi:10.1523/JNEUROSCI.3672-12.2013

Davatzikos, C., Bhatt, P., Shaw, L. M., Batmanghelich, K. N., & Trojanowski, J. Q. (2011). Prediction of MCI to AD conversion, via MRI, CSF biomarkers, and pattern classification. *Neurobiology of Aging*, *32*(12), 2322.e19-2322.e27. doi:10.1016/j.neurobiolaging.2010.05.023

Do Carmo, S., & Cuello, A. C. (2013). Modeling Alzheimer's disease in transgenic rats. *Molecular Neurodegeneration*, *8*(1), 8-37. doi:10.1186/1750-1326-8-37

Drago, V., Babiloni, C., Bartrés-Faz, D., Caroli, A., Bosch, B., Hensch, T., ... & Venturi, L. (2011). Disease tracking markers for Alzheimer's disease at the prodromal (MCI) stage. *Journal of Alzheimer's Disease*, *26*(s3), 159-199. doi:10.3233/JAD-2011-0043

Echeverria, V., Ducatenzeiler, A., Dowd, E., Jänne, J., Grant, S. M., Szyf, M., ... & Hartmann, T. (2004). Altered mitogen-activated protein kinase signaling, tau hyperphosphorylation and mild spatial learning dysfunction in transgenic rats expressing the β-amyloid peptide intracellularly in hippocampal and cortical neurons. *Neuroscience*, *129*(3), 583-592. doi:10.1016/j.neuroscience.2004.07.036

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Hu, L., Morris, K. S., ... & Kramer, A. F. (2009). Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus*, *19*(10), 1030-1039. doi:10.1002/hipo.20547

Erickson, K. I., Raji, C. A., Lopez, O. L., Becker, J. T., Rosano, C., Newman, A. B., ... & Kuller, L. H. (2010). Physical activity predicts gray matter volume in late adulthood The Cardiovascular Health Study. *Neurology*, 75(16), 1415-1422. doi:10.1212/WNL.0b013e3181f88359

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., ... & Wojcicki, T. R. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences*, *108*(7), 3017-3022. doi:10.1073/pnas.1015950108

Erten-Lyons, D., Woltjer, R. L., Dodge, H., Nixon, R., Vorobik, R., Calvert, J. F., ... & Kaye, J. (2009). Factors associated with resistance to dementia despite high Alzheimer disease pathology. *Neurology*, *72*(4), 354-360. doi:10.1212/01.wnl.0000341273.18141.64

Folkesson, R., Malkiewicz, K., Kloskowska, E., Nilsson, T., Popova, E., Bogdanovic, N., ... & Benedikz, E. (2007). A transgenic rat expressing human APP with the Swedish Alzheimer's disease mutation. *Biochemical and Biophysical Research Communications*, *358*(3), 777-782. doi:10.1016/j.bbrc.2007.04.195

Forloni, G., Artuso, V., La Vitola, P., & Balducci, C. (2016). Oligomeropathies and pathogenesis of Alzheimer and Parkinson's diseases. *Movement Disorders*, *31*(6), 771-781. doi:10.1002/mds.26624

Gandy, S., Almeida, O. P., Fonte, J., Lim, D., Waterrus, A., Spry, N., ... & Martins, R. N. (2001). Chemical andropause and amyloid-β peptide. *JAMA*, *285*(17), 2195-2196. doi:10.1001/jama.285.17.2195-a

García-Mesa, Y., Pareja-Galeano, H., Bonet-Costa, V., Revilla, S., Gómez-Cabrera, M. C., Gambini, J., ... & Sanfeliu, C. (2014). Physical exercise neuroprotects ovariectomized 3xTg-AD mice through BDNF mechanisms. *Psychoneuroendocrinology*, *45*, 154-166. doi:10.1016/j.psyneuen.2014.03.021

Gauthier, S., Reisberg, B., Zaudig, M., Petersen, R. C., Ritchie, K., Broich, K., ... & Cummings, J. L. (2006). Mild cognitive impairment. *The Lancet*, *367*(9518), 1262-1270. doi:10.1016/S0140-6736(06)68542-5

Glenner, G. G., & Wong, C. W. (1984). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and Biophysical Research Communications*, *120*(3), 885-890. doi:10.1016/S0006-291X(84)80190-4

Gray, S. L., Anderson, M. L., Hubbard, R. A., LaCroix, A., Crane, P. K., McCormick, W., ... & Larson, E. B. (2013). Frailty and incident dementia. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences*, *68*(9), 1083-1090. doi:10.1093/gerona/glt013

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, *297*(5580), 353-356. doi:10.1126/science.1072994

Hansson, O., Zetterberg, H., Buchhave, P., Andreasson, U., Londos, E., Minthon, L., & Blennow, K. (2007). Prediction of Alzheimer’s disease using the CSF Aβ42/Aβ40 ratio in patients with mild cognitive impairment. *Dementia and Geriatric Cognitive Disorders*, *23*(5), 316-320. doi:10.1159/000100926

Henderson, V. W., Benke, K. S., Green, R. C., Cupples, L. A., & Farrer, L. A. (2005). Postmenopausal hormone therapy and Alzheimer’s disease risk: interaction with age. *Journal of Neurology, Neurosurgery & Psychiatry*, *76*(1), 103-105. doi:10.1136/jnnp.2003.024927

Holloszy, J. O., & Larsson, L. (1995). Motor units: remodeling in aged animals. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *50*(Special Issue), 91-95. doi:10.1093/gerona/50A.Special_Issue.91

Irvine, K., Laws, K. R., Gale, T. M., & Kondel, T. K. (2012). Greater cognitive deterioration in women than men with Alzheimer's disease: a meta-analysis. *Journal of Clinical and Experimental Neuropsychology*, *34*(9), 989-998. doi:10.1080/13803395.2012.712676

Jin, M., Shepardson, N., Yang, T., Chen, G., Walsh, D., & Selkoe, D. J. (2011). Soluble amyloid β-protein dimers isolated from Alzheimer cortex directly induce Tau

hyperphosphorylation and neuritic degeneration. *Proceedings of the National Academy of Sciences, 108*(14), 5819-5824. doi:10.1073/pnas.1017033108

Joo, I. L., Lai, A. Y., Bazzigaluppi, P., Koletar, M. M., Dorr, A., Brown, M. E., ... & Stefanovic, B. (2017). Early neurovascular dysfunction in a transgenic rat model of Alzheimer's disease. *Scientific Reports, 7*, 46427. doi:10.1038/srep46427

Ke, H. C., Huang, H. J., Liang, K. C., & Hsieh-Li, H. M. (2011). Selective improvement of cognitive function in adult and aged APP/PS1 transgenic mice by continuous non-shock treadmill exercise. *Brain Research, 1403*, 1-11. doi:10.1016/j.brainres.2011.05.056

Kirkitadze, M. D., Bitan, G., & Teplow, D. B. (2002). Paradigm shifts in Alzheimer's disease and other neurodegenerative disorders: the emerging role of oligomeric assemblies. *Journal of Neuroscience Research, 69*(5), 567-577. doi:10.1002/jnr.10328

Kivipelto, M., Ngandu, T., Fratiglioni, L., Viitanen, M., Kåreholt, I., Winblad, B., ... & Nissinen, A. (2005). Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Archives of Neurology, 62*(10), 1556-1560. doi:10.1001/archneur.62.10.1556

Klein, W. L., Krafft, G. A., & Finch, C. E. (2001). Targeting small Aβ oligomers: the solution to an Alzheimer's disease conundrum?. *Trends in Neurosciences, 24*(4), 219-224. doi:10.1016/S0166-2236(00)01749-5

Kloskowska, E., Pham, T. M., Nilsson, T., Zhu, S., Öberg, J., Codita, A., ... & Folkesson, R. (2010). Cognitive impairment in the Tg6590 transgenic rat model of Alzheimer's disease. *Journal of Cellular and Molecular Medicine, 14*(6b), 1816-1823. doi:10.1111/j.1582-4934.2009.00809.x

Kretner, B., Trambauer, J., Fukumori, A., Mielke, J., Kuhn, P. H., Kremmer, E., ... & Steiner, H. (2016). Generation and deposition of Aβ43 by the virtually inactive presenilin-1 L435F mutant contradicts the presenilin loss-of-function hypothesis of Alzheimer's disease. *EMBO Molecular Medicine,*8, 458-465. doi:10.15252/emmm.201505952

Kumar, A., & Singh, A. (2015). A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacological reports, 67*(2), 195-203. doi:10.1016/j.pharep.2014.09.004

Leem, Y. H., Lim, H. J., Shim, S. B., Cho, J. Y., Kim, B. S., & Han, P. L. (2009). Repression of tau hyperphosphorylation by chronic endurance exercise in aged transgenic mouse model of tauopathies. *Journal of Neuroscience Research*, *87*(11), 2561-2570. doi:10.1002/jnr.22075

Leranth, C., Petnehazy, O., & MacLusky, N. J. (2003). Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *Journal of Neuroscience*, *23*(5), 1588-1592. doi:10.1523/JNEUROSCI.23-05-01588.2003

Maass, A., Düzel, S., Goerke, M., Becke, A., Sobieray, U., Neumann, K., ... & Ahrens, D. (2015). Vascular hippocampal plasticity after aerobic exercise in older adults. *Molecular Psychiatry*, *20*(5), 585-593. doi:10.1038/mp.2014.114

MacDonald, S. W., Hundza, S., Love, J. A., DeCarlo, C. A., Halliday, D. W., Brewster, P. W., ... & Dixon, R. A. (2017). Concurrent indicators of gait velocity and variability are associated with 25-year cognitive change: a retrospective longitudinal investigation. *Frontiers in Aging Neuroscience*, *9*, 17. doi:10.3389/fnagi.2017.00017

Maple-Grødem, J., Chung, J., Lunde, K. A., Tzoulis, C., Tysnes, O. B., Pedersen, K. F., & Alves, G. (2018). Alzheimer disease associated variants in SORL1 accelerate dementia development in Parkinson disease. *Neuroscience Letters*, *674*, 123-126. doi:10.1016/j.neulet.2018.03.036

Matura, S., Fleckenstein, J., Deichmann, R., Engeroff, T., Füzéki, E., Hattingen, E., ... & Tesky, V. A. (2017). Effects of aerobic exercise on brain metabolism and grey matter volume in older adults: results of the randomized controlled SMART trial. *Translational Psychiatry*, *7*(7), e1172. doi:10.1038/tp.2017.135

McArdle, W. D., Katch, F. I., & Katch, V. L. (2010).Individual differences and measurement of energy capacity *Exercise physiology: Nutrition, Energy, and Human Performance* (8th ed.). Baltimore, MD: Lippincott Williams & Wilkins.

McDonald, R. J., Craig, L. A., & Hong, N. S. (2010). The etiology of age-related dementia is more complicated than we think. *Behavioral Brain Research*, *214*(1), 3-11. doi:10.1016/j.bbr.2010.05.005

Misra, C., Fan, Y., & Davatzikos, C. (2009). Baseline and longitudinal patterns of brain atrophy in MCI patients, and their use in prediction of short-term conversion to AD: results from ADNI. *Neuroimage*, *44*(4), 1415-1422. doi:10.1016/j.neuroimage.2008.10.031

Munetomo, A., Hojo, Y., Higo, S., Kato, A., Yoshida, K., Shirasawa, T., ... & Kawato, S. (2015). Aging-induced changes in sex-steroidogenic enzymes and sex-steroid receptors in the cortex, hypothalamus and cerebellum. *The Journal of Physiological Sciences*, *65*(3), 253-263. doi:10.1007/s12576-015-0363-x

Muñoz-Moreno, E., Tudela, R., López-Gil, X., & Soria, G. (2018). Early brain connectivity alterations and cognitive impairment in a rat model of Alzheimer's disease. *Alzheimer's Research & Therapy*, 10(1), 16. doi:10.1186/s13195-018-0346-2

Nagamatsu, L. S., Weinstein, A. M., Erickson, K. I., Fanning, J., Awick, E. A., Kramer, A. F., & McAuley, E. (2016). Exercise mode moderates the relationship between mobility and basal ganglia volume in healthy older adults. *Journal of the American Geriatrics Society*, *64*(1), 102-108. doi:10.1111/jgs.13882

Nead, K. T., Gaskin, G., Chester, C., Swisher-McClure, S., Dudley, J. T., Leeper, N. J., & Shah, N. H. (2016). Androgen deprivation therapy and future Alzheimer's disease risk. *Journal of Clinical Oncology*, *34*(6), 566-571. doi:10.1200/JCO.2015.63.6266

Nouchi, R., Taki, Y., Takeuchi, H., Sekiguchi, A., Hashizume, H., Nozawa, T., ... & Kawashima, R. (2014). Four weeks of combination exercise training improved executive functions, episodic memory, and processing speed in healthy elderly

people: evidence from a randomized controlled trial. *Age, 36*(2), 787-799. doi:10.1007/s11357-013-9588-x

Olichney, J. M., Taylor, J. R., Gatherwright, J., Salmon, D. P., Bressler, A. J., Kutas, M., & Iragui-Madoz, V. J. (2008). Patients with MCI and N400 or P600 abnormalities are at very high risk for conversion to dementia. *Neurology, 70*(19 Part 2), 1763-1770. doi:10.1212/01.wnl.0000281689.28759.ab

Pentkowski, N. S., Berkowitz, L. E., Thompson, S. M., Drake, E. N., Olguin, C. R., & Clark, B. J. (2018). Anxiety-like behavior as an early endophenotype in the TgF344-AD rat model of Alzheimer's disease. *Neurobiology of Aging, 61*, 169-176. doi:10.1016/j.neurobiolaging.2017.09.024

Phung, T. K. T., Waltoft, B. L., Laursen, T. M., Settnes, A., Kessing, L. V., Mortensen, P. B., & Waldemar, G. (2010). Hysterectomy, oophorectomy and risk of dementia: a nationwide historical cohort study. *Dementia and Geriatric Cognitive Disorders, 30*(1), 43-50. doi:10.1159/000314681

Pollack, M., Phaneuf, S., Dirks, A., & Leeuwenburgh, C. (2002). The role of apoptosis in the normal aging brain, skeletal muscle, and heart. *Annals of the New York Academy of Sciences, 959*(1), 93-107. doi:10.1111/j.1749-6632.2002.tb02086.x

Puzzo, D., Piacentini, R., Fa, M., Gulisano, W., Puma, D. D. L., Staniszewski, A., ... & Fraser, P. (2017). LTP and memory impairment caused by extracellular Aβ and Tau oligomers is APP-dependent. *Elife, 6*, e26991. doi:10.7554/eLife.26991

Rapoport, M., Dawson, H. N., Binder, L. I., Vitek, M. P., & Ferreira, A. (2002). Tau is essential to β-amyloid-induced neurotoxicity. *Proceedings of the National Academy of Sciences, 99*(9), 6364-6369. doi:10.1073/pnas.092136199

Roberson, E. D., Scearce-Levie, K., Palop, J. J., Yan, F., Cheng, I. H., Wu, T., ... & Mucke, L. (2007). Reducing endogenous tau ameliorates amyloid ß-induced deficits in an Alzheimer's disease mouse model. *Science, 316*(5825), 750-754. doi:10.1126/science.1141736

Roberts, H. C., Syddall, H. E., Sparkes, J., Ritchie, J., Butchart, J., Kerr, A., ... & Sayer, A. A. (2013). Grip strength and its determinants among older people in different healthcare settings. *Age and Ageing*, *43*(2), 241-246. doi:10.1093/ageing/aft118

Rorabaugh, J. M., Chalermpalanupap, T., Botz-Zapp, C. A., Fu, V. M., Lembeck, N. A., Cohen, R. M., & Weinshenker, D. (2017). Chemogenetic locus coeruleus activation restores reversal learning in a rat model of Alzheimer's disease. *Brain*, *140*(11), 3023-3038. doi:10.1093/brain/awx232

Rosario, E. R., Chang, L., Head, E. H., Stanczyk, F. Z., & Pike, C. J. (2011). Brain levels of sex steroid hormones in men and women during normal aging and in Alzheimer's disease. *Neurobiology of Aging*, *32*(4), 604-613. doi:10.1016/j.neurobiolaging.2009.04.008

Salvatore, M. F., Terrebonne, J., Fields, V., Nodurft, D., Runfalo, C., Latimer, B., & Ingram, D. K. (2016). Initiation of calorie restriction in middle-aged male rats attenuates aging-related motoric decline and bradykinesia without increased striatal dopamine. *Neurobiology of Aging*, *37*, 192-207. doi:10.1016/j.neurobiolaging.2015.10.006

Sheppard, J. M., & Young, W. B. (2006). Agility literature review: Classifications, training and testing. *Journal of Sports Sciences*, *24*(9), 919-932. doi:10.1080/02640410500457109

Shukitt-Hale, B., Mouzakis, G., & Joseph, J. A. (1998). Psychomotor and spatial memory performance in aging male Fischer 344 rats. *Experimental Gerontology*, *33*(6), 615-624. doi:10.1016/S0531-5565(98)00024-2

Smith, L. A., & McMahon, L. L. (2018). Deficits in synaptic function occur at medial perforant path-dentate granule cell synapses prior to Schaffer collateral-CA1 pyramidal cell synapses in the novel TgF344-Alzheimer's Disease Rat Model. *Neurobiology of Disease*, *110*, 166-179. doi:10.1016/j.nbd.2017.11.014

Sternäng, O., Reynolds, C. A., Finkel, D., Ernsth-Bravell, M., Pedersen, N. L., & Dahl Aslan, A. K. (2015). Grip strength and cognitive abilities: associations in old

age. *Journals of Gerontology Series B: Psychological Sciences and Social Sciences*, *71*(5), 841-848. doi:10.1093/geronb/gbv017

Taekema, D. G., Ling, C. H., Kurrle, S. E., Cameron, I. D., Meskers, C. G., Blauw, G. J., ... & Maier, A. B. (2012). Temporal relationship between handgrip strength and cognitive performance in oldest old people. *Age and Ageing*, *41*(4), 506-512. doi:10.1093/ageing/afs013

Takahashi, M., Miyata, H., Kametani, F., Nonaka, T., Akiyama, H., Hisanaga, S. I., & Hasegawa, M. (2015). Extracellular association of APP and tau fibrils induces intracellular aggregate formation of tau. *Acta Neuropathologica*, *129*(6), 895-907. doi:10.1007/s00401-015-1415-2

Talantova, M., Sanz-Blasco, S., Zhang, X., Xia, P., Akhtar, M. W., Okamoto, S. I., ... & Kang, Y. J. (2013). Aβ induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. *Proceedings of the National Academy of Sciences*, *110*(27), E2518-E2527. doi:10.1073/pnas.1306832110

Tsai, Y., Lu, B., Ljubimov, A. V., Girman, S., Ross-Cisneros, F. N., Sadun, A. A., ... & Wang, S. (2014). Ocular changes in TgF344-AD rat model of Alzheimer's disease. *Investigative Ophthalmology & Visual Science*, *55*(1), 523-534. doi:10.1167/iovs.13-12888

Um, H. S., Kang, E. B., Koo, J. H., Kim, H. T., Kim, E. J., Yang, C. H., ... & Cho, J. Y. (2011). Treadmill exercise represses neuronal cell death in an aged transgenic mouse model of Alzheimer's disease. *Neuroscience Research*, *69*(2), 161-173. doi:10.1016/j.neures.2010.10.004

Umpierre, D., Ribeiro, P. A., Kramer, C. K., Leitão, C. B., Zucatti, A. T., Azevedo, M. J., ... & Schaan, B. D. (2011). Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. *JAMA*, *305*(17), 1790-1799. doi:10.1001/jama.2011.576

Vercauteren, F. G., Clerens, S., Roy, L., Hamel, N., Arckens, L., Vandesande, F., ... & Cuello, A. C. (2004). Early dysregulation of hippocampal proteins in transgenic rats with Alzheimer's disease-linked mutations in amyloid precursor protein and

presenilin 1. *Molecular Brain Research, 132*(2), 241-259. doi:10.1016/j.molbrainres.2004.10.012

Verghese, J., Lipton, R. B., Katz, M. J., Hall, C. B., Derby, C. A., Kuslansky, G., ... & Buschke, H. (2003). Leisure activities and the risk of dementia in the elderly. *New England Journal of Medicine, 348*(25), 2508-2516. doi:10.1056/NEJMoa022252

Verhoeven, K. J., Jansen, J. J., van Dijk, P. J., & Biere, A. (2010). Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytologist, 185*(4), 1108-1118. doi:10.1056/NEJMoa022252

Voelcker-Rehage, C., Godde, B., & Staudinger, U. M. (2011). Cardiovascular and coordination training differentially improve cognitive performance and neural processing in older adults. *Frontiers in human Neuroscience, 5*(26), 1-12. doi:103389/fnhum.2011.00026

Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature Protocols, 1*(2), 848-858. doi:10.1038/nprot.2006.116

Voorhees, J. R., Remy, M. T., Cintrón-Pérez, C. J., El Rassi, E., Khan, M. Z., Dutca, L. M., ... & Pieper, A. A. (2018). (−)-P7C3-S243 Protects a Rat Model of Alzheimer's Disease From Neuropsychiatric Deficits and Neurodegeneration Without Altering Amyloid Deposition or Reactive Glia. *Biological Psychiatry, 84*(7), 488-498. doi:10.1016/j.biopsych.2017.10.023

Voss, M. W., Prakash, R. S., Erickson, K. I., Basak, C., Chaddock, L., Kim, J. S., ... & Wójcicki, T. R. (2010). Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. *Frontiers in Aging Neuroscience, 2*, 32. doi:10.3389/fnagi.2010.00032

Voss, M. W., Erickson, K. I., Prakash, R. S., Chaddock, L., Kim, J. S., Alves, H., ... & Olson, E. A. (2013). Neurobiological markers of exercise-related brain plasticity in older adults. *Brain, Behavior, and Immunity, 28*, 90-99. doi:10.1016/j.bbi.2012.10.021

White, H., Pieper, C., Schmader, K., & Fillenbaum, G. (1996). Weight change in Alzheimer's disease. *Journal of the American Geriatrics Society*, *44*(3), 265-272. doi:10.1111/j.1532-5415.1996.tb00912.x

Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., & Selkoe, D. J. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature*, *398*(6727), 513-517. doi:10.1038/19077

Woolley, C. S., & McEwen, B. S. (1992). Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. *Journal of Neuroscience*, *12*(7), 2549-2554. doi:10.1523/JNEUROSCI.12-07-02549.1992

Yasojima, K., McGeer, E. G., & McGeer, P. L. (2001). Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. *Brain Research*, *919*(1), 115-121. doi:10.1016/S0006-8993(01)03008-6

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), 426-432. doi:10.1016/j.nbd.2009.06.002

APPENDIX

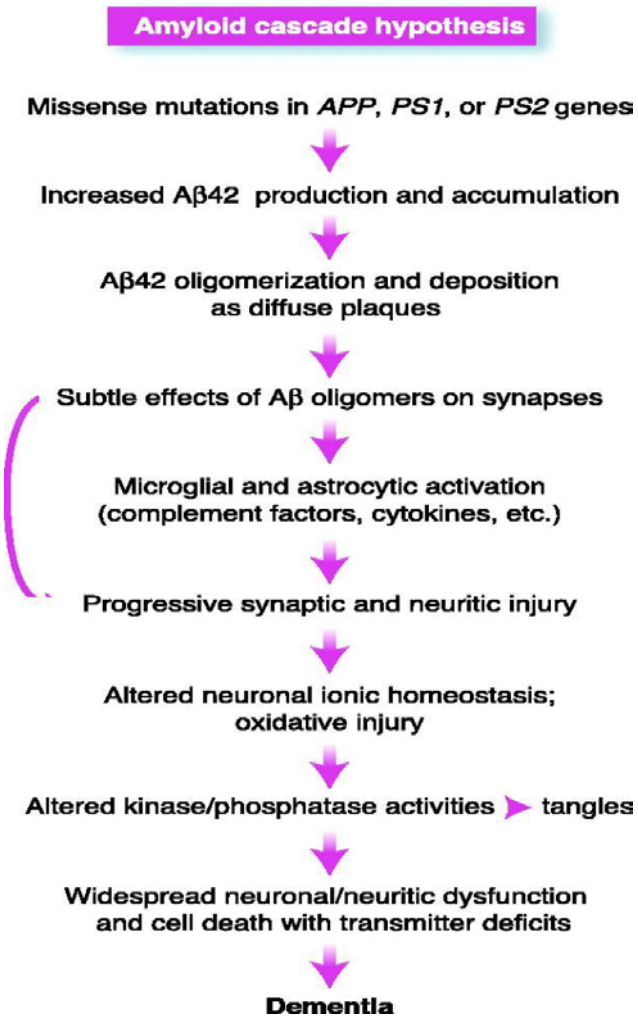

Figure A.1 Amyloid Cascade Hypothesis
Hardy & Selkoe (2002).