Summer 5-5-2017

SEX SPECIFIC SPATIAL NAVIGATION AND MEMORY IMPAIRMENT IN THE TGF344-AD RAT MODEL OF ALZHEIMER'S DISEASE

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by

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THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science
Psychology

The University of New Mexico
Albuquerque, New Mexico

July, 2017

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ABSTRACT

Spatial navigation and memory are impaired in early stages of Alzheimer's disease (AD), and may be a defining behavioral marker of preclinical AD. Nevertheless, limitations of diagnostic criteria for prodromal AD and within animal models of AD make characterization of preclinical AD difficult. A new rat model (TgF344-AD) of AD overcomes many of these limitations, though spatial navigation and memory has not been comprehensively assessed. This study aimed to characterize. Using three paradigms of the Morris Water Maze, spatial navigation and memory were assessed in TgF344-AD (n=16) and Fischer 344 (n=12) male and female rats over three time points. TgF344-AD females exhibited navigational deficits at 4.5 months while TgF344-AD males show impairment at 10.5 months. Furthermore, TgF344-AD males demonstrate acute reference memory impairment at 10.5 months whereas TgF344-AD females are unimpaired. Across all time points, cued navigation, spatial working memory and reference memory remain largely intact between subjects. Overall, these results indicated TgF344-AD rats exhibit comparable deficits to those found in individuals with MCI and provides further evidence of sexual dimorphisms of AD.

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Introduction

Alzheimer's disease (AD) is the most common form of dementia in the United States and is characterized by progressive cognitive decline and neurodegeneration such as amyloid plaque deposition, neurofibrillary tangles, cell loss and inflammation (Alzheimer's Association, 2017). Over time, AD pathology spreads throughout the Papez circuit, initiating in anterior thalamus and entorhinal cortex (Braak & Braak, 1995), and mounting evidence suggests it could take up to 20 years before the onset of symptoms (Vos, et al., 2013; Bertens, Knol, Scheltens, Visser, & Alzheimer's Disease Neuroimaging Initiative, 2015). Indeed, middle-aged cognitively normal carriers of the APOE4 allele, a genetic risk factor of AD, exhibit decreased Papez circuit functional connectivity between regions involved in learning and memory (Li et al., 2014) and neurovascular dysfunction has recently been found to best predict of late-onset Alzheimer's disease (Iturria-Medina, et al., 2016). The first symptoms to manifest include impairments in spatial navigation and spatial memory as well as neuropsychiatric disorders such as depression and anxiety (Cushman, Stein & Duffy, 2008; Delpoly et al., 2007; Lithfous et al., 2013; Nathan et al., 2017; Ng et al., 2017). These symptoms typically corresponds to a diagnosis of mild cognitive impairment (MCI), which has been attributed to the prodromal phase of general dementia (Petersen et al., 2004), and about 8-16% of individuals with this diagnosis progress to AD (Dubois, et al., 2016).

The long preclinical period poses a significant challenge to elucidating early mechanisms and markers in individuals with AD (Dubois, Padovani, Scheltens, Rossi, & Dell'Agnello, 2016; Graham, Bonito-Oliva, & Sakmar, 2017). Both biological and

behavioral markers associated with AD have been identified, though the sensitivity and specificity for predicting progression to AD widely vary (Dubois et al., 2007). Biomarkers, obtained from cerebrospinal fluid or using magnetic resonance imaging, may improve prediction of cognitive decline, though these have yet to be validated for clinical use (Boccardi et al., 2017; Galasko & Shaw, 2017). The characterization of MCI subtypes has identified that individuals with the amnesic form (aMCI) have an increased risk of progressing to AD (Winblad et al., 2004; Petersen, 2004). Individuals with aMCI exhibit indications of AD-like pathology on MRI, such as progressive ventricular expansion, decreased volumes in the medial and inferior temporal lobes, and white matter changes in the fornix (Jack et al., 2009; Whitwell et al., 2008; Oishi, Mielke, Albert, Lyketsos & Mori, 2012). However, diagnostic criteria for MCI or aMCI variably predict conversion to AD (Dubois et al., 2007) and not all individuals with aMCI progress to AD (Gainotti, Quaranta, Vita & Marra, 2014). Animal models with AD-like pathology have provided significant insight into preclinical AD, though these findings are limited to aspects of the disease state (Mhillaj, Cuomo, & Mancuso, 2017; Webster, Bachstetter, Nelson, Schmitt, & Van Eldik, 2014; Carmo & Cuello, 2013; Elder, Sosa & Gasperi, 2010; Lecanu & Papadopoulous, 2013; Hane, Lee & Leonenko, 2017). Nevertheless, behavioral profiles of individuals with putative preclinical or prodromal AD have revealed significant deficits in spatial cognition.

The regions that exhibit the earliest pathological markers of AD have been linked to spatial navigation and spatial orientation (Jankowski et al., 2013; Hargreaves, Yoganarasimha, & Knierim, 2007), and impairments of both allocentric (environmental-centered) and egocentric (body-centered) navigation and spatial memory is a promising

distinctive marker of AD (Laczo et al., 2010; Verghese, Lipton, & Ayers, 2017; Allison, Fagan, Morris & Head, 2016). Medial temporal lobe structures have been implicated in allocentric navigation whereby individuals locate a goal location relative to distal landmarks (Burgess, 2006). Posterior parietal and retrosplenial cortices have been implicated in egocentric navigation that involves navigation using the goal location relative to the body's position (Burgess, 2006). Using a CSF biomarker to determine preclinical status, Allison and colleagues (2016) found differences in navigational strategies between individuals with preclinical and early-AD who were tested in a virtual reality environment. Specifically, these researchers found allocentric navigation deficits in preclinical AD, but both egocentric and allocentric navigation impairments in early-AD. Furthermore, allocentric memory deficits have been found to contribute to topographical disorientation in AD (Burgess, Trinkler, King, Kennedy & Cipolotti, 2006) and allocentric memory is significantly impaired in aMCI patients performing an analogue of the water maze task (Laczo et al., 2010). Animal models of AD-like pathology have also exhibited spatial impairments. Several types of spatial memory deficits are observed in various mouse models of AD-like pathology as early as 3-5 months (Webster, Bachstetter, Nelson, Schmitt, & Van Eldik, 2014). Furthermore, cell types that contribute to spatial representations of place and distance, such as place cells in the hippocampus and grid cells in the medial entorhinal cortex, are dysfunctional in mouse models of AD-like pathology (Mably, Gereke, Jones, & Lee Colgin, 2016; Fu et al., 2017). For spatial navigation and memory to be used as a predictive factor in the progression to AD, further characterization in a comprehensive model of AD is required.

Recently, Cohen and colleagues (2013), were the first to develop a rat model with AD-like pathology (TgFF344-AD) that comprehensively demonstrates pathology found in humans. With the inclusion of the Swedish form of mutant amyloid precursor protein gene (*APP*) and human mutant presenilin 1 exon 9 (PS1ΔE9), these rats develop progressive signs of amyloid dysfunction, neuroinflammation, hyperphosphorylation of endogenous tau, cerebral amyloidangiopathy and cell death. Furthermore, recent findings in TgF344-AD rats revealed neurovascular dysfunction that precedes the onset of significant plaque and tangle formation (Joo al., 2017). Given that no rodent model of AD-like pathology has shown a similar pathological profile of AD-like as comprehensive as TgF344-AD rats, these rats show significant promise for elucidating a more translatable profile of preclinical AD. Currently, only one study has assessed spatial memory in TgF344-AD rats, however; this was limited to one assessment prior to the onset of significant pathology and did not distinguish between sexes (Cohen et al., 2013).

This study thus aimed to assess spatial navigation and memory in a comprehensive animal model of AD at preclinical time points. Furthermore, this study utilized a longitudinal design to capture within subject changes across multiple time points. Given that significant pathology is observed at 16 months in TgF344-AD rats with subtle changes occurring at 6 months (Cohen et al., 2013), subjects in this study were assessed at 4.3-5.5mo, 8.7-9.9mo and 10.3-11.5mo. At each time point, allocentric and egocentric navigation as well as spatial reference and working memory were assessed using three variants of the Morris Water Maze (Morris, 1984; Vorhees, & Williams, 2006; Harker & Wishaw, 2002). Additionally, both male and female subjects were used to address influence of sex on presentation of AD given females exhibit a disproportional higher

rate of progression to AD and increased severity of clinical dementia (for review see Mielke, Vemuri & Rocca et al., 2014). The results indicated that allocentric navigation impairment is evident in female TgF344-AD rats earlier than TgF344-AD males, while egocentric navigation remains intact and does not differ across time points. Reference memory strengthened across five days of learning and spatial working memory were intact in TgF344-AD rats across time points. However, male TgF344-AD rats demonstrated acute reference memory deficit after one day of learning at 10.5 months whereas female TgF344-AD rats do not differ from controls. These results indicate that the TgF344-AD rat model exhibit spatial impairments comparable to individuals with putative preclinical AD before 10.5 months of age and further supports findings of sex differences in AD.

Methods

Subjects

Sixteen TgF344-AD (Tg) rats (Rat Resource & Research Center, Columbia, MO), expressing mutant human amyloid precursor protein (*APPsw*) and presenilin 1 (*PS1ΔE9*), and 12 wild type Fischer 344 (WT) rats counterbalanced for sex served as subjects. Subjects were pair housed and kept on a 12 hour light/dark cycle. Food and water was provided ad libitum throughout the duration of the study. Animal care practices and experiments were approved by the University of New Mexico Institutional Animal Care and Use Committee and adhered to the APA ethical principles of animal use.

Experimental Design

A longitudinal mixed design was used to assess change in reference and spatial working memory as well as a cued task for visual acuity. Subjects were placed into

groups by genotype (Tg/WT and further grouped by sex (male/female). Figure 1a. summarizes the temporal order of all measures. At the start of testing, subjects' age ranged from 4.30-5.7, 7.5-8.9, and 10.3-11.7 for time point 1, 2, and 3, respectively. Tasks assessing spatial memory were delivered in the same temporal order at each time point, starting with reference memory, working memory and visual acuity. Procedures were maintained between time points and experimenters did not change throughout the duration of the study. All subjects were naïve to experimentation prior to the start of testing.

Apparatus and Testing Room

A single circular pool (150 diameter, 48 cm high) with a white inner wall was situated on a wooden frame (50 cm high) and utilized at all time points. The escape platform consisted of a 16 cm x 16 cm plastic top surface covered with a metal grate and a height of 25 cm. The pool was filled with water (20 - 22°C) until the level reached ~2.5cm above the top of the platform. Non-toxic white paint was used to make the water opaque. Various objects (movie posters, thin particle board hangings) and furniture (desks & bookshelves) were placed in fixed locations throughout the room to serve as visual cues. The testing room was modified at each time point to control for context dependent memory effects, including the use of a curtain and different lighting conditions. An overhead camera was fastened above the pool to record swim paths.

Morris Water Task

The MWM is a classic experiment used for testing spatial memory in rodents, whereby subjects learn to navigate to an escape platform (Morris, 1984; Vorhees, & Williams, 2014). Subjects were placed in the pool facing the wall at one of four

equidistant drop points. Drop location was randomized using the *randperm* function in Matlab (Mathworks, Natick, MA). Drop location varied between time point/trials/days, but was maintained between subjects. Time to reach the platform, escape latency, was recorded by an experimenter at the end of each trial. Subjects were run in group of seven for each task. Platform locations for all tasks were shifted to new spatial positions at each time point. In addition, video recordings of all trials were obtained and stored on an external drive until tracking and analysis could be performed.

Cued platform. A cued-platform test was performed to verify intact visual processing. In this task, a 9 cm diameter black ball with a white horizontal stripe attached to a metal rebar that extended 11.5 cm above the water was placed in the center of the platform. This task consisted of eight trials total. If the platform was not found within 60 seconds, the experimenter guided the subject to the platform by hand.

Reference memory learning. Subjects underwent 20 total learning trials evenly dispersed across five days. Experimental parameters surrounding drop location and time to find the platform were identical to those used during the cued platform task. Once the platform was found, the subject remained on the platform for 10 seconds to allow for room cue learning. After the end of the learning trials, subjects underwent a 60 second probe trial whereby the platform was removed from the pool.

Matching-to-place. To test for spatial working memory as well as acute reference memory, subjects learned the location of a platform that moved to a new location each day for five days (Harker & Wishaw, 2002; Wishaw, 1985b). In this task, subjects were provided two trials to learn the location of the platform. On trial one, subjects had a maximum of 180 seconds to find the platform and were guided to the platform if the

maximum time was met. Subjects then remained on the platform for 10 seconds and were returned to their home cage for 30 seconds. Immediately after, subjects were returned to the pool for trial two.

Path Analysis

Upon completion of data collection, video files were preprocessed and each subjects' location was manually tracked by blinded coders. Raw video files were first converted to JPEG images using a custom script in Matlab. Image files were then imported into FIJI (NIH, Bethesda, MD) for tracking analysis using the Manual Track plugin. Blind coders obtained the X-Y coordinates of each subjects' nose position, which allowed for sensitive tracking of the subjects' paths.

Tracking coordinates were further processed using a custom Matlab script. First, XY coordinates were resampled to 10 frames/second as lighting differences caused the video capture sample rate to fluctuate slightly between time points. Coordinates were then converted to centimeters, rotated to correct for video angle aliasing and smoothed using runline (Chronux). Processed coordinates were then used to obtain various measures of search error via cumulative search error and average search error (Gallagher, Burwell, & Burchinal, (1993). Briefly, search error was measured by measuring the average distance across 1s between the animal's location and the center of the platform location. In order to control for different drop locations during learning, total search error minus the distance from the drop location and the center of the platform was performed to compute cumulative search error. Since drop location distances was comparable in the probe trial, search error was instead averaged across the entire session. In addition to average search error, a preference score was calculated for probe trials in order to control

for locomotion differences. Preference score equals the average difference in dwell time between the target quadrant and all other quadrants (Harker & Wishaw, 2002).

Statistical Analysis

All statistical analyses were performed using SPSS version 23 (IBM, Armonk, NY). For reference memory learning and matching-to-place, mixed model ANOVAs were used to assess differences within or between time points. Within subject factors consisted of sex (female, male) and genotype (Tg, WT) while between subject factors were day (day 1, day 2, day 3, day 4, day 5) , trial (Trial 1, Trial 2) or time point (T1, T2, T3). A Greenhouse-Geiser correction was applied to data that did not meet the assumption of sphericity. Two-way ANOVAs were used to compare performance on the cued platform task within a time point using genotype and sex as between subject factors. Both significant (p <.05) and trending non-significant (n.s.) (.1>p>.05) results are reported.

Results

One subject was excluded from the analyses for time point 3 after developing glaucoma. Furthermore, one subject died prior to time point 3 cued platform and thus was not included in the analysis. Average swim speed was observed to be different between time points, $F(1.58,85.69)$=85.7, p<.001. Pairwise comparisons revealed significant differences in swim speeds between each time point, whereby the highest average swim speed was observed at time point 3 (figure 1c.), $F(2,22)$=85.7. Sex differences were also observed, $F(1,23)$=58.4, p<.001, as females exhibited decreased swim speeds (M =28 sec, SEM=.56sec) relative to males (M=34, SEM=.59sec).

Comparable performance of TgF344-AD and F344 rats on Cued Platform Task

No significant group differences were observed for sex, genotype or sex*genotype interaction for escape latency on the cued platform, indicating visual acuity and egocentric navigation was similar across subjects. However, a main effect of time point was observed, $F\ (1,23)$=13.27, p=.001, whereby escape latency was significantly decreased at the third time point relative to prior time points, $F\ (2,22)$=8.99, p=.001. Despite changes in performance across time points, these results verify visual acuity and egocentric navigation was comparable between subjects.

TgF344-AD Demonstrate Sustained and Dynamic Navigational Deficits during Reference Memory Learning

All subjects learned the platform location over the course of training as indicated by a significant main effect of day (Table 1). In addition, all rats were able to retain task demands as indicated by progressively lower Day 1 escape latencies across time points, F=(2,46)=25.10, p<.001, with no significant differences between sex or genotype.

Time Point 1. The median age for all subjects at the start of testing was 4.8 months. Results of time point 1 are summarized in Figure 2a-b. Female Tg subjects were differentially impaired relative to male Tg subjects and WT controls. Specifically, Tg females exhibited the highest search error over the last three days of training (M=4687cm, SEM=387cm) compared to WT females (M=3804cm, SEM=387cm), WT males (M=4371cm, SEM=387cm) and Tg males (M=3347cm, SEM=335cm), contributing to a significant Genotype*Sex interaction (Figure 2b), $F\ (1,24)$=6.96, p=.014. This could have contributed to the trend that females obtained asymptotic escape latencies before males at time point 1 but search error, which is not sensitive to swim speed, was

increased in females relative to males, though this did not reach significance *F(1.51,36.25)*=3.28, *p*=.062 (Figure 2a.).

Time Point 2. The median age for all subjects at the start of testing was 8.0 months. Results of time point 2 are summarized in Figure 2c-d. Again, groups did not differ in their time to reach the platform (Table 1). At time point 2, groups continued to display differences in search error with a main effect of Sex, *F(1,24)*=4.61, *p*=.042, and trending main effect of Genotype, *F(1,24)*=3.85, *p*=.061(Figure 2c-d). Though the Genotype*Sex interaction was not significant at this time point, *F(1,24)*=.002, *p*=.97, Tg females continued to hold the highest search error (*M*=4351 cm, *SEM*=451 cm) relative to all other groups (*Ms*≤3375 cm, *SEMs*≤521 cm).

Time point 3. The median age for all subjects at the start of testing was 10.8 months. Results of time point 3 are summarized in Figure 2e-f. At time point 3, time to reach the platform and search error were different between Tg and WT subjects. Specifically, Tg subjects longer overall to reach the platform (*M*=10, *SEM*=.59) relative to WT controls (*M*=8 *SEM*=.71), *F*(1,23)=4.27, *p*=.05. Though female Tg subjects took longer overall to reach the platform (*M* =12sec, SEM=.83 sec versus *Ms*≤9 sec, *SEMs*≤1sec), this did not reach significance, *F*(1,23)=1.65, *p*=.211. Consistent with this finding, search error was also significantly different between Tg and WT subjects, *F*(1,23)=10.46, *p*=.004, whereby Tg subjects searched further from the platform (*M*=3117,*SEM*=212) relative to WT controls (*M*=2039cm,*SEM*=257cm) (Figure 2f).

Overall, these results suggest a dynamic navigational impairment leading to increased search error in Tg females starting as early as 4.8 mo and Tg males as early at 10.8 mo relative to WT controls.

Reference Memory Learning is Intact in TgF344-AD rats

Tests of search error, preference score and time in platform location during the probe trial indicated no sex or genotype differences across all time points (Figure 3,4), indicating Tg rats learned the platform location comparably to WT controls at the end of training. Females also tended to swim slower than males in the first two time points, but not the second. However, age effects were observed whereby both preference score and time in platform location demonstrated significant decreases between time points, $F(2,46)=6.09$, $p=.005$ and $F(2,46)=16.84$, $p<.001$, respectively, though no difference in search error was observed, $F(2,46)=1.46$, p=n.s. Planned contrasts indicated the decreases for preference score and time in platform area were significantly decreased between time point 1 and the latter time points $F(1,23)=28.41$, $p<.001$, but the decrease between time point 2 and time point 3 was not significant, $F(1,23)=.25$, $p=.625$. Thus, although group differences were observed during learning, all subjects exhibited similar reference memory during the probe trials across time points.

TgF344-AD Males exhibit Acute Reference Memory Impairment but Spared Working Memory at 10.5mo

All subjects were able to find the location of the platform on the second trial throughout all time points, $Fs(1,24)\geq 25.02$, $ps<.001$ (Figure 5). However, at time point 3, a significant trial*sex*genotype interaction was observed, $F(1,23)=5.29$, $p=.031$. Simple effects analysis with Bonferroni correction ($\alpha=.0125$) revealed that Tg males did not remember the previous days platform location as indicated by non-significant trial 1 and trial 2 differences, $F(1,23)=.53$, $p=.473$. However, WT females also demonstrated non-significant differences after alpha correction, $F(1,23)=5.59$, $p=.027$. Thus, Tg males, and

WT females to a lesser degree, exhibit differential reference memory compared to Tg females and WT males after 10.5 mo of age.

Discussion

This study identified spatial navigation and reference memory impairment across different time points in TgF344-AD rats. Evidence of allocentric navigation impairment and reference memory impairment was found in TgF344-AD rats relative to WT. Sex differences were also found between TgF344-AD rats. First, navigation to the platform was dynamically impaired in TgF344-AD subjects during reference memory learning. Specifically, at 4.5mo, TgF344-AD females demonstrated navigational impairment as revealed by increased search error relative to all groups. This impairment diminished around 8.5mo as TgF344-AD females exhibited comparable search error to WT females. However, at 8.5mo, TgF344-AD males and females start to converge whereby both groups demonstrate trending increased but non-significant search error relative to WT controls. At 10.5mo of age, both male and female TgF344-AD subjects took longer to reach the platform and exhibited significant search error relative to WT controls, but no sex differences were observed. Furthermore, all subjects also retained memory of task demands consistent with previous studies of prior experience on subsequent performance (Vicens, Redolat, & Carrasco, 2002; Van Groen, Kadish & Wyss, 2002). Probe testing further revealed all subjects incurred comparable search error and spent an equal amount of time in the platform location and target quadrant. Thus, after five days of training, all subjects were able to retain the platform location despite differential navigation abilities. Surprisingly, despite no differences in reference memory probe test measures, TgF344-AD males showed impaired acute reference memory on the matching-to-place task

relative to TgF344-AD females, although WT females also demonstrated subtle but statistically significant impairments as well. This finding demonstrates a dissociation between the development of spatial memory impairment in TgF344-AD males and females. Finally, neither TgF344-AD males nor females exhibited spatial working memory, visual processing or egocentric navigation impairment relative to WT controls. Overall, these results provide evidence of preclinical navigation impairments in male and female TgF344-AD rats prior to the onset of significant pathology (i.e. Cohen et al., 2013).

The differences observed between TgF344-AD males and females contributes further evidence of sex differences associated with AD. Human studies indicate the prevalence and rate of conversion to AD is higher in females (Gao et al., 1998; Mielke et al., 2014; Pike, 2017) Furthermore, various mouse models of AD-like pathology have identified increased cognitive deficits in females relative to males (Clinton et al., 2007; Granger, Franko, Taylor, Messier, George-Hyslop, & Bennett, 2016; King et al., 1999; Melnikova, Park, Becker, Lee, Cho, Sayyida, et al., 2016). Consistent with these findings, TgF344-AD females are impaired earlier than males, but these impairments become non-significant as the disease progress. Clinton and colleagues (2007) found similar results to those presented here, but also identified an elevated stress response in young but not old females after water maze testing. Moreover, recent work by Bangasser et al (2016) found females may be more sensitive to AD pathology as overexpression of corticotrophin-releasing factor results in elevated amyloid beta dysfunction relative to males. Though it is possible the search error deficits observed at 4.5 months in TgF344-AD females might correspond to an elevated stress response, a separate study found elevated anxiety in

TgF344-AD subjects at ~5.5mo but no significant differences between sex (Pentkowski, Berkowitz, Thompson, Drake, Olguin & Clark, manuscript submitted for publication). Therefore, navigational impairment at 4.5 months is likely not due to anxiety. Finally, this study did not characterize histopathological markers of AD, but pathological markers of AD in TgF344-AD rats have not been shown to vary between sexes (Cohen et al., 2013; Joo al., 2017). However, these characterizations may not address possible effects of prior experience. Given increased stress response has been found in 3xTg-AD female mice after MWM training (Clinton et al., 2007), future studies using TgF344-AD rats might look at whether MWM training modulates AD pathology differentially depending on sex.

Overall spatial impairment in TgF344-AD found here also closely correspond to those observed in individuals with preclinical or prodromal AD (Guariglia & Nitrini, 2009; Pai, & Jacobs, 2004). Although reference memory remained intact across all time points, TgF344-AD subjects, particularly females, exhibited search error impairment indicative of allocentric navigation impairments. This is consistent with impairments observed in individuals with early AD (Serino, Cipresso, Morganti, & Riva, 2014). Deficits observed in this study were mainly associated with allocentric navigation and acute reference memory, as spatial working memory and egocentric navigation (i.e. cued platform) remained intact. Thus, it is likely the preclinical phase of TgF344-AD rats lies prior to 10.5mo, and more global spatial navigation and memory impairment would be observed later on. Prior characterization of pathological markers of AD in TgF344-AD supports this notion. Specifically, TgF344-AD rats develop neuropathic changes as early as 6mo of age (Cohen et al., 2013) and neurovascular dysfunction at 9 months (Joo, et al.,

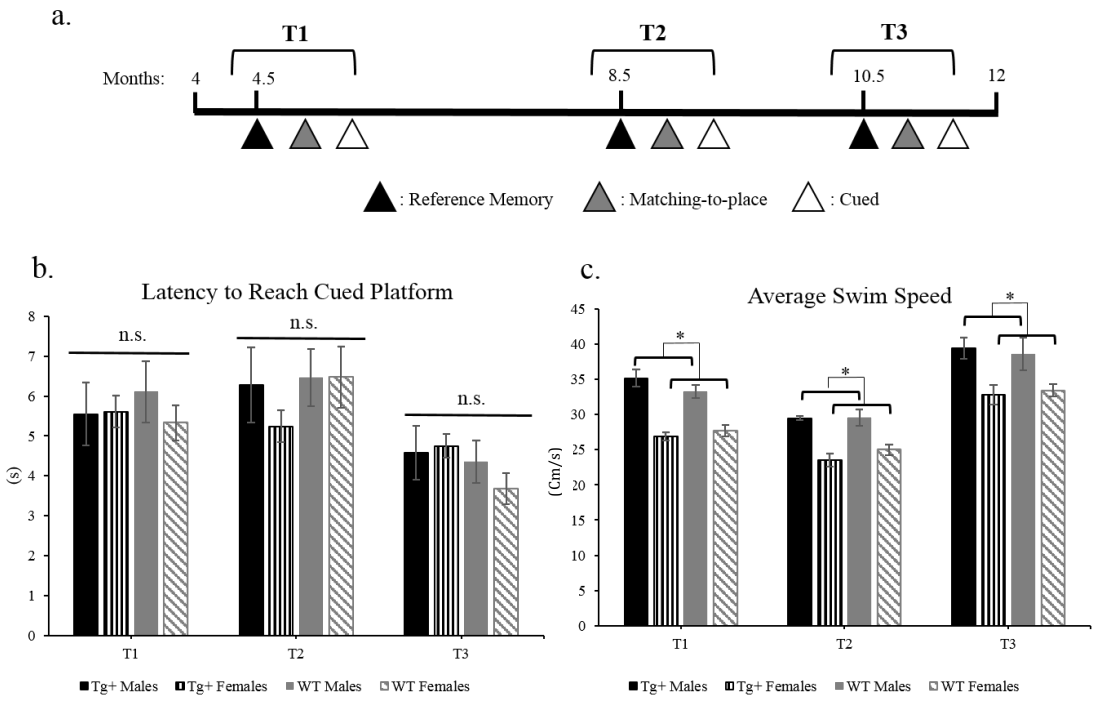
2017), while significant amyloid plaques, inflammation, tauopathy and cell loss occurs at 16 months. Thus, the differences observed in this study at 4.5 month & 8.5 month time points may indicate subtle effects of the burgeoning AD pathology as no memory deficits were detected. Furthermore, the subtle differences observed at 10.5 months in TgF344-AD males could be considered the MCI phase, though further characterization of pathology and behavior is needed.

Future studies using TgF344-AD rats should focus on elucidating the temporal association between neurodegeneration and spatial memory impairment. The hippocampus has been a strong focus in AD research, despite various Papez circuit structural involvement in AD (Aggleton, Pralus, Nelson & Hornberger, 2016). Specifically, future studies should identify when and where the initiation of pathology occurs in TgF344-AD rats. Cohen and colleagues (2013) identified inflammation and initial tau changes at 6 months of age in the cingulate cortex and hippocampus. However, earliest incidence of pathology in humans is associated with the entorhinal cortex and anterior dorsal thalamic nucleus (Braak & Braak, 1991). Whether TgF344-AD rats exhibit AD pathology in these structures at 6 months or earlier is unknown and warrants investigation. This is particularly important given cell types coding for direction (i.e. Head Direction Cells) and distance (i.e. grid cells) are found in the anterior dorsal thalamus (Taube, 1995) and medial entorhinal cortex (Hafting, Fyhn, Molden, Moser, & Moser, 2005) , respectively. Future electrophysiological studies in these regions might elucidate whether deficits of spatial representation contribute to TgF344-AD navigational impairment prior to 10.5 months.

Several limitations of this study are worth addressing. Testing spatial memory in the water maze task might not have been sensitive enough to detect subtle differences, particularly as subjects retained long-term memory of task demands in this study. Thus, the use of the radial arm maze, which is more taxing and is less sensitive to prior experience than the water maze, might allow for more robust characterization of spatial working memory in TgF344-AD rats (Hodges, 1996). In addition, hormone cycles were not tracked during the study for females. Although estrous has been implicated in sex differences of AD, many studies find impairments due to estrous after 19 months of age.

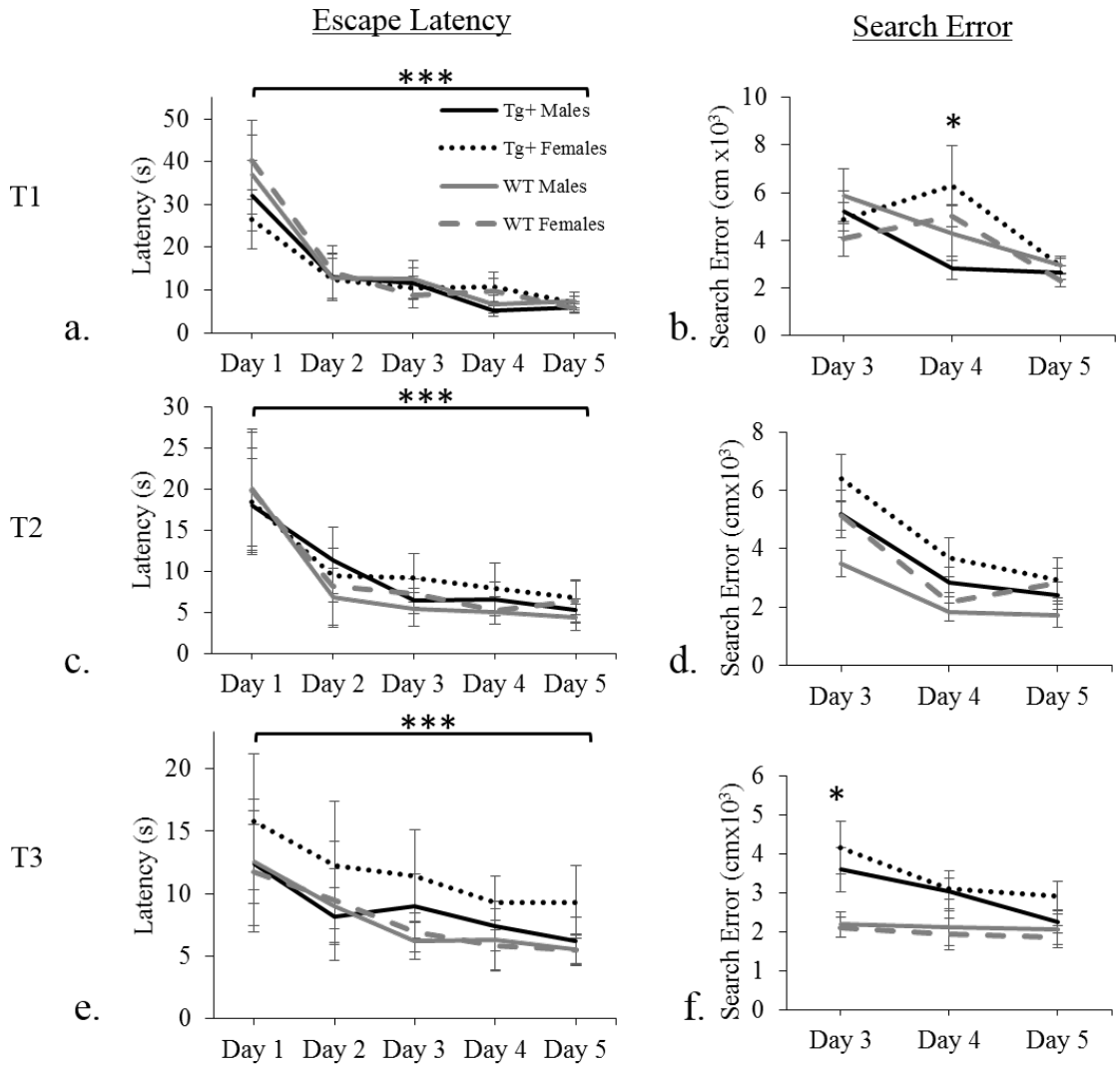
Figures

Figure 1. Study timeline and summary results for cued task and swim speed



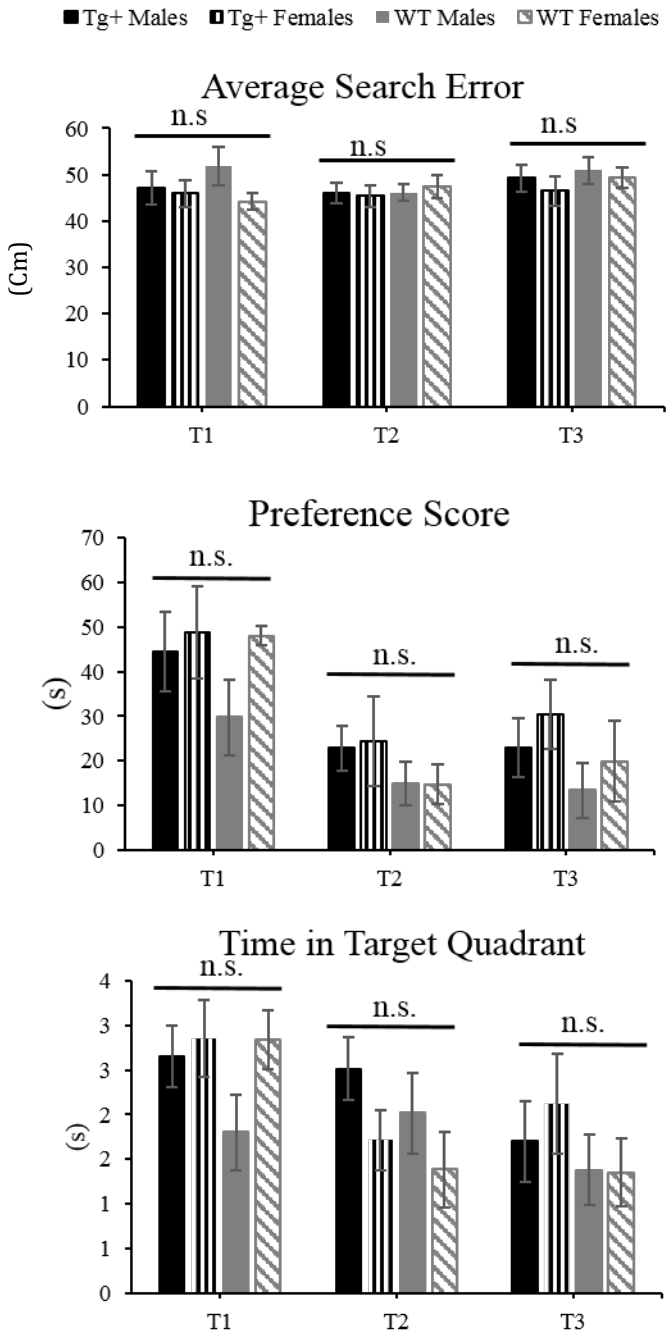
Note. Figure (a) summarizes administration of water tasks across each time point. Bar graphs display average time to reach the platform (in seconds) (b) and average swim speed (centimeters per second) (c) between groups and across time points. n.s.=p>.05, *=p<.001, T1=time point 1, T2=time point 2, T3=time point 3.

Figure 2. Summary Results for Reference Memory Learning



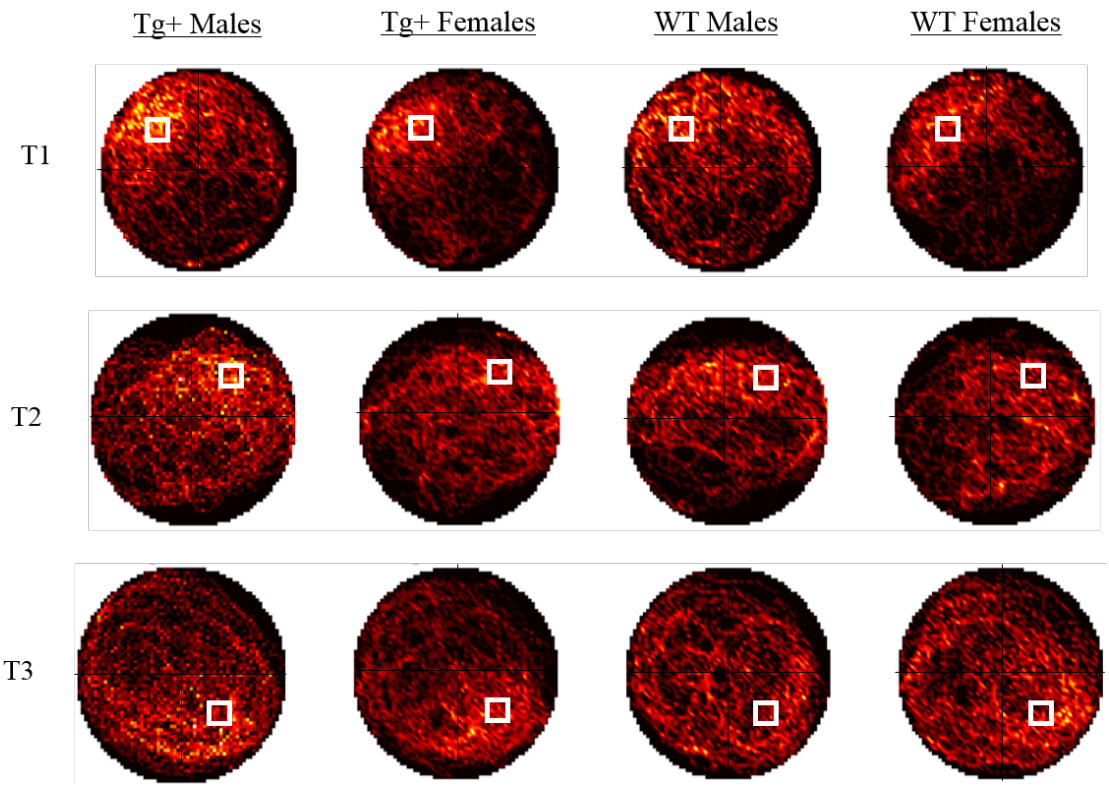
Note. Line graphs display averages by day between groups. Figure (a,c,e) summarize time to reach the platform (latency in seconds) while (b,d,f) summarize search error (centimeters) for the final three days of learning trials. *=p<.05m ***p<.001, T1=time point 1, T2=time point 2, T3=time point 3.

Figure 3. Reference Memory Probe Trial Summary Results



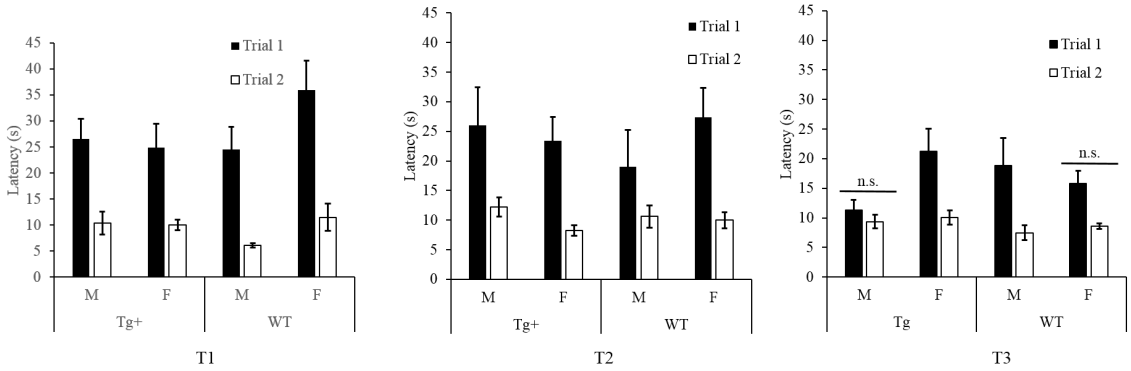
Note. Bar graphs display mean scores between groups for all time points. n.s.=p>.05.

Figure 4. Heat Maps of Swim Paths during Reference Memory Probe Trial



Note. Heat maps indicate dwell time (seconds) 2.5cm bins across entire 60 second probe trial. Warmer colors indicate increasing time in area. White square represents prior location of platform during learning trials. T1=time point 1, T2=time point 2, T3=time point 3.

Figure 5. Summary Results for Matching-to-Place task



Note. Bar graphs demonstrate average time to reach the platform for trial 1 and trial 2 in seconds. Trial 1 and Trial 2 differences were significant between trials in the first two time points as expected. Non-significant differences (p>.0125, Bonferroni corrected) were observed in the last time point for transgenic males and wild type control females. T1=time point 1, T2=time point 2, T3=time point 3.

Table 1. Day effects from 2 (Genotype) x 2 (Sex) x 5 (Day) Mixed ANOVA of Reference Memory Learning

Source	*df*	*MS*	*F*	*p*	η_p^2
		Time Point 1			
Day	1.913	2630.593	55.478	>.001	.698
Error	45.911	99.150			
		Time Point 2			
Day	2.253	1315.419	47.134	>.001	.663
Error	54.079	27.908			
		Time Point 3			
Day	2.420	356.156	12.830	>.001	.358
Error	55.653	27.759			

Note. ANOVA table only indicated errors associated with main effect of Day. *df*=degrees of freedom, *MS*=mean squared error, *F*=f statistic, *p*= p-value, η_p^2=Partial eta squared.

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