THE EFFECT OF ESTROGEN DEFICIENCY ON LEARNING AND MEMORY IN
TWO NOVEL RAT MODELS OF ALZHEIMER'S DISEASE

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DIANA KLAKOTSKAIA

Dr. Todd Schachtman, Dissertation Supervisor

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

THE EFFECT OF ESTROGEN DEFICIENCY ON LEARNING AND MEMORY IN
TWO NOVEL RAT MODELS OF ALZHEIMER'S DISEASE

presented by Diana Klakotskaia,

a candidate for the degree of Doctor of Philosophy,

and hereby certify that, in their opinion, it is worth of acceptance.

Professor Todd R. Schachtman

Professor David C. Geary

Associate Professor Yuksel Agca

Associate Professor Matthew J. Will

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF FIGURES	iv
LIST OF TABLES	vii
LIST OF ABBREVIATIONS	viii
ABSTRACT	ix
Chapter	
1. INTRODUCTION.....	1
Female Gender as a Risk Factor for AD	2
Characteristics of Transgenic Mouse Models of AD.....	4
Behavioral Markers of AD.....	5
Issues with Tg Mouse Models of AD	6
Rationale	8
Current Rat Models.....	9
2. EXPERIMENT 1.....	10
Materials and Methods.....	12
Results and Discussion	16
3. EXPERIMENT 2.....	26
Materials and Methods.....	28
Results and Discussion	31
4. EXPERIMENT 3.....	44
Materials and Methods.....	46
Results and Discussion	49
5. GENERAL DISCUSSION.....	58
6. REFERENCES.....	66
7. VITA	94

LIST OF FIGURES

Figure	Page
1. Figure 1, The effect of genotype on acquisition error performance in the Barnes maze during Experiment 1	17
2. Figure 2, The effect of genotype on acquisition latency performance in the Barnes maze during Experiment 1	17
3. Figure 3, The effect of genotype on overall error performance in the Barnes maze during Experiment 1	18
4. Figure 4, The effect of genotype on overall latency performance in the Barnes maze during Experiment 1	19
5. Figure 5, The effect of genotype on reversal error performance in the Barnes maze during Experiment 1	20
6. Figure 6, The effect of genotype on reversal latency performance in the Barnes maze during Experiment 1	20
7. Figure 7, The effect of genotype on time spent in open arms and time ratio in the elevated plus maze during Experiment 1	23
8. Figure 8, The effect of genotype on arm alternations and entry ratio in the elevated plus maze during Experiment 1	23
9. Figure 9, The effect of sex on time spent in open arms and time ratio in the elevated plus maze during Experiment 1	24
10. Figure 10, The effect of sex on arm alternations and entry ratio in the elevated plus maze during Experiment 1	24
11. Figure 11, Objects used in the novel object recognition task	29
12. Figure 12, The effect of genotype on object and side discrimination in the novel object recognition task during Experiment 2	32
13. Figure 13, The effect of genotype on object and side recognition indices in the novel object recognition task during Experiment 2	33
14. Figure 14, T The effect of age on object and side discrimination in the novel object recognition task during Experiment 2	34

15. Figure 15, The effect of age on object and side recognition indices in the novel object recognition task during Experiment 2	34
16. Figure 16, The effect of genotype on acquisition error performance in the Barnes maze during Experiment 2	36
17. Figure 17, The effect of genotype on acquisition latency performance in the Barnes maze during Experiment 2	37
18. Figure 18, The effect of genotype on overall error performance in the Barnes maze during Experiment 2	37
19. Figure 19, The effect of genotype on overall latency performance in the Barnes maze during Experiment 2	39
20. Figure 20, The effect of age on retention performance in the Barnes maze during Experiment 2	40
21. Figure 21, The effect of genotype on reversal error performance in the Barnes maze during Experiment 2	41
22. Figure 22, The effect of age on reversal performance in the Barnes maze during Experiment 2	42
23. Figure 23, The effect of genotype on reversal latency performance in the Barnes maze during Experiment 2	42
24. Figure 24, The effect of genotype on object and side discrimination in the novel object recognition task during Experiment 3	49
25. Figure 25, The effect of genotype on object and side recognition indices in the novel object recognition task during Experiment 3	50
26. Figure 26, The effect of estrogen on object and side discrimination in the novel object recognition task during Experiment 3	50
27. Figure 27, The effect of estrogen on object and side recognition indices in the novel object recognition task during Experiment 3	51
28. Figure 28, The interaction of genotype by estrogen on acquisition error performance over training days in the Barnes maze during Experiment 3	52
29. Figure 29, The interaction of genotype by estrogen on acquisition latency performance over training days in the Barnes maze during Experiment 3	53

30. Figure 30, The effect of genotype on overall performance in the Barnes maze during Experiment 3	53
31. Figure 31, The effect of estrogen on overall latency performance in the Barnes maze during Experiment 3	54
32. Figure 32, The interaction of genotype by estrogen on reversal error performance over training days in the Barnes maze during Experiment 3	56
33. Figure 33, The interaction of genotype by estrogen on reversal latency performance over training days in the Barnes maze during Experiment 3	56

LIST OF TABLES

Table	Page
1. Table 1, Rats used in Experiment 1	13
2. Table 2, Rats used in Experiment 2	28
3. Table 3, Rats used in Experiment 3	47

LIST OF ABBREVIATIONS

A β :	Amyloid-Beta Peptides
ACUC:	Animal Care and Use Committee
AD:	Alzheimer's Disease
ANOVA:	Analysis of Variance
APP:	Amyloid Precursor Protein
CEE:	Conjugate Equine Estrogen
CTL:	Control
ELITE:	Early Versus Late Intervention Trial with Estradiol
HRT:	Hormone Replacement Therapy
KEEPS:	Kronos Early Estrogen Prevention Study
MPA:	Medroxyprogesterone Acetate
MWM:	Morris Water Maze
nTg:	Non-Transgenic
OvX:	Ovariectomized
PS1:	Presenlin-1
PS2:	Presenlin-2
SEM:	Standard Error of the Mean
SERM:	Selective Estrogen Receptor Modulator
Tg:	Transgenic
WHIMS:	Women's Health Initiative Memory Study
WHIMS-Y:	Women's Health Initiative Memory Study of Younger Women

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Todd R. Schachtman, Dissertation Supervisor

ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disorder that results in synaptic and neuronal loss in regions of the brain responsible for memory and cognition. To date, transgenic animal models have played a crucial role in our understanding of the underlying mechanisms of the disease. In the present studies, behavioral performance was assessed in two strains of transgenic rats that overexpress human beta amyloid precursor protein. After behavioral deficits were established, females were used to assess the effect of estrogen deficiency on these deficits. Previous research suggests an increased vulnerability for females to Alzheimer disease-like pathology; while the underlying causes for this are unclear, it has been suggested that estrogens have numerous neuroprotective effects and that the loss of estrogens during aging and menopause may accelerate disease-like pathology. Both types of transgenic rats showed behavioral deficits compared to age-matched non-transgenic control rats and estrogen deficiency exacerbated behavioral symptoms reflective of Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects regions of the brain responsible for memory and cognition (Alzheimer, 1987 reprinted from 1907; Ballard & Walker, 1999). AD affects more than 5.4 million people in the United States (Reitz, 2012) and an estimated 35.6 million people worldwide (Wimo et al., 2013). The prevalence of AD is expected to rise with the anticipated increase in population life expectancy (Hebert, Scherr, Bienias, Bennett, & Evans, 2003).

AD is a growing public-health concern as there are currently no known cures to treat the disease (Reitz, 2012). Current pharmacological interventions for AD are effective at treating the symptoms of the disease, but they offer no ability to prevent or delay neurodegeneration. One hopeful approach to address neurodegeneration is to develop an appropriate animal model of the disease and to use it to test the efficacy of emerging therapies. Animal models mimic aspects of the disorder, including the pathological, physiological, or behavioral symptoms of the human disease (Sabbagh, Kinney, & Cummings, 2013 2013). The ideal model should mimic multiple aspects of the disease, including its etiology and a time-dependent progression of the pathology; it should also involve similar structures to that of the human pathology (Do Carmo & Cuello, 2013). One reason for the lack of progress towards developing a cure is that there are currently no animal models that completely capture the full spectrum of AD pathology. This introduction briefly summarizes the most successful mouse models and addresses the development of a new rat model to study AD, with a focus on elucidating the link between gender and AD.

Female Gender as a Risk Factor for AD

Although age is the most significant risk factor for the development of AD, age-related loss of sex steroid hormones is also an established risk factor in both men and women (Li & Singh, 2014; Pike, Carroll, Rosario, & Barron, 2009 & Barron, 2009; Vest & Pike, 2013). While the relationships between sex steroid hormones and AD are not fully understood, findings from both human and animal studies show that depletion of estrogen and progesterone in women and androgens in men increase the vulnerability of the aging brain to AD pathogenesis (Vest & Pike, 2013). Apart from declining levels of circulating sex steroid hormones, female gender may also be an inherent risk factor for AD. Nearly two-thirds of patients with AD are women (Gao, Hendrie, Hall, & Hui, 1998; Hebert, Weuve, Scherr, & Evans, 2013). Not only is there is a higher number of female patients with AD than male patients with AD (Andersen et al., 1999; Bachman et al., 1992; Brayne et al., 1995; Brookmeyer, Gray, & Kawas, 1998; Farrer et al., 1997), but the incidence, which refers to the number of new cases within a certain time period, of AD is also higher in women (Andersen et al., 1999; Jorm & Jolley, 1998; Miech et al., 2002; Ruitenberg, Ott, van Swieten, Hofman, & Breteler, 2001; Hofman, & Breteler, 2001). However, these differences in the prevalence and incidence rates of AD are difficult to interpret due to gender differences in life expectancy. Nonetheless, AD pathology and AD-related cognitive decline are greater in women than in men (Barnes et al., 2005; Buckwalter, Sobel, Dunn, Diz, & Henderson, 1993; Corder et al., 2004; Henderson & Buckwalter, 1994; Read et al., 2006; Ruitenberg et al., 2001; Sinforiani et al., 2010; Swaab, Chung, Kruijver, Hofman, & Ishunina, 2001). Furthermore, women with AD tend to have relatively greater difficulty with cognitive skills that are viewed as

female-advantaged, such as verbal fluency, naming, and verbal episodic memory (Henderson & Buckwalter, 1994; Ripich, Petrill, Whitehouse, & Zoil, 1995).

It has been suggested that women may be disproportionately affected by some genetic and environmental risk factors. For example, there is a stronger association of sporadic AD with the apolipoprotein E $\epsilon 4$ allele, a major genetic risk factor for AD, in women than in men. This association correlates with memory impairments and greater hippocampal and cortical atrophy (Altmann, Tian, Henderson, Greicius, & Alzheimer's Disease Neuroimaging Initiative, 2014; Damoiseaux, Prater, Miller, & Greicius, 2012; Farrer et al., 1997; Fleisher et al.; Liu et al., 2010; Payami et al., 1994), as well as higher amyloid plaque and neurofibrillary tangle burden in the brains of women than of men who are carriers of the $\epsilon 4$ allele (Corder et al., 2004). While the underlying causes for the increased vulnerability for females to AD-like pathology are unclear, Grimm, Mensah-Nyagan, and Eckert (2016) argue that there is a close relationship between age-related decreases in steroid levels and the age-related increases in oxidative stress. Mitochondrial dysfunction is suggested to underlie these deficits in brain metabolism (Long, He, Shen & Li, 2012; Nilsen, 2008; Viña & Lloret, 2010). Hence, because the loss of estrogens is most drastic during menopause, women may have a higher vulnerability to oxidative stress at this period of time, which can promote the onset of neurodegenerative disorders. Overall, research suggests that estrogens have numerous neuroprotective effects relevant to the prevention of AD and that the loss of estrogens in females during aging and the menopausal period may accelerate AD-like pathology (Pike et al., 2009).

Characteristics of Transgenic Mouse Models of AD

To date, the most successful and widely used animal models of AD have been transgenic (Tg) mice. Tg mouse models have played a crucial role in our understanding of the genetics underlying the disease. All Tg models exhibit one or more of the four physiological symptoms of the disorder seen in humans: (1) Senile amyloid plaques caused by extracellular deposits of amyloid- β peptides (A β), (2) intracellular deposits of abnormally hyperphosphorylated tau protein in the form of neurofibrillary tangles, (3) chronic neuroinflammation, and (4) widespread neuronal loss (Dickson & Vickers, 2001; Selkoe, 2001). Tg models mimic AD by overexpressing genes that have been associated with the familial form of the disease. For instance, mutations in the amyloid precursor protein gene (APP) on chromosome 21, the presenilin-1 gene (PS1) on chromosome 14, and the presenilin-2 gene (PS2) on chromosome 1, cause early-onset autosomal dominant AD (St George-Hyslop, 2000); and thus, Tg reproduction of AD pathology is based on the overexpression or transfection of mutant forms of these genes.

Historically, mice have been preferred as an animal model in this line of research mainly because the tools for mouse genome manipulation are widely available (Abbott, 2004; Tesson et al., 2005). Initial Tg mouse lines carried mutations of APP, thus the vast majority of Tg animal models of AD exhibit a massive and often rapid production and accumulation of A β peptides (Games et al., 1995; Hsiao et al., 1996; Kokjohn & Roher, 2009). Subsequent models have relied on PS mutations (Duff et al., 1996), tau mutations (Lewis et al., 2000), or a combination of two or three mutations (Holcomb et al., 1998; Lewis et al., 2001; Oddo et al., 2003). In general, mouse models bearing APP and/or PS mutations have provided valuable information about the mechanisms involved in AD and

the role of A β pathology (Sabbagh et al., 2013). A brief history of AD animal model research involving behavior is reviewed below.

Behavioral Markers of AD

Behavioral deficits in AD are caused by progressive synaptic and neuronal loss, especially in the temporal and parietal regions of the brain. One of the earliest brain regions affected in AD is the hippocampus, and as expected, impaired performance is observed on many hippocampal-dependent tasks, especially spatial learning and memory tasks. Hippocampal-dependent spatial navigation is highly conserved across mammalian species (Squire, 1992) and navigational ability is often assessed using mazes in rodents. Consistent with the AD phenotype, research using Tg mice has consistently demonstrated AD-related impairments on a variety of spatial learning measures, including the Morris water maze (Blázquez, Cañete, Tobeña, Giménez-Llort, & Fernández-Teruel, 2014; Cañete, Blázquez, Tobeña, Giménez-Llort, & Fernández-Teruel, 2015), radial arm maze (Stepanichev et al., 2004; Stewart, Cacucci, & Lever, 2011; Wright et al., 2013) and the Barnes maze (Attar et al., 2013; Stover, Campbell, Van Winssen, & Brown, 2015; Walker et al., 2011; Walker et al., 2015). The Morris water maze (MWM) is a hippocampal-dependent spatial learning and memory task that does not rely on appetitive motivation (Morris, 1984), which makes it particularly well-suited to investigate learning in animal models of AD (Billings, Green, McGaugh, & LaFerla, 2007; Hsiao et al., 1996; Kinney, Starosta, & Crawley, 2003). Similar to the MWM, the Barnes maze is also a hippocampal-dependent spatial learning task, but is considered to be less stressful as it does not involve the stress of forced swimming (Barnes, 1979; Rosenfeld & Ferguson,

2014). As such, this maze can be used with aged animals that sometimes develop locomotor difficulties. Tests assessing contextual memory are also widely used to measure hippocampal function (Dineley, Xia, Bui, Sweatt, & Zheng, 2002). Other tasks, such as the radial arm maze, novel object recognition, and passive avoidance are also useful in examining cognitive deficits consistent with AD in rodents. Several comprehensive overviews of the literature on memory and spatial learning deficits in Tg mouse models have been published (Ashe, 2001; Foley, Ammar, Lee, & Mitchell, 2015; Kobayashi & Chen, 2005; Webster, Bachstetter, Nelson, Schmitt, & Van Eldik, 2014).

Issues with Tg Mouse Models of AD

As mentioned, many mouse models of AD have been created, but none of them fully replicate the extensive cellular, biochemical, and behavioral pathology of AD patients (Kokjohn & Roher, 2009; Sabbagh et al., 2013). For example, many of the Tg APP mouse models fail to develop neurofibrillary tangles (Colton et al., 2008; Oddo et al., 2003; Padmanabhan, Levy, Dickson, & Potter, 2006; Wilcock et al., 2008). On the other hand, Tg mice with altered tau expression that develop AD-like tangles and cellular loss do not develop A β deposition (Sabbagh et al., 2013). Although more sophisticated double and triple Tg mouse models have provided insights into the interactions between A β and tau pathologies (Mastrangelo & Bowers, 2008; Oddo et al., 2003; Sabbagh et al., 2013; Stover et al., 2015; Wilcock, 2010), many still have drawbacks. In humans, for instance, high levels of neuronal A β can be present for some time, sometimes decades, before the appearance of any cognitive symptoms (Bateman et al., 2012; Price & Morris, 1999). This increased A β load does not lead to pathology until there is an additional

disturbance like stroke, hypertension, or type 2 diabetes. Not surprisingly, multiple pathologies appear to reduce the threshold at which AD changes are clinically manifest as cognitive impairment (Schneider, Arvanitakis, Bang, & Bennett, 2007). In contrast to human AD, most mouse models exhibit behavioral deficits before they develop significant plaque pathology (Beauquis, Vinuesa, Pomilio, Pavía, & Saravia, 2014; Eriksen & Janus, 2007; Games, Buttini, Kobayashi, Schenk, & Seubert, 2006; Sterniczuk, Antle, Laferla, & Dyck, 2010; Wright et al., 2013), which fails to accurately mimic the disease course of human AD. To further complicate the issue, there is evidence that neither plaques nor tangles are the proximate cause of neuronal dysfunction; instead, they may reflect the fundamental processes that accelerate synapse loss and eventually lead to neuronal death. In fact, A β load often does not correlate with cognitive impairment (Arriagada, Growdon, Hedley-Whyte, & Hyman, 1992; Berg, McKeel, Miller, Baty, & Morris, 1993; Foley et al., 2015); instead, cognitive impairment is more strongly related to excessive synapse loss (Terry, 2006), an AD pathology many current mouse models (e.g., PDAPP, Tg2576) lack despite having extensive amyloid deposition (Irizarry et al., 1997; Irizarry, Soriano, & McNamara, 1997). It seems as though neuronal loss can be induced in mice only by the combination of multiple mutations that individually are sufficient to cause the disease in humans (Casas et al., 2004; Schmitz et al., 2004; Oakley et al., 2006).

Even more importantly, however, cognitive deficits in Tg mice tend to be mild and resemble the cognitive deficits of prodromal, or early stage, AD more than of AD dementia. Additionally, cognitive assessment in mice can be quite challenging. Learning is slow in mice (Abbott, 2004) and their behavior is often variable and thus, may not

always be indicative of their true abilities. Behavioral performance underlies cognitive assessment in animals, and the fact that performance does not always reflect acquired knowledge makes it difficult to accurately determine the effects of interventions on cognition, a major objective of this field of research.

Rationale

The failure of mouse models to capture all the major features of the disorder highlights the necessity to develop superior models of the AD pathology. Now that genome manipulation technologies have been developed for non-mouse species, it may be time to consider other animals as possible models of the disease, which may allow for new evidence regarding underlying pathology.

Similar to Tg mouse models, Tg rat models are also based on the overexpression or transfection of mutant APP and PS1 genes. Moreover, rat models offer numerous advantages over mouse models. Rats are physiologically, genetically, and morphologically more similar to humans than mice (Gibbs et al., 2004; Jacob & Kwitek, 2002; Lin, 1995; Yang et al., 2004). Additionally, rats' larger bodies and brain size facilitate many experimental procedures including neurosurgery and neuroimaging (Tesson et al., 2005). More importantly, rat behavior is far better characterized. Rats have finer and more accurate motor coordination than mice and exhibit more complex learning and social behaviors (Do Carmo & Cuellar, 2013; Iannaccone & Jacob, 2009). Consequently, rat models of AD allow for a more innovative and accurate assessment of the impact of pathology and novel therapies on cognition and behavior. As a result, rats are increasingly being used to mimic neurodegenerative pathologies including

Parkinson's (Inden et al., 2011; Nuber et al., 2013; Taravini et al., 2011), Huntington's (von Hörsten et al., 2003), and even AD (Do Carmo & Cuello, 2013). However, although rat models may more accurately generate the physiological characteristics of AD, an open question in the published literature is whether Tg rat models will also display the appropriate behavioral deficits of AD.

Current Rat Models

In an attempt to generate a more accurate model of AD, Agca and colleagues (2008) have created a novel line of Tg rats that overexpress human APP with Swedish and Indiana mutations, which are genetic variations that have been linked to cause early onset AD in humans. Similar to human AD, this Tg line does not always develop significant pathology unless there is an additional disturbance (Rosen et al., 2012). To this extent, although this Tg line produces high levels of neuronal APP, it fails to produce A β plaques. In order to increase AD pathogenesis to more accurately model human AD, the APP line was used to generate a double Tg line that overexpresses both the APP as well as the PS1 transgene. To characterize these new rat models' behavioral resemblance to AD pathology, both models of Tg rats underwent behavioral assessment in the present series of experiments. Once characterized, these models were used to further investigate the interaction of gender and AD.

EXPERIMENT 1

The purpose of Experiment 1 was to examine the spatial learning and memory deficits and anxiety levels associative with two novel rat models of AD. To assess spatial learning and memory, the Barnes maze was used (Barnes, 1979). This maze takes advantage of rodents' natural aversion to bright open spaces and tendency to spend more time in darker enclosed areas. More specifically, animals escape from a mildly aversive brightly lit circular platform surface to a small, dark recessed chamber located under one of 20 holes around the perimeter of the platform. Latency to locate the escape chamber is measured on each trial during each testing session. The Barnes maze procedure is able to tap into three distinct aspects of spatial learning and memory. First, animals must learn the initial location of escape box and improved performance with each trial is indicative of normal learning and memory. After learning the initial location of the escape chamber, the animals can be given a retention interval after which memory for the location can again be measured. Lastly, the animals can also be given reversal training in which the location of the escape chamber is moved and the rate of learning of the new location can again be measured.

The Barnes maze is a hippocampal-dependent task that been shown to be sensitive to both hippocampal damage and to cognitive decline in AD rodents. Previous research shows that Tg animals display poor spatial learning in the Barnes maze task compared to their wild-type littermates in a variety of AD mouse models, including TgCRND8 (Walker et al., 2011), 3xTg (Attar et al., 2013), and APPswe/PS1 Δ E9 (O'Leary & Brown, 2009; Reiserer, Harrison, Syverud, & McDonald, 2007). Although very few studies have investigated sex differences in cognitively normal rats in the Barnes maze, findings

suggest no sex differences in performance on this task (Bucci, Chiba, & Gallagher, 1995). However, there is evidence that sex hormone levels can influence spatial learning performance, such that females perform better during proestrus when estrogen levels are high (Warren & Juraska, 1997). Nonetheless, Tg female mice are reported to show poorer behavioral performance than Tg males (Carroll et al., 2010; Clinton et al., 2007; King et al., 1999; Pistell, Zhu, & Ingram, 2008). Furthermore, Tg mouse models of AD also exhibit sex differences in AD-like neuropathology; most commonly, female mice show earlier and more robust A β accumulation than males (Andersen et al., 1999; Callahan et al., 2001; Carroll et al., 2010; Halford & Russell, 2009; Hirata-Fukae et al., 2008; Lee, Cole, Palmiter, Suh, & Koh, 2002; Sturchler-Pierrat & Staufenbiel, 2000). The observance of greater behavioral impairment and pathology in females across multiple Tg mouse models suggest that females are inherently more vulnerable than males to AD pathogenesis (Vest & Pike, 2013). Thus, in line with previous research, it was predicted that both AD rat models (APP and APP+PS1) would show spatial learning and memory deficits compared to age-matched non-Tg (nTg) controls, and that double Tg (APP+PS1) rats would show the most severe impairments. Additionally, it was predicted that larger memory impairments would be observed in the female Tg rats compared to the male Tg rats.

A speed-accuracy trade off in Barnes maze performance is one potential problem that can be difficult to control. Animals that are presumably anxious may complete the task more quickly (enter the escape box) but make a larger number of errors while doing so. To check whether such a speed-accuracy trade off occurs in the Barnes maze behavioral data, an additional test, the elevated plus maze, was administered as a measure

to assess anxiety levels (Handley & Mithani, 1984; Pellow, Chopin, File, & Briley, 1985; Walf & Frye, 2007). This commonly used task consists of two enclosed arms and two open arms arranged in the shape of a plus. Similar to the Barnes maze, the elevated plus maze also takes advantage of rodents' natural aversion to open spaces and animals that spend less time in the open arms relative to the enclosed arms are said to have higher levels of anxiety. The anxiety test was exploratory in nature and no predictions were made because the literature on anxiety disturbances in Tg mice has been mixed. For example, many studies have found evidence for increased anxiety-like behaviors and hyperactivity in Tg animals (Beauquis et al., 2014; Blázquez et al., 2014; Pietropaolo, Feldon, & Yee, 2014; Sterniczuk et al., 2010), while others have found evidence for anxiolytic-like behaviors (Lok et al., 2013) and reduced exploratory behavior (Hsiao et al., 1995; Moechars et al., 1999). Several reviews of similar findings have been published (Ashe, 2001; Webster et al., 2014). Although the relationship between anxiety levels and AD is not entirely clear, this behavioral assessment was included to ensure that any observed spatial learning and memory performance differences were not caused by differences in anxiety levels given the aversive motivational nature of the Barnes maze.

Materials and Methods

Animals. Male and female APP+PS1 ($n = 12$), APP ($n = 11$), and nTg Fischer 344 ($n = 12$) rats aged between 8 and 12.5 months (mean = 9.8) were used (see Table 1). Tg rats were bred as previously described (Agca et al., 2008) and Fischer 344 controls were bred from parents that were obtained from Harlan (Indianapolis, IN, USA). All

procedures were performed in accordance with the University of Missouri's Animal Care and Use Committee (ACUC) guidelines and the ILAR Guide for the Care and Use of Laboratory Animals. The rats were housed in pairs in conventional cages at 20–25°C in a controlled lighting environment and provided *ad libitum* access to water and standard pelleted rodent chow.

	Gender	
	Number of Males	Number of Females
Genotype		
APP+PS1	$n = 6$	$n = 6$
APP	$n = 6$	$n = 5$
nTg (CTL)	$n = 6$	$n = 6$

Table 1. Rats used in Experiment 1. A total of 35 rats were used.

Behavioral Assessment. Each rat was handled at least four times prior to behavioral treatments to reduce anxiety due to handling during testing. On each day that behavioral testing occurred, rats were transported to the testing room from the colony room in their home cages and acclimated in the testing room for a minimum of 30 min prior to the first trial. All animals were examined in the Barnes maze first, followed by the elevated plus maze.

Barnes Maze. The maze consists of a grey circular platform 122 cm in diameter, surrounded by a wall that was 30.5 cm in height. The maze was elevated 83.8 cm above the floor by a stand. Twenty holes measuring 10.2 cm in diameter were evenly spaced around the perimeter. A rectangular grey escape box (28 cm in length x 12.7 cm wide x

7.6 cm deep at the area closest to the floor of the maze tapering to 16.5 cm deep) could be placed beneath any hole. The escape box included an entry ramp that provided easy entry access for the rat. A black curtain was hung around the maze to surround the apparatus and ensure that rats only used the visual cues provided in the maze, instead of distal cues within the testing room, to navigate around the maze. Proximal cues were more likely to remain constant, within subjects and across subjects, during the course of training. Four visual cues consisting of various shapes (triangle, square, circle, cross) were placed at evenly spaced intervals on the inside of the maze walls. Two 86-W, 120-V floodlights producing 1690 lumens were hung above the platform to brightly light the maze in order to create a potentially aversive environment to help motivate the rats to escape from the brightly lit, open surface in favor of the dark environment of the escape box. One light was hung 68.5 cm on one side of the maze, while the other was hung 137 cm from the other side.

Each rat was assigned an escape hole; assigned holes were alternated across rats to eliminate odor cues for consecutively tested rats. The escape box location remained constant for any individual rat across test trials. Behavioral testing consisted of 2 shaping trials (2 trials/day) and 6 acquisition trials (2 trials/day) over a period of 3 days. Shaping trials were performed exactly like the acquisition trials except that the data were not used in the statistical analysis. Before shaping trials, each rat was pre-exposed to the goal box for 90 sec. The entrance of the goal box was covered with the grey, opaque start box (a 23 cm x 23 cm box) to prevent escape from the goal box into the maze.

An acquisition trial began by placing the rat under the start box positioned in the center of the platform. After 30 sec, the box was lifted and the rat had a maximum of 5 min (300 sec) to find and enter the escape box. Live observers recorded latency (time it

takes for the rat to enter the escape box) and total errors (nose-pokes into non-escape holes). If the rat did not enter the escape box within 5 min, it was gently guided there by the experimenter's hand. After 30 sec, the rat was removed from the escape box and returned to its home cage. The platform and escape box were cleaned after every trial with a 20% ethanol solution. Rats were then allowed to rest in their home cage in the testing room for 30 min before starting their second daily trial. After the third day, testing abated for 3 days, after which retention was evaluated with two additional trials (1 day), conducted exactly like those just described. The following day, the rats were given reversal training, in which the rats were given trials identical to those described above, except that the escape box was moved to a new location in the opposite quadrant. Reversal training continued for a total of three days.

Elevated Plus Maze. The elevated plus maze was made of grey-painted wood. The square center area of the maze, which could lead to any of the four arms, measured 14 cm by 14 cm. Each of the four arms was 43.2 long from the center area. The two enclosed arms had sidewalls and an endwall that were 20 cm in height; the open arms had sidewalls that were 3.75 cm high and there was no wall at the end of these arms. The maze was elevated 57.2 above the floor. Each rat was placed in the central platform of the maze, and allowed to freely explore for 10 min. Time spent in the open arms, time spent in closed arms, number of entries into open arms, and number of entries into closed arms was recorded. After each trial, the maze was cleaned with 20% ethanol solution.

Data Analysis. Behavioral analyses were conducted using analysis of variance (ANOVA) in SPSS (SPSS 21, SPSS Inc., Chicago USA). For the Barnes maze, the average of each animal's performance scores each day was used in the repeated-measures analysis. Day was used as the within-subjects factor and Genotype and Sex were used as between-group factors. Data from the first acquisition day were not included in the analysis. For the elevated plus maze, Genotype and Sex were used as the between-group factors. Differences were considered significant at $p < 0.05$ for all analyses. Multiple comparisons were conducted using Tukey HSD tests and Greenhouse-Geisser corrections were used for violations in sphericity.

Results and Discussion

Barnes Maze.

Acquisition. Analysis of errors and latency during acquisition did not produce a significant main effect of Genotype, Sex, or Day ($ps > 0.09$); there were also no significant interactions between Day and Sex, Day and Genotype, nor three-way interaction ($ps > 0.08$). Overall, these results suggest that the groups did not differ in learning during the acquisition trials (see Fig. 1 for errors and Fig. 2 for latency). Although it could be argued that no learning occurred during this phase as there was no main effect of Day, we know this is not the case because the animals made fewer errors and had shorter latencies during the acquisition test days than on the shaping day, as shown in Figures 1 and 2, respectively.

Figure 1. The effect of genotype on acquisition error performance in the Barnes maze during Experiment 1. All values are expressed as daily group means $\pm$ standard error of the mean (SEM). There were no significant main effects or interactions during acquisition; however, overall error performance on the acquisition training trials was qualitatively better than performance during the prior shaping trials.

Figure 2. The effect of genotype on acquisition latency performance in the Barnes maze during Experiment 1. All values are expressed as daily group means $\pm$ SEM. There were no significant main effects or interactions during acquisition; however, latency performance on the acquisition training trials was qualitatively better than performance during the prior shaping trials for each group.

Retention. Analysis of errors during the probe trials following the retention interval revealed no significant main effect of Genotype or Sex nor an interaction ($ps > 0.18$), as shown in Figure 3. Analysis of latencies during this phase produced a significant main effect of Genotype ($p < 0.04$), but no main effect of Sex or an interaction ($ps > 0.22$). Specific comparisons revealed that APP+PS1 rats had significantly longer latencies than both CTL rats ($p < 0.05$) but not APP rats ($p < 0.09$), as shown in Figure 4. APP and Control rats did not differ ($p > 0.97$) in latency scores. Thus, the memory deficit evidenced by longer latencies was larger for APP+PS1 rats than APP rats, consistent with our hypothesis.

Figure 3. The effect of genotype on overall error performance in the Barnes maze during Experiment 1. The acquisition and reversal bars represent overall group means $\pm$ SEM of all three training days, while the retention bars represent group means $\pm$ SEM of two probe trials. There were no significant main effects or interactions in error performance

during any of the three stages of testing; however, APP+PS1 rats had a non-significant tendency to make more errors than APP and CTL rats in all phases of the task.

Figure 4. The effect of genotype on overall latency performance in the Barnes maze during Experiment 1. The acquisition and reversal bars represent overall group means $\pm$ SEM of all three training days, while the retention bars represent group means $\pm$ SEM of two probe trials. There was a significant main effect of Genotype during retention, indicating that APP+PS1 rats had significantly longer latencies than Control rats ($p < 0.05$) but not APP rats ($p > 0.08$). * indicates $p < 0.05$.

Reversal. During reversal learning, analysis of errors produced no significant interactions or main effects of Sex or Genotype ($ps > 0.09$); but there was a significant main effect of Day ($p < 0.02$), suggesting that error performance differed over training days, with animals producing more errors on training day 2. Although group differences did not reach conventional levels of significance, inspection of Figure 5 reveals that APP+PS1 rats tended to show poorer error performance than APP animals ($p < 0.08$). Analysis of latencies produced no significant main effects or interactions ($ps > 0.10$), but inspection of Figure 6 suggests that APP+PS1 rats tended to show poorer latency

performance than both APP rats ($p < 0.15$) and Control rats ($p < 0.08$) as indicated by their higher latency times.

Figure 5. The effect of genotype on reversal error performance in the Barnes maze during Experiment 1. All values are expressed as daily group means $\pm$ SEM. There was no significant main effect of Genotype, but there was a main effect of Day, suggesting that animals produced more errors on training day 2 than on days 1 and 3.

Figure 6. The effect of genotype on reversal latency performance in the Barnes maze during Experiment 1. All values are expressed as daily group means $\pm$ SEM. There were no significant main effects or interactions during reversal training, but APP+PS1 rats had a tendency to have longer latencies than both APP rats ($p < 0.15$) and Control rats ($p < 0.08$).

Overall, these results support the initial hypotheses that nTg Controls would outperform Tg rats and that APP+PS1 rats would perform worse than APP rats on this behavioral task. However, statistically significant group differences were only found for one measure, which may be due to lack of power to detect significant group differences because of small sample sizes. Another factor that may have contributed to these findings stems from the age ranges examined in this study, which may not have been optimally selected; it is possible that the AD animals used in this study were too young to show large memory deficits and it is likely that older rats would show larger group differences on this behavioral task, as older rats are likely to also have increased AD-like neuropathology. Nevertheless, although not statistically significant, the trends observed in the data do correspond to the initial hypotheses and should be evaluated further.

A possible advantage of this model over previous animal models of AD is that this model may better mimic the timing of disease onset of human AD. Many mouse models develop aggressive AD-like pathology as early as 3 months of age (PDAPP: Dodart, Mathis, Saura, Bales, Paul, & Ungerer, 2000; TgCRND8: Janus et al., 2000 and Chishti et al., 2001); and as previously stated, many of these mouse models often develop behavioral impairments before the onset of wide-spread plaque development (Beauquis, Vinuesa, Pomilio, Pavía, & Saravia, 2014; Eriksen & Janus, 2007; Games, Buttini, Kobayashi, Schenk, & Seubert, 2006; Sterniczuk, Antle, Laferla, & Dyck, 2010; Wright et al., 2013). Consequently, this time course and disease progression do not correspond to human AD, in which the onset is much later in life and in which substantial plaque pathology is probably present for some time before cognitive symptoms first appear (Price & Morris, 1999). The rat models of AD used in this experiment have been shown

to develop significant AD-like pathology, including neurofibrillary tangles in the APP rats and chromatolysis in hippocampal neurons and neuronal loss in CA3 region of hippocampus in the APP+PS1 rats, when examined at 18-19 months of age (Agca, Klakotskaia, Schachtman, Chan, Lah, & Agca, 2016). However, it is currently unclear whether these neuropathologies are also present at the younger age range used in the current experiment. Previous studies with these models suggest that plaque formation in these animals is dependent on environmental challenges (Rosen et al., 2012), so it is likely that there was little to no significant AD-like neuropathology at the younger age ranges used in the current study. Larger memory deficits are expected with the onset of more severe pathology; consequently, future studies should evaluate behavior in older animals.

Elevated Plus Maze. Analysis of performance produced four measures: (1) total time spent in the open arms (Open Arms); (2) the ratio of time spent in the open arms relative to the closed arms (Time Ratio); (3) the number of arm alternations (Alternations); and (4) the ratio of entries into the open arms relative to the closed arms (Entry Ratio).

The analysis revealed no significant main effect of Genotype (Fig. 7 and 8) or interaction for any of the four measures ($p > 0.20$). There was however a significant main effect of Sex for Open Arms ($p < 0.01$), Alternations ($p < 0.001$), and Entry Ratio ($p < 0.02$), but not Time Ratio ($p > 0.76$). Females spent significantly more time in the open arms than the male rats, alternated between the open and the closed arms significantly

more frequently than male rats, and made significantly more entries into open arms relative to closed arms than males, as shown in Figures 9 and 10.

Figure 7. The effect of genotype on time spent in open arms and time ratio in the elevated plus maze during Experiment 1. All values are expressed group means $\pm$ SEM. There were no significant Genotype main effects or interactions.

Figure 8. The effect of genotype on arm alternations and entry ratio in the elevated plus maze during Experiment 1. All values are expressed group means $\pm$ SEM. There were no significant Genotype main effects or interactions.

Figure 9. The effect of sex on time spent in open arms and time ratio in the elevated plus maze during Experiment 1. All values are expressed group means $\pm$ SEM. There was a significant main effect of Sex, indicating that female rats spent significantly more time in the open arms than male rats ($p < 0.01$). There was no sex difference in the ratio of time spent in the open arms relative to the closed arms ($p > 0.76$). ** indicates $p < 0.01$.

Figure 10. The effect of sex on arm alternations and entry ratio in the elevated plus maze during Experiment 1. All values are expressed group means $\pm$ SEM. There was a significant main effect of Sex, such that females made significantly more arm alternations ($p < 0.001$) and significantly more entries into open arms relative to closed arms ($p < 0.05$) than males. * indicates $p < 0.05$ and *** indicates $p < 0.001$.

Although no predictions were made for the elevated plus maze, these results do support previous behavioral studies using nTg rats. For instance, Johnston and File (1991) also found that female rats showed a reduced aversion to the open arms of the elevated plus maze compared to male rats. However, these authors were unable to make a firm conclusion concerning sex differences in anxiety levels because the results of the different anxiety tests they used did not lead to effects in the same direction. Additionally, previous studies have also found that behavioral indices of anxiety can vary across stages of the estrous cycle in rats (Mora, Dussaubat, & Díaz-Véliz, 1996), and that anxiety levels are lower in the proestrus stage than the diestrus stage (Marcondes, Miguel, Melo, & Spadari-Bratfisch, 2001), when serum estradiol levels are higher. Although estrous cycles were not monitored in this study, it is possible that estrous stage may have influenced the results of both of these tasks. In any case, the lack of a Genotype main effect confirms that differential anxiety levels did not influence the observed spatial learning and memory performance in the Barnes Maze.

EXPERIMENT 2

In an attempt to further investigate AD in females, Experiment 2 examined spatial learning and memory and recognition memory deficits in the two novel models of AD using only female rats. Given that Experiment 1 was expected to produce a learning deficit in the Tg rats but no such deficit was observed, Experiment 2 examined whether performance will be poor in more aged rats. The Barnes maze was used to measure spatial learning and memory and similar to the previous experiment, it was predicted that both AD rat models would show spatial learning and memory deficits compared to age-matched nTg controls, and that double Tg rats (APP+PS1) would show the most severe impairments.

In addition to spatial reference memory, a different type of memory, specifically recognition memory, which has also been extensively shown to be impaired in various mouse models of AD (Alkam et al., 2008; Dodart, Mathis, & Ungerer, 1997; Greco et al., 2010; Hale & Good, 2005; Jardanhazi-Kurutz et al., 2010; Taglialatela, Hogan, Zhang, & Dineley, 2009; Yuede et al., 2009; Zhang et al., 2012), was assessed in this experiment. To assess recognition memory, the novel object recognition task was used (Ennaceur & Delacour, 1988). This test is based on the spontaneous behavior of rodents to interact more with a novel object than with a familiar one. The task consists of two phases. In the training phase, rats are allowed to explore two identical objects. In the testing phase, one of the two identical objects is replaced by a novel object. Rats that explore the novel object for a longer time period are said to have better memory of the familiar object,

whereas rats that explore the novel and familiar objects for the same amount of time, are said to have a poorer recall for the familiar object (Antunes & Biala, 2012).

The novel object recognition task has been used to detect memory deficits in many mouse models of AD, including single, double, and triple Tg models, as well as other models (see Grayson et al., 2015 for a review). However, it is important to point out that some studies have failed to find a difference in recognition memory between Tg animals and wild-type controls (Ardiles et al., 2012; Cheng, Low, Logge, Garner, & Karl, 2014; Davis, Easton, Eacott, & Gigg, 2013; Heneka et al., 2006; Huang et al., 2006; Middei, Daniele, Caprioli, Ghirardi, & Ammassari-Teule, 2006; Scullion, Kendall, Marsden, Sunter, & Pardon, 2011). The lack of deficits could be explained by the wide range of protocols used (e.g., varying intertrial intervals) and the nature of the animals studied (e.g., strain, gender, age, etc.). Despite inconsistent results in the literature across AD models, the novel object recognition task is dependent on the integrity of the temporal region in rodents (Ennaceur, Neave, & Aggleton, 1996; Mumby, Pinel, & Dastur, 1993) and requires prefrontal cortical input (Ennaceur, Neave, & Aggleton, 1997), which corresponds to the brain regions affected in human AD (Delacourte, David, Sergeant, Buee, & Wattez, 1999; Whitwell, Przybelski, Weigand, & Knopman, 2007). Thus, a good animal model of AD should ideally show deficits in the novel object recognition task. Therefore, it was predicted that both AD rat models would also show recognition memory impairments compared to age-matched nTg controls, and that double Tg rats would show the most severe impairments.

Materials and Methods

Animals. Female APP+PS1 ($n = 12$), APP ($n = 17$), and nTg Fischer 344 ($n = 17$) rats aged 12 months and 14 months were used (see Table 2). Similar to Experiment 1, Tg rats were bred as previously described (Agca et al., 2008) and Fischer 344 controls were bred from parents obtained from Harlan (Indianapolis, IN, USA). All procedures were performed in accordance with the University of Missouri's ACUC guidelines and the ILAR Guide for the Care and Use of Laboratory Animals. All unspecified details were the same as those in Experiment 1.

	Age	
	Number of 12-mo Rats	Number of 14-mo Rats
Genotype	APP+PS1	$n = 5$
	APP	$n = 7$
	nTg (CTL)	$n = 7$

Table 2. Rats used in Experiment 2. A total of 46 rats were used.

Behavioral Assessment. Each rat was handled at least four times prior to any behavioral treatment to reduce anxiety due to handling during testing. Each day on which behavioral testing occurred, rats were transported to the testing room from the colony room in their home cages and acclimated in the testing room for a minimum of 30 min prior to the first trial. All animals underwent the novel object recognition task first, followed by the Barnes maze.

Novel Object Recognition Task. Two identical copies of two pairs of objects were used. The heights of the objects averaged 11.5 ± 1.5 cm (mean $\pm$ SEM), the diameters averaged 7.5 ± 1 cm, and all objects weighed in excess of 400 g to prevent displacement. One object was made of hexagonal shapes consisting of gray plastic and the other was a small clear round vase filled with marbles, as pictured in Figure 11. The objects were placed in a 50.5 x 39.1 x 32.1 (l x w x h) cm white acrylic rectangle-shaped chamber 2.5 cm from the two short-side walls. The objects were washed with 20% ethanol solution after each exposure to a rat.

Figure 11. Objects used in the novel object recognition task. Two identical pairs of objects were used in this task. The object pictured on the left was a small clear round-shaped vase filled with marbles and the object pictured on the right was made of gray plastic and consisted of three layered hexagonal shapes.

The novel object recognition task procedure was as follows. On Day 1, the rats were placed in the chamber (without objects) for a 20-min acclimation session. Day 2 involved two sessions: A training session and a test session. During the training session, the rats were placed in the apparatus with two identical objects for 3 min. The second session, the test session, occurred 15 min after the training session. The rats spent this 15

min retention period in the home cage. The rats were then placed in the chamber for 3 additional min with one object that was used in the training session and a novel object. The amount of time spent interacting with each object, as well as, time spent in each half of the chamber were recorded. Interaction with an object was defined as the rat's head being oriented towards the object and the rat's nose being within 3 cm of the object. Trials were video recorded and scored by observers that were blind to the treatment status of the rats.

Barnes Maze. A similar Barnes maze procedure was used as in Experiment 1, with a few modifications to shorten the duration of the assessment. First, the animals were only given a maximum of 3 min (180 sec) to find and enter the escape box, rather than 5 min (300 sec). Additionally, all animals were given three trials per day, instead of two trials per day, again with 30 min intertrial intervals. Lastly, similar to Experiment 1, there was an initial shaping day (again, data from these 3 trials were not included in the analysis), which was followed by three additional days of acquisition training. However, in this experiment, the retention interval was increased from three days to 14 days to produce a greater retention effect. The retention day was followed by three reversal training days, where the location of the target box was moved to the opposite quadrant.

Data Analysis. Behavioral analyses were conducted using analysis of variance (ANOVA) in SPSS (SPSS 21, SPSS Inc., Chicago USA). For the Barnes maze, daily latency and error performance was averaged each day and used in the repeated-measures analysis. Day was used as the within-subjects factor and Genotype and Age were used as

between-group factors. For the novel object recognition task, Genotype and Age were used as between-group factors. An object discrimination index, calculated for each rat, was expressed as $(T_N - T_F)$, where T_N and T_F were the time spent during trial 2 with the novel object and familiar object, respectively; a corresponding score was calculated for side discrimination (Antunes & Biala, 2012). Additionally, an object recognition index, calculated for each rat, was expressed as the ratio $(T_N \times 100)/(T_F + T_N)$; a corresponding score was calculated for side recognition (Antunes & Biala, 2012). Differences were considered significant at $p < 0.05$ for all analyses. Multiple comparisons were conducted using Tukey HSD tests and Greenhouse-Geisser corrections were used for violations in sphericity.

Results and Discussion

Novel Object Recognition Task. There were no significant Genotype differences in object discrimination or side discrimination during the training trial ($ps > 0.05$; data not shown), which indicated that there were no differences in object or side preferences between the identical pair of objects. However, there was a significant main effect of Age in object and side discrimination during the training trial ($ps < 0.05$), suggesting that younger animals spent more time with the left object and on the left side of the chamber when given two identical items, which may have influenced the results of the subsequent test trial. Analysis of object discrimination, side discrimination, object recognition index, and side recognition index, during the test trial yielded a main effect of Genotype ($ps < 0.05$). Post hoc comparisons indicated that the APP rats had a significantly larger

preference for the new object on the test trial than the Controls ($p < 0.05$); in contrast, the Control rats had a significant preference for the old object, as shown in Figure 12. Paradoxically, these results suggest better memory in APP rats than in Control rats. APP+PS1 also tended to have a preference for the new object, but their preference was not significantly different from Controls ($p > 0.14$). Although there was a significant main effect of Genotype for side discrimination ($p < 0.05$), post hoc comparisons did not reveal any significant group differences ($ps > 0.05$), but suggested that Controls had a non-significant preference for the old side compared to both of the other groups ($ps < 0.07$).

Figure 12. The effect of genotype on object and side discrimination in the novel object recognition task during Experiment 2. All values are expressed group means $\pm$ SEM. There was a significant main effect of Genotype, indicating a significantly larger preference for the old object in the Control rats compared to the APP rats. Control rats also had a non-significant preference for the old side than both the APP and APP+PS1 animals ($ps < 0.07$). * indicates $p < 0.05$.

For the recognition indices, an index above 50% indicates a novel object/side preference, an index below 50% indicates a familiar object/side preference, and an index of 50% indicates no preference. Similar to the object and side discrimination indices,

APP rats had a significantly stronger preference for the novel object than the Controls ($p < 0.03$) according to this index, and therefore, outperformed Controls on this task, as shown in Figure 13. To explore whether differential exploratory behavior could be responsible for the differences in object discrimination, the times spent not exploring either object were also compared between the groups and no differences were seen ($p > 0.05$; data not shown). Lastly, there were no significant age differences between the 12-month old and 14-month old animals (Figs. 14 and 15) or an interaction between Genotype and Age for any of the measures ($ps > 0.18$).

Figure 13. The effect of genotype on object and side recognition indices in the novel object recognition task during Experiment 2. All values are expressed group means $\pm$ SEM. APP rats had a significantly stronger preference for the novel object than the Control rats. Although there were no significant group differences in side recognition, Control rats showed a non-significant preference for the familiar side. * indicates $p < 0.05$.

Figure 14. The effect of age on object and side discrimination in the novel object recognition task during Experiment 2. All values are expressed group means $\pm$ SEM. There were no significant group differences.

Figure 15. The effect of age on object and side recognition indices in the novel object recognition task during Experiment 2. All values are expressed group means $\pm$ SEM. There were no significant group differences.

There was no evidence of poor memory using the novel object recognition task for Tg rats. In fact, unexpectedly, the APP and APP+PS1 rats exhibited behavior that would be indicative of better memory relative to Control animals. These results are surprising and do not support previous findings in the literature. Although numerous

previous studies have failed to find a difference in recognition memory between Tg animals and wild-type controls (Cheng, Low, Logge, Garner, & Karl, 2014; Davis, Easton, Eacott, & Gigg, 2013; Heneka et al., 2006; Huang et al., 2006; Middei, Daniele, Caprioli, Ghirardi, & Ammassari-Teule, 2006; Scullion, Kendall, Marsden, Sunter, & Pardon, 2011) as stated earlier, there is no evidence in the literature for a reversed effect like the one observed in this study. Although anxiety was not measured in this study, Experiment 1 found that females showed significantly less anxiety in the elevated plus maze than males, which might suggest a lack of anxiety toward novel objects in all of the animals used in this study. For example, Frick and Gresack (2003) found that male C57BL/6 mice showed a strong preference for the novel object in a similar paradigm, whereas females exhibited no preference. Such sex differences could be explained by sexual dimorphisms in a number of relevant brain regions like the neocortex, hippocampus, and septum (Baker & Kim, 2002; Clark, Zola, & Squire, 2000; Ennaceur & Aggleton, 1997). However, this explanation would suggest no group differences on any of the novel object recognition task measures, so it does not explain the significant differences observed in this study. It is also possible that the preference for the novel object in the Tg animals was due to decreased neophobia; however, it is important to note that this is inconsistent with previous studies that have found more neophobia to stimuli in AD rats than wild-type littermates (España et al., 2010; Hsiao et al., 1995; Moechars et al., 1999).

Lastly, it is also likely that these results are contingent upon the specific protocol used in this study. For example, it is possible that the 20-min acclimation session before the training trial was too long, which may have caused habituation with the chamber and

thus less exploration of either of the objects or sides on the subsequent trials the following day. Thus, subsequent studies may wish to use a shorter acclimation period.

Barnes Maze.

Acquisition. Analysis of errors and latency during acquisition produced a main effect of both Genotype ($p < 0.05$) and Day ($p < 0.01$), but there was no significant effect of Age for either measure ($p > 0.23$). Additionally, there was a significant three-way interaction for errors ($p < 0.05$), but not latency ($p > 0.24$) and no other significant interactions ($p > 0.28$). Both error and latency performance significantly improved across days as shown in Figures 16 and 17, and Control animals made significantly fewer errors than APP ($p < 0.002$) and APP+PS1 animals ($p < 0.02$), as shown in Figure 18.

Figure 16. The effect of genotype on acquisition error performance in the Barnes maze during Experiment 2. All values are expressed as daily group means $\pm$ SEM. Error performance significantly improved over days ($p < 0.01$).

Figure 17. The effect of genotype on acquisition latency performance in the Barnes maze during Experiment 2. All values are expressed as daily group means $\pm$ SEM. Latency performance improved over days ($p < 0.001$).

Figure 18. The effect of genotype on overall error performance in the Barnes maze during Experiment 2. The acquisition and reversal bars represent overall group means $\pm$ SEM of all three training days, while the retention bars represent group means $\pm$ SEM of three probe trials. There was a significant main effect of Genotype for all phases of the testing, such that nTg rats made significantly fewer errors than Tg rats. However, Control animals did not differ from APP animals during retention. * indicates $p < 0.05$ and ** indicates $p < 0.01$.

Surprisingly, however, Controls also had significantly longer latencies than APP+PS1 animals ($p < 0.04$), as shown in Figure 19. The trend was different than what was expected, such that Control rats showed the longest latencies and the APP+PS1 rats showed the shortest latencies during acquisition. This finding may have stemmed from the modified Barnes maze procedure used in this experiment. The protocol in this experiment used shorter trials with more trials per day than the previous experiment. The shorter trial length may have substantially reduced the average entry latencies of the Tg animals in a way that benefited their overall latency performance. Further, the additional exposure to the maze via the extra trial per day may have had a detrimental effect on the performance of the nTg Control animals. It is possible that the Control animals were no longer sufficiently aversively stimulated by the Barnes Maze environment and thus unmotivated to complete the maze. This explanation seems plausible given that the Controls only had longer latencies but did not produce many errors. Subsequent studies may consider adding additional aversive stimuli to the testing environment such as white noise or a fan to improve motivation in the Control rats.

Figure 19. The effect of genotype on overall latency performance in the Barnes maze during Experiment 2. The acquisition and reversal bars represent overall group means $\pm$ SEM of all three training days, while the retention bars represent group means $\pm$ SEM of three probe trials. There was a significant main effect of Genotype during acquisition, indicating that Control rats had significantly longer latencies than APP+PS1 rats ($p < 0.05$) but not APP rats ($p > 0.05$). There were no significant group differences during retention or reversal training. * indicates $p < 0.05$.

Retention. Error performance during the retention test produced significant main effects of Genotype ($p < 0.004$; Fig. 18) and Age ($p < 0.02$), as well as an interaction between Genotype and Age ($p < 0.004$). There was also a significant main effect of Age ($p < 0.05$) but not Genotype ($p > 0.55$; Fig. 19) for latency. Post hoc comparisons indicated that APP+PS1 rats made statistically more errors than both APP ($p < 0.03$) and Control animals ($p < 0.001$) and that 14-month old rats made significantly more errors ($p < 0.02$) and had significantly longer latencies than 12-month old rats ($p < 0.05$), as shown in Figures 18 and 20 respectively.

Figure 20. The effect of age on retention performance in the Barnes maze during Experiment 2. All bars are expressed as group means $\pm$ SEM. There was a significant main effect of Age for both errors and latencies, suggesting that 14-month old rats performed significantly worse than 12-month old rats. * indicates $p < 0.05$.

Reversal. Analysis of error performance during reversal training found a significant interaction between Day and Genotype ($p < 0.02$) and a main effect of Day ($p < 0.001$), indicating that errors for all groups declined with reversal training as shown in Figure 21. There was also a significant main effect of Genotype ($ps < 0.04$) and Age ($ps < 0.05$) for both measures, suggesting that Control animals made significantly fewer errors than both APP ($p < 0.001$) and APP+PS1 animals ($p < 0.003$) as shown in Figure 18, and that 14-month old animals made significantly more errors and had significantly longer latencies than 12-month old rats ($ps < 0.05$) as shown in Figure 22; the Age effect, however, did not vary as a function of Genotype for either measure ($ps > 0.46$). Post hoc comparisons did not find any significant Genotype differences for latencies, but suggested that APP+PS1 rats had a non-significant tendency to have shorter latencies than both the Control ($p < 0.09$) and the APP rats ($p < 0.12$), as shown in Figure 23. Again, this effect in the latency data may stem from differential motivation to complete the maze as

suggested earlier. Although anxiety levels were not found in Experiment 1, other studies have found that AD animals tend to have higher levels of anxiety (Beauquis et al., 2014; Blázquez et al., 2014; Pietropaolo, Feldon, & Yee, 2014; Sterniczuk et al., 2010) and thus, it is possible that AD animals continue to be motivated to escape the aversive nature of the Barnes maze even after so many training trials, whereas the Control animals may learn that the maze environment is no longer fear-inducing after being exposed to it on so many previous trials.

Figure 21. The effect of genotype on reversal error performance in the Barnes maze during Experiment 2. All bars are expressed as group means $\pm$ SEM. There was a significant main effect of Day, indicating that the number of errors made declined with reversal training ($p < 0.001$).

Figure 22. The effect of age on reversal performance in the Barnes maze during Experiment 2. All bars are expressed as group means $\pm$ SEM. There was a significant main effect of Age, indicating that older animals made significantly more errors and had significantly longer latencies than younger animals ($p < 0.05$). * indicates $p < 0.05$.

Figure 23. The effect of genotype on reversal latency performance in the Barnes maze during Experiment 2. All bars are expressed as group means $\pm$ SEM. Although there were no significant group differences, APP+PS1 rats had a non-significant tendency to have shorter latencies than both the Control ($p < 0.09$) and the APP rats ($p < 0.12$).

Overall, in terms of error performance, APP and APP+PS1 rats showed poor memory for the location of the escape box during acquisition, retention, and reversal in

the Barnes maze. This result is consistent with many spatial learning findings using rodent models of AD (Blázquez, Cañete, Tobeña, Giménez-Llort, & Fernández-Teruel, 2014; Cañete, Blázquez, Tobeña, Giménez-Llort, & Fernández-Teruel, 2015; Stepanichev et al., 2004; Stewart, Cacucci, & Lever, 2011; Wright et al., 2013), including performance in the Barnes maze (Attar et al., 2013; O'Leary & Brown, 2009; Reiserer, Harrison, Syverud, & McDonald, 2007; Stover, Campbell, Van Winssen, & Brown, 2015; Walker et al., 2011; Walker et al., 2015).

EXPERIMENT 3

After examining the behavioral characteristics of these two novel rat models for AD and having established that they are good models of the disorder using the previous experiments, the purpose of this final study was to use these models to further elucidate the link between gender and AD. As such, Experiment 3 examined the effects of decreased endogenous estrogen levels in females on memory performance.

Although the role of sex steroids in brain development and reproductive behavior has long been recognized, the importance of these steroids on older adult brain function has only recently been examined (Ball & Birge, 2002). In addition to its role as a reproductive hormone, estrogen has been shown to play neuroprotective and neuromodulatory role in the brain. For example, estrogen is involved in the control of neuronal functions such as neuronal proliferation, neuronal survival, and neuronal plasticity (Bhavnani, 2003; Brann, Dhandapani, Wakade, & Mahesh, 2007). Furthermore, estrogen receptors are expressed in brain regions vulnerable to AD, such as the hippocampus and the neocortex (Galea et al., 2013; Lee, Jiang, Han, Shin, & Choi, 2014; Shughrue, Scrimo, Lane, Askew, & Merchenthaler, 1997). Evidence also shows that estrogen can play an important neuroprotective role in AD as it may reduce A β levels, or prevent them from rising, in the presence of pathological triggers (Anastasio, 2013). Hence, it has been suggested that the reduction of estrogen levels during aging, especially during the postmenopausal period in women, induces progressive neurodegeneration in the brain and results in cognitive impairment (Brann et al., 2007; Fukuzaki et al., 2008; Paganini-Hill & Henderson, 1994).

Experimental animals provide a model for investigating age-related effects of estrogen on brain function. Previous research has found that hippocampal estradiol treatment in middle-aged and aged female rodents increases levels of synaptophysin, which has been associated with improved spatial reference memory performance (Frick, Fernandez, & Bulinski, 2002). Estradiol treatment can also block the induction of long-term depression (Foster, Sharrow, Kumar, & Masse, 2003), which may be the mechanism through which estradiol improves memory in aging females. In addition, the literature on estrogen deficiency has found that ovariectomy in middle-aged rats results in a decline in both spatial and non-spatial memory performance (Heikkinen et al., 2004; Markowska & Savonenko, 2002; Sato et al., 2003; Wallace, Luine, Arellanos, & Frankfurt, 2006) and active avoidance behavior (Singh, Meyer, Millard, & Simpkins, 1994). Physiologically, estrogen deficiency through ovariectomy has been found to result in the production of neurofibrillary tangles and amyloid deposition in rats (Anukulthanakorn, Malaivijitnond, Kitahashi, Jaroenporn, & Parhar, 2013). Furthermore, there is evidence to suggest that such cognitive deterioration can be prevented by estrogen replacement. Estradiol administration to ovariectomized rats is associated with enhanced performance on a number of tasks (Cost, Williams-Lee, Fustok, & Dohanich, 2012; Gibbs et al., 2004; Iivonen et al., 2006; Luine, Jacome, & Maclusky, 2003; Markowska & Savonenko, 2002; Rhodes & Frye, 2004; Singh et al., 1994), further highlighting the potential benefits of estrogen.

These multiple effects of estrogen on brain function seem to suggest an effective strategy to prevent and treat AD. To further characterize our novel rat models as valuable models of AD, this study examined the effects of estrogen deficiency in these models on

spatial learning and memory and recognition memory. Both AD models and nTg controls underwent either an ovariectomy or sham surgery, after which, spatial learning and memory and recognition memory were assessed. As before, the Barnes maze and the novel object recognition task were used to assess behavior. Assessment occurred approximately 6 months after the surgeries based on previous findings that have shown that deterioration in memory performance is not evident until at least 3 to 4 months after ovariectomy (Singh et al., 1994). It was predicted that Tg (APP and APP+PS1) animals would show greater memory deficits than nTg controls, and that the APP+PS1 rats would have the most severe memory impairments on both tasks. Additionally, it was predicted that ovariectomized animals would show more deficits than their sham controls, and that ovariectomy would have the largest detrimental effect on the APP rats. The reasoning underlying this hypothesis was that the APP+PS1 animals were expected to have the worst overall behavioral performance, possibly near floor level; thus, there may be no room for their performance to decline any further. Estrogen deficiency was predicted to not have much of an effect on the control animals as they do not have any AD pathology. Therefore, estrogen deficiency was expected to have the largest effect on the single Tg (APP) animals.

Materials and Methods

Animals. Similar to the previous experiments, female APP+PS1 ($n = 25$), APP ($n = 21$), and nTg Fischer 344 ($n = 28$) rats were used. Tg rats were bred as previously described (Agca et al., 2008) and Fischer 344 controls were obtained from Harlan

(Indianapolis, IN, USA) at 3 weeks of age. To manipulate estrogen deficiency at six weeks of age, half of the animals in each group underwent an ovariectomy surgery while the other half underwent a sham surgery (see Table 3). All procedures were performed in accordance with the University of Missouri's ACUC guidelines and the ILAR Guide for the Care and Use of Laboratory Animals. All unspecified details were the same as those in Experiment 2.

Genotype	Estrogen	
	Number of Sham Rats	Number of OvX Rats
APP+PS1	$n = 12$	$n = 13$
APP	$n = 12$	$n = 9$
nTg (CTL)	$n = 14$	$n = 14$

Table 3. Rats used in Experiment 3. A total of 74 rats were used.

Behavioral Assessment. Behavioral testing occurred starting at 8 months of age, or 6.5 months after surgeries. Each rat was handled at least four times prior to any behavioral treatment to reduce anxiety due to handling during testing. Each day on which behavioral testing occurred, rats were transported to the testing room from the colony room in their home cages and acclimated in the testing room for a minimum of 30 min prior to the first trial. The behavioral treatment order (Barnes maze and novel object recognition Task) was counterbalanced for all rats.

Novel Object Recognition Task. A similar novel object recognition task procedure was used as in Experiment 2, with one minor modification. Instead of a 20-min acclimation

session on Day 1, rats received a 10-min acclimation session as to ensure high levels of activity during subsequent sessions.

Barnes Maze. A similar Barnes maze procedure was used as in Experiment 1, with a few modifications. First, the animals were again given a maximum of 5 min (300 sec) to find and enter the escape box. Additionally, all animals were given two trials per day, again with 30 min intertrial intervals. Lastly, there was an initial training day with only one trial (data from this trial were not included in the analysis), which was followed by four additional days of acquisition training. Additionally, the retention interval in this experiment was 14 days. The retention day was followed by four reversal training days where the location of the target box was moved to the opposite quadrant.

Data Analysis. Behavioral analyses were conducted using analysis of variance (ANOVA) in SPSS (SPSS 21, SPSS Inc., Chicago USA). For the Barnes maze, the average of each animal's performance scores each day was used in the repeated-measures analysis. Day was used as the within-subjects factor and Genotype and Estrogen were used as between-group factors. For the novel object recognition task, Genotype and Estrogen were used as between-group factors. Differences were considered significant at $p < 0.05$ for all analyses. Multiple comparisons were conducted using Tukey HSD tests and Greenhouse-Geisser corrections were used for violations in sphericity.

Results and Discussion

Novel Object Recognition Task. There were no significant Genotype differences in object discrimination or side discrimination during the training trial ($ps > 0.90$; data not shown); however, there was a significant main effect of Estrogen in side discrimination during the training trial, suggesting that Sham animals had a preference for the left side of the chamber and OvX animals had a preference for the right side during the initial training trial, which may have affected the subsequent test trial results. During the test trial, there were no significant main effects of Genotype ($ps > 0.53$; Fig. 24 and 25) or Estrogen ($p > 0.68$; Fig. 26 and 27), or any interactions ($ps > 0.17$) for any of the four variables.

Figure 24. The effect of genotype on object and side discrimination in the novel object recognition task during Experiment 3. All values are expressed group means $\pm$ SEM. There were no significant group differences.

Figure 25. The effect of genotype on object and side recognition indices in the novel object recognition task during Experiment 3. All values are expressed group means $\pm$ SEM. There were no significant group differences.

Figure 26. The effect of estrogen on object and side discrimination in the novel object recognition task during Experiment 3. All values are expressed group means $\pm$ SEM. There were no significant group differences.

Figure 27. The effect of estrogen on object and side recognition indices in the novel object recognition task during Experiment 3. All values are expressed group means $\pm$ SEM. There were no significant group differences.

Although these results do not support the initial hypothesis, they do align with previous findings in the literature that have failed to find differences in recognition memory between Tg animals and wild-type controls (Ardiles et al., 2012; Cheng, Low, Logge, Garner, & Karl, 2014; Davis, Easton, Eacott, & Gigg, 2013; Heneka et al., 2006; Huang et al., 2006; Middei, Daniele, Caprioli, Ghirardi, & Ammassari-Teule, 2006; Scullion, Kendall, Marsden, Sunter, & Pardon, 2011). This outcome suggests that the novel object recognition task may not be a sensitive measure to gauge the behavioral impairments observed in these rat models of AD.

Barnes Maze.

Acquisition. Analysis of errors and latencies during acquisition produced a main effect of Day ($p < 0.001$), indicating that performance improved over training trials, as shown in Figures 28 and 29. There was also a significant main effect for Genotype in error

performance ($p < 0.02$) but not in latency performance ($p > 0.61$), revealing that Controls made significantly fewer errors than APP rats ($p < 0.04$), but not APP+PS1 animals ($p > 0.07$), as seen in the leftmost acquisition bars in Figure 30. There was also a significant main effect of Estrogen in latency performance ($p < 0.04$) but not in error performance ($p > 0.53$), suggesting that Sham animals had significantly lower latencies than OvX animals during acquisition, as shown in the acquisition latency bars (fourth set of bars from the left) in Figure 31. Overall, Controls made statistically fewer errors and had numerically shorter latencies than Tg rats during the acquisition phase, which is consistent with the initial hypothesis that Control rats would outperform Tg rats and supports previous Tg research. Additionally, the finding that OvX animals would perform worse than Shams was also expected and consistent with previous spatial memory research (Heikkinen et al., 2004; Markowska & Savonenko, 2002; Sato et al., 2003; Wallace, Luine, Arellanos, & Frankfurt, 2006).

Figure 28. The interaction of genotype by estrogen on acquisition error performance

over training days in the Barnes maze during Experiment 3. All values are expressed as daily group means $\pm$ SEM. Error performance significantly improved over days ($p < 0.001$).

Figure 29. The interaction of genotype by estrogen on acquisition latency performance over training days in the Barnes maze during Experiment 3. All values are expressed as daily group means $\pm$ SEM. Latency performance significantly improved over days ($p < 0.001$).

Figure 30. The effect of genotype on overall performance in the Barnes maze during

Experiment 3. The acquisition and reversal bars represent overall group means $\pm$ SEM of all four training days, while the retention bars represent group means $\pm$ SEM of two probe trials. Control rats made significantly fewer errors than APP ($p < 0.04$) but not APP+PS1 rats ($p < 0.07$) during acquisition. During retention, APP rats made significantly more errors ($p < 0.04$) and had longer latencies ($p = 0.05$) than Controls, but not APP+PS1 rats ($ps > 0.14$). During reversal, APP+PS1 animals made significantly more errors than Control animals ($p < 0.002$) but not APP animals ($p > 0.14$) and had significantly longer latencies than APP animals ($p < 0.03$) but not Controls ($p > 0.31$). * indicates $p < 0.05$ and ** indicates $p < 0.01$.

Retention. Both error and latency performance during the retention test produced significant main effects of Genotype ($ps < 0.05$; Fig. 30), but not Estrogen ($ps > 0.68$; Fig. 31), and no significant interactions ($ps > 0.23$). Post hoc comparisons that collapsed over estrogen indicated that APP rats made significantly more errors ($p < 0.04$) and had statistically longer latencies ($p = 0.05$) than Control animals during the retention test, as shown in Figure 30. APP+PS1 rats did not differ from the other two groups on these two measures ($ps > 0.14$).

Figure 31. The effect of estrogen on overall latency performance in the Barnes maze during Experiment 3. The acquisition and reversal bars represent overall group means $\pm$

SEM of all four training days, while the retention bars represent group means $\pm$ SEM of two probe trials. There was a significant main effect of Estrogen in latency during both acquisition and reversal, showing that OvX animals had significantly longer latencies than Sham animals ($p < 0.05$). There were no significant estrogen differences for latency performance during retention or for error performance in any phase of training. * indicates $p < 0.05$.

Reversal. Analysis of error performance during reversal training found significant main effects of Day ($p < 0.005$; Fig. 32) and Genotype ($p < 0.002$; Fig. 30) and two-way interactions between Day and Estrogen and Genotype and Estrogen ($ps < 0.05$). Analysis of latency performance during reversal training found significant main effects of Day ($p < 0.002$; Fig. 33) and Estrogen ($p < 0.05$; Fig. 31), and a three-way interaction ($p < 0.001$). It was found that OvX animals had significantly longer latencies than Sham animals ($p < 0.05$), as seen in the rightmost bars of Figure 31. Additionally, post hoc comparisons revealed that APP+PS1 animals made significantly more errors than Control animals ($p < 0.002$) but not APP animals ($p > 0.14$), and had significantly longer latencies than APP animals ($p < 0.03$) but not Controls ($p > 0.31$), as shown in Figure 30.

Figure 32. The interaction of genotype by estrogen on reversal error performance over training days in the Barnes maze during Experiment 3. All values are expressed as daily group means $\pm$ SEM. Error performance significantly improved over days ($p < 0.005$).

Figure 33. The interaction of genotype by estrogen on reversal latency performance over training days in the Barnes maze during Experiment 3. All values are expressed as daily group means $\pm$ SEM. Latency performance significantly improved over days ($p < 0.002$).

In addition to replicating the genotype differences from the previous experiments, Experiment 3 found that estrogen deficiency exacerbated AD symptoms in the Tg rat models, as evidenced by longer latencies in the Barnes maze. This finding is consistent with previous studies of neuronal cell cultures and rodent models that propose a protective effect of estrogen in AD (e.g. Anastasio, 2013; Behl, Widmann, & Holsboer, 1995; Xu et al., 1998). More importantly, however, it was found that estrogen deficiency had a larger detrimental effect on the APP rats than the APP+PS1 rats, as predicted; the effect is most clearly illustrated in Figures 32 and 33. As previously discussed, this could be due to the fact that both APP+PS1 groups performed closer to floor level due to their more severe AD-like impairments. In many cases, the APP sham animals performed comparably to the two Control groups, and therefore, the detrimental effect of estrogen deficiency could be seen most clearly in this genotype group. This finding implies that the APP Tg model may be useful in testing the detrimental effects of certain treatments on AD onset and progression. On the other hand, the APP+PS1 model may be better suited towards evaluating the effectiveness of therapies that might benefit AD symptoms and progression.

GENERAL DISCUSSION

As human life expectancy increases, the prevalence of AD is expected to rise (Hebert et al, 2003); this makes AD a growing public-health concern, as there are no known cures to treat the disease (Reitz, 2012). One reason for the lack of progress towards developing a cure is that there are currently no animal models that completely capture the full-spectrum of human AD pathology, which makes it difficult to evaluate the efficacy of new therapies. This paper addressed the development of two new Tg rat models for the disease and evaluated whether they display the behavioral deficits of AD.

The results of the present studies and other physiological studies with these Tg rats suggest that these models may be more accurate models of the disease than the Tg models that are currently widely-used in the literature. The combined results of Experiment 1 and 2 found that these models showed significant memory deficits in performance in the Barnes maze. Error performance tended to be a more consistent measure of spatial learning and memory rather than latency, possibly due to genotype motivational differences. It was also found that the 12-14 month age range used in Experiment 2 was more sensitive to genotype differences than the 8-12 month age range used in Experiment 1. More specifically, Experiment 1 found significant genotype differences only during the retention phase of the Barnes maze in the 8-12 month old rats, whereas the Experiment 2 found significant genotype differences in all three phases of the Barnes maze for 12-14 month old rats. This finding highlights the importance of measuring memory ability across the entire lifespan of the animal and correlating

memory loss with molecular markers appearing at different stages of disease (Ashe, 2001).

As previously mentioned, histological examinations of these models have found that the APP rats display significant neurofibrillary tangles and that the APP+PS1 rats show chromatolysis and neuronal loss in the hippocampus (Agca et al., 2016) at 18-19 months of age. These physiological markers are consistent with human AD pathology (Ball, 1977; Gómez-Isla et al., 1997; West, 1993; West, Coleman, Flood, & Troncoso, 1994). Another advantage of the present APP rat model is that it is able to mimic the gradual timing of disease onset more accurately than current mouse models, most of which develop aggressive early-onset AD-like pathology. A previous study using the present APP rats suggested that this model does not exhibit A β plaque formation unless there is an additional environmental challenge (Rosen et al., 2012), similar to how human AD patients may not display clinical symptoms until there is an additional physiological disturbance, such as a stroke. Additionally, the only AD mouse models that develop neurofibrillary tangles to date are those that express mutant forms of tau, even though no cases of human AD are linked to mutations in tau (as reviewed by Ashe, 2001). Thus, the observance of neurofibrillary tangles in an APP-alone Tg rat is noteworthy and may be the largest indication that the present APP rat model may have distinct advantages over models currently used in the field.

The present models, nevertheless, are not without fault. As discussed earlier, A β burden is often present in human patients with AD for some time before the manifestation of clinical impairments (Bateman et al., 2012; Price & Morris, 1999). Unfortunately, the present APP model does not naturally develop A β plaques, which does not correspond

with the time-dependent progression of pathology of human AD. However, it is important to highlight that the relationship between A β burden and cognitive symptoms is by no means clear (Foley et al., 2015), and therapeutic approaches to reduce A β production or A β aggregation have thus far been disappointing (Doody et al., 2013; Holmes et al., 2008; Salloway et al., 2014). Hence, this model may provide new insights into this puzzling issue. There is another disadvantage to the present APP rat model. As previously mentioned, cognitive impairment seems to be strongly related to excessive neuronal loss (Terry, 2006), an AD feature many current single transgenic mouse models (i.e., PDAPP, Tg2576) lack despite having extensive amyloid deposition (Irizarry, McNamara, Fedorchak, Hsiao, & Hyman, 1997; Irizarry et al., 1997). This disadvantage, unfortunately, is also true for the current APP model, as previous examinations of their brain tissue have not yet shown evidence of significant neuronal loss (Agca et al., 2016). Evidence of substantial neuronal loss has only been observed in the APP+PS1 rats (Agca et al., 2016).

Despite these drawbacks, the present Tg rat models do provide a slightly different framework than existing models and have the potential to add new perspectives to the existing AD literature. As such, these models were used to investigate the link between sex and AD. Due to the abundance of studies that suggest a neuroprotective effect of estrogen, the influence of estrogen deficiency via ovariectomy on AD cognitive symptoms was evaluated. It was found that estrogen deficiency exacerbated AD symptoms in the Tg rat models, as evidenced by longer latencies in the Barnes maze. More importantly, however, estrogen deficiency had a larger detrimental effect on the APP rats, as predicted. This finding implicates differential utility of each of the Tg

models. The single Tg APP model may be useful in testing the detrimental effects of certain treatments on AD onset and progression. On the other hand, the double Tg APP+PS1 model may be better suited towards evaluating the effectiveness of therapies that might benefit AD symptoms and progression.

Although there is mounting evidence from cellular and animal models, as well as human observational studies (e.g., Rocca, Grossardt, & Shuster, 2011), that speaks to the neuroprotective effects of estrogen in AD, including the present study, clinical human trial data remain controversial. At first, studies showed a promising effect of the use of hormone replacement therapy (HRT; Fillit, 2002; Paganini-Hill & Henderson, 1996; Tang et al, 1996), which involves the administration of synthetic estrogen and progesterone to replace a woman's depleting hormone level, in postmenopausal women to reduce the risk of AD or delay the onset of the disease. However, the beneficial effects of HRT have been challenged by the results of the Women's Health Initiative Memory Study (WHIMS), a study that was ended early due to reported detrimental effects of long-term HRT use (Manson et al., 2013; Shumaker et al., 2003). WHIMS participants were recruited from among participants in the Women's Health Initiative trials, who were ages 65 to 79 years and without dementia at baseline. The results found that for women with a uterus that received combined estrogen-progesterone therapy, there was a two-fold increase in dementia after 4.2 years of HRT, as well as, increased risks for breast cancer, pulmonary embolism, and stroke (Taylor & Manson, 2011). For women without a uterus that received estrogen-alone therapy, incidence of dementia did not differ significantly between the two treatment groups. A more recent randomized, double-blind, placebo-controlled trial using raloxifene, an oral selective estrogen receptor modulator (SERM),

found no cognitive benefit in postmenopausal women with mild to moderate AD after 12 months of treatment (Henderson et al., 2015). These findings further the debate regarding the risk-benefit ratio of hormone therapy.

There have been several important criticisms of the WHIMS study. The first is directed towards the synthetic nature of the hormones administered, as well as, the route of administration and dose. Critics have argued that transdermal administration (e.g., Asthana et al., 1999) and the use of natural hormones are more effective than the conjugate equine estrogen (CEE) and medroxyprogesterone acetate (MPA) used in the study (e.g. Kaari et al., 2006). However, the largest criticism focuses on the age of the participants of the WHIMS study. Estrogen therapy may not have universal utility in treating or preventing dementia and a growing number of studies support the existence of the “critical window of opportunity” (Gibbs, 2000b; Resnick & Maki, 2000; Resnick & Henderson, 2002; see Maki, 2013 and Rocca et al., 2011 for an overview), which states that there is a limited period after menopause during which hormone therapy is effective. The primary criticism of the WHIMS study is that the intervention may have been initiated after the critical window of opportunity and at a point at which the brain was no longer responsive to sex hormones. According to this argument, hormone therapy exerts its beneficial effects only if begun near the time of menopause. Since most participants of the WHIMS study were many years beyond menopause, this hypothesis could explain the absence of a beneficial neural effect of hormone therapy. Further, the WHIMS findings may be valid for women with similar characteristics to those of the trial participants but may not generalize to much younger women who initiate hormone therapy much closer to the time of menopause (Henderson, 2006).

The critical window notion of an age-related loss in brain responsiveness to estrogen is supported by several animal studies. One study found that estradiol and estradiol plus progesterone replacement was associated with improved spatial memory in a delayed match task in OvX animals when administered within 3 months post-OvX, but not when treatment was delayed 10 months post-OvX (Gibbs, 2000a). Additionally, estradiol given immediately after OvX improved spatial memory performance in the radial arm maze in rats, but had no significant effect when given 5 months after OvX (Daniel, Hulst, & Berbling, 2006). These data suggest that the brain can show reduced hormone responsiveness after an extended period of hormone depletion, however, further work is required to elucidate the key factors and mechanisms underlying these effects (Pike et al., 2009).

More recent human studies have found similar beneficial effects of initiating estrogen-based hormone therapy close to the onset of menopause. One longitudinal study by Bove and colleagues (2014) reported that earlier surgical menopause was associated with faster decline in global cognition and increased AD neuropathology. More importantly, the use of HRT for at least 10 years in those with surgical menopause, when initiated within a 5-year postmenopausal window, was associated with decreased decline in cognition. Another study reported by Wroolie, Kenna, Williams, and Rasgon (2015) used women aged between 49-69 who had initiated HRT close to menopause onset and had been taking HRT for at least one year. All women underwent cognitive assessment before randomization to continue or discontinue HRT. They found that women who continued HRT performed better on cognitive tests at the 2-year follow-up assessment than those that discontinued therapy. Additionally, women that used 17β -E, whether

randomized to continue or discontinue treatment, showed better performance than women that used CEE. These results suggest that continuation of HRT seems to protect cognition in women with a heightened risk for AD when initiated close to menopause onset, which lends further support to the window of opportunity hypothesis.

Current ongoing prospective randomized placebo-controlled trials will formally test the critical window of opportunity hypothesis. The Kronos Early Estrogen Prevention Study (KEEPS; ClinicalTrials.gov Identifier: NCT00154180) looks at the effect of five years of treatment with cyclic micronized progesterone in combination with transdermal estradiol or CEE on cognitive function and cardiovascular disease in women aged 42 to 58 years within 36 months of their final menstrual period. The Early Versus Late Intervention Trial with Estradiol (ELITE; ClinicalTrials.gov Identifier: NCT00114517) tests the timing hypothesis by looking at the effects of oral estradiol plus a vaginal progesterone gel for on cognitive function in both younger (< 6 years since menopause) and older postmenopausal women (10 or more years since menopause). Lastly, the Women's Health Initiative Memory Study of Younger Women (WHIMS-Y; ClinicalTrials.gov Identifier: NCT01124773) is an ancillary study to the Women's Health Initiative hormone therapy trial and assesses the impact of CEE and CEE+MPA on cognitive function in women who were aged 50-54 at enrollment in WHI. The results of these trials will provide evidence in support or against the optimal window of opportunity hypothesis.

Overall, the present findings suggest that the novel Tg rats used in these studies may be better models of human AD than other models currently used in the literature and may provide new insights into the underlying mechanisms of the disease. The

development of more accurate animal models is essential to the ability to test the efficacy of novel therapeutics before the evaluation of therapies in human clinical trials. The vast number of human clinical trial outcomes that do not align with animal findings underscore the importance of developing accurate animal models for AD. Despite promising results in animal models, there has been a failure of phase III trials of anti-A β therapies (Drachman, 2014), such as A β immunization with AN1792 or bapineuzumab and treatment with gamma-secretase inhibitor, semagacestat. The semagacestat trial was terminated because participants in the active treatment arm were found to have poorer cognitive outcomes (Doody et al., 2013) and the bapineuzumab trial found no improvement in clinical outcomes in patients with AD (Salloway et al., 2014). Additionally, the AN1792 trial was halted when some participants developed autoimmune encephalopathy and there was no treatment effect on disease progression (Gilman et al., 2005). The failure of these therapies in human trials implies that a firmer understanding of basic mechanisms underlying the disease is needed before large scale clinical trials are devised. A better understanding of the disease and its progression can be gained from the use of animal models that better mimic the human disease. Moreover, testing new therapies in more accurate animal models prior to the initiation of human clinical trials can help save not only substantial amounts of time and resources, but also human lives.

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VITA

Diana Klakotskaia is a neuroscientist that studies the behavioral and neural mechanisms of learning and memory. More specifically, she is interested in what leads to cognitive decline in normal aging and conditions like Alzheimer's, type 2 diabetes, heart failure, and traumatic brain injury. Her research focuses on the cognitive benefit of lifestyle changes, specifically, modifications in diet, nutritional supplement intake, and exercise regimens and their ability to ameliorate age-related decline. She has extensive experience with rodent behavioral assessment including but not limited to the use of the Barnes maze, T-maze, Y-maze, elevated plus maze, novel object recognition, conditioned taste aversion, open-field, beam walking test, and a variety of operant conditioning paradigms.

Diana Klakotskaia received the St. Dominic Scholarship, a full-tuition merit-based award, to attend Providence College in Providence, RI at the undergraduate level. She graduated magna cum laude from the Liberal Arts Honors Program with B.A. in Psychology in May 2011. While at Providence College, she worked in the Affective Neuroscience Lab under the supervision of Dr. Christopher M. Bloom. While working with Dr. Bloom, she received a summer undergraduate research fellowship from the Rhode Island Institutional Development Award Network for Excellence in Biomedical Research (RI-INBRE) to evaluate the effects of systematically manipulated stress levels on observed behavior in a rat model of non-suicidal self-injurious behavior.

Diana's experience with animal research motivated her to pursue a graduate degree at the University of Missouri in Columbia, MO. During her studies, she worked

in the Conditioning and Neuroscience Lab under the direction of Dr. Todd R. Schachtman to investigate information processing in animals using a variety of behavioral techniques. During her time at Mizzou, Diana served as President of the MU Graduate Peer Mentoring Program, Vice President of the Graduate Association for Students in Psychology, and as a member of MU's Department of Psychological Sciences' Web and Scholarly Communications Committee. Diana was awarded the Department of Psychological Sciences' Graduate Student Excellence Award in 2017 and has been granted multiple recognitions for her oral presentation skills and public outreach service, as well as funding to present her research findings at scientific conferences around the nation. She received her M.A. in Psychology with a focus on Cognition and Neuroscience in August 2013 and her Ph.D. in Psychology and a graduate certificate in Neuroscience in May 2017.