Impaired Spatial Reorientation in the 3xTg-AD Mouse Model of Alzheimer's Disease

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Abstract:

In early Alzheimer's disease (AD) spatial navigation is impaired; however, the precise cause of this impairment is unclear. Recent evidence suggests that getting lost in new surroundings is one of the first impairments to emerge in AD. It is possible that getting lost in new surroundings represents a failure to use distal cues to get oriented in space. Therefore, we set out to look for impaired spatial orientation in a mouse model of amyloidosis (3xTg-AD). We assessed spatial reorientation in male and female 3xTg-AD mice. For this task, mice were trained to shuttle to the end of a track and back to an enclosed start box to receive a water reward. Then, mice were trained to stop in an unmarked reward zone to receive a brain stimulation reward. The time required to remain in the zone for a reward was increased across training, and the track was positioned in a random start location for each trial. Thus, distal but not local cues were informative. We found that 6-month female 3xTg-AD mice were impaired, but 6-month male 3xTg-AD mice were not, when compared to non-Tg age/sex-matched controls. Male and female mice had only intracellular pathology at this age; however, consistent with the lack of impairment in male mice, less pathology was observed. Finally, 3-month female mice also had less intracellular pathology than 6-month female mice, and were not impaired at spatial reorientation. Thus, AD may cause spatial disorientation as a result of impaired use of landmarks to maintain spatial orientation.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting over 35 million people in the world and the leading cause of dementia (Billings et al 2005, Forner et al 2017). AD involves the formation of amyloid beta (Aβ) plaques followed by tau pathology in the form of neurofibrillary tangles (Billings et al 2005). A critical problem for developing treatments that prevent AD symptomology is that Aβ and tau pathology progress substantially before serious cognitive symptoms appear, leading to a diagnosis long after neurological damage has been done (McDade & Bateman 2017). Individuals with AD have multiple cognitive symptoms including memory and navigational impairments (Henderson et al 1989, Weintraub et al 2012). In fact, one of the earliest cognitive symptoms to arise in AD is spatial disorientation, particularly in new surroundings (Allison et al 2016, Henderson et al 1989, Weintraub et al 2012).

Mouse models designed to mimic the amyloidosis associated with AD are also impaired at spatial navigation (Attar et al 2013, Liu et al 2013, Marlatt et al 2013). Impaired spatial coding has also been reported with less stable spatial maps in 3xTg-Ad mice compared to non-transgenic (non-Tg) mice (Cayzac et al 2015, Mably et al 2017), though the precise cause of this deficit in navigation is unclear. Some studies suggest that navigation is the primary impairment (Cañete et al 2015, Davis et al 2017, Stover et al 2015), while others suggest the impairments may actually be a consequence of failure to retain information that was learned the previous day (i.e., a memory impairment; Billings et al 2005).

Navigational studies in AD have often used tasks designed to engage allocentric (map-like) navigational strategies; however, there is also evidence for egocentric navigational impairments in AD. AD can be differentiated from frontotemporal dementia (FTD) based on the presence of an additional egocentric deficit, while allocentric (map-like) impairments are present in both FTD

and also AD patients (Tu et al 2017). Consistent with these findings the parietal cortex (PC), which is thought to play a role in egocentric (e.g., viewer dependent) navigational strategies (Clark et al 2018, Nitz 2009, Nitz 2006, Nitz 2012, Whitlock et al 2012, Wilber et al 2014, Wilber et al 2017), is also dysfunctional in mouse models and humans with AD (Khan et al 2014). This dysfunction in PC was attributed to changes in a PC-hippocampal network. Emerging evidence from modeling and experimental studies suggests that this same PC-hippocampal brain network may translate the viewer-dependent (egocentric) representations of landmarks into allocentric (world-centered) coordinates for navigation (Clark et al 2018, Deshmukh & Knierim 2013, McNaughton et al 1995, Wilber et al 2014).

The ability to update knowledge of current location using surrounding landmarks would be critical for navigating new surroundings, this is precisely the deficit observed in AD (Allison et al 2016). Here we test the hypothesis that AD may specifically affect the ability to use distal cues to get oriented in space. In order to address this question, we selected a *spatial reorientation task* that was previously used to show deficits in aged rats that were linked to impaired updating of the hippocampal place field map (Rosenzweig et al 2003).

We use a well-characterized triple transgenic mouse model (APPSwe, PS1M146V, and tauP301L) that expresses three major genes associated with familial AD. The 3xTg-AD mouse exhibits plaque and tangle pathologies with a distribution pattern comparable to that observed in humans (Mesulam 1999). In humans with AD, loss of synaptic density precedes neuronal degeneration (DeKosky & Scheff 1990) and is a better predictor of memory loss than plaques and tangles (Flood & Coleman 1990, Masliah et al 2001). The 3xTg-AD mouse model also mimics these synaptic changes (Oddo et al 2003).

Methods

3xTg-AD male and female mice and age-matched non-transgenic controls (n=5/sex) were grouped housed (2 to 4 per cage) in 12:12 h light/dark cycles until the beginning of the experiment. Both 3xTg-AD mice (originally obtained directly from Dr. LaFerla), and non-transgenic controls of the same background strain as 3xTg-AD mice, were bred in our vivarium. We assessed two age groups both early in disease progression: 6-months and 3-months (n=5/age/sex). All experimental procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by both the University of California, Irvine and Florida State University Animal Care and Use Committees.

Pre-training

Mice were water deprived to no less than 80% of their beginning body weight and given food *ad libitum*. Mice were then trained to shuttle to a black barrier at the end of a linear track and back for a water reward (*alternation training*). This barrier was positioned so that there was a black background behind it (from the view of the mouse) on the wall. This track has the capability of moving its starting position, so the length of the track can be variable (**Fig. 1**). Starting position was randomly selected from 9 possible start locations for both *alternation training* and *spatial reorientation training* (9 possible start locations spaced over a range of 84 cm – 104 cm from the end barrier; distances listed in cm are approximations used for visualization purposes). After reaching criterion (either reaching asymptote, ± 6 total runs, 3 out of 4 days, or a total of 50 or more runs down the track and back), the mouse was scheduled for surgery to implant stimulating electrodes, and continued with *alternation training* every other day until the day before surgery when water deprivation ceased.

Stimulating Electrode Implantation

Once criteria were met on the *alternation training* task, mice underwent surgery to implant bilateral stimulating electrodes targeting the left and right medial forebrain bundle (MFB; -1.9 mm posterior to bregma, 0.8 lateral, 4.8 mm below dura). The implantation of these stimulating electrodes allowed for intracranial self-stimulation (Carlzon & Chartoff, 2007).

Stimulation Parameters

Following a 1 week recovery period, mice were placed in a custom 44 x 44 x 44 cm box with a circular, nose poke port (Med Associates) mounted on the left half of one wall of the chamber. First, mice were shaped using manual stimulations to approach the nose poke port, then for touching the port, and finally to automatically trigger brain stimulation with beam breaks by nose poking. Following shaping, a custom MATLAB program delivered one 500 ms brain stimulation reward for each beam break in the nose poke port. Over the course of one week,

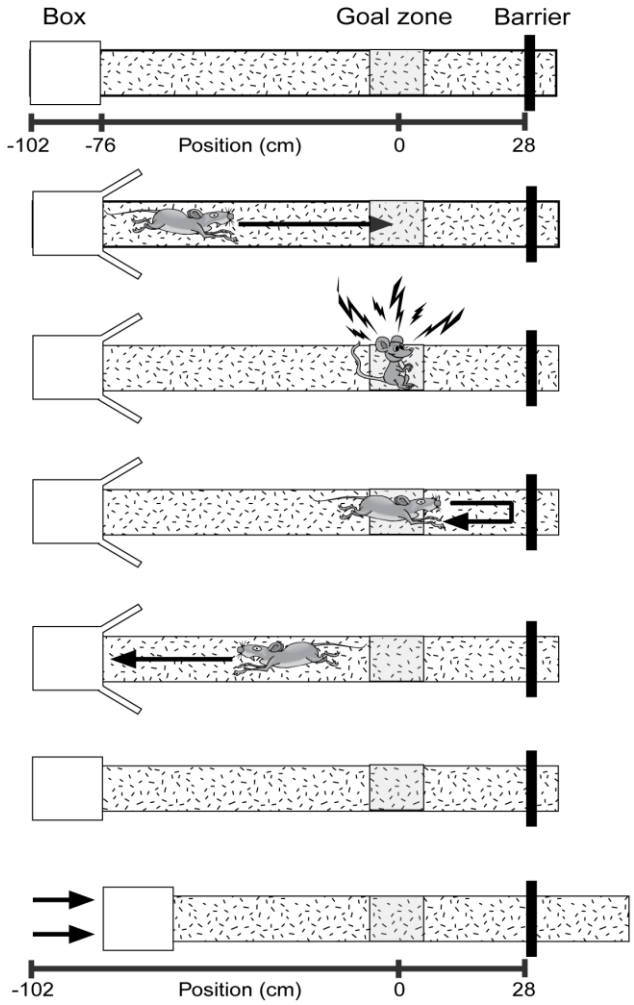

Figure 1. Spatial reorientation task. The rewarded location (grey box) is always fixed within the real room; however, the start box "moves" between trials. The sequence of events for each trial are illustrated (*top to bottom*). Each trial ends with the track being moved to a new randomly selected start location while the mouse is consuming a water reward. Thus each trial begins with the mouse "lost" with respect to the position of the grey reward zone in the room. After leaving the start box, the mouse gets position estimates initially from both self-motion (i.e., distance from the start box). As the mouse moves down the track, the position estimation is updated using room-cues. If position is successfully updated using room cues then the mouse will stop in the reward location for the required delay and obtain a brain stimulation reward. Adapted from Rosenzweig et al. (2003).

settings were adjusted (171-201 Hz frequency, 50-70 μA current, electrode wire combinations) to achieve maximal response rate. No attempt was made to balance responding across genotype or age; however, we did track and compare responding (nose poke rate) across genotype and age to ensure that differences in reward strength were not likely to contribute to the observed effects. We found no significant effects of genotype (3xTg-AD vs non-Tg) on response rate at either age (3-months or 6-months) or for either sex ($ts_{(8)} < -0.09$, $p > 0.41$).

Spatial Reorientation Training

Once mice were responding maximally (the minimum response rate was 0.8 pokes/min), mice underwent one additional refresher *alternation training* session, then *spatial reorientation training* commenced (adapted from the Rosenzweig et al 2003 task for rats). Throughout the remainder of training and testing, mice shuttled back and forth for a water reward that was delivered in the start box and consumed while the track was moved to the next randomly selected starting location (9 possible start locations spaced over a range of 84 cm – 104 cm from the end barrier; random lists were generated using Random.org, random with repeats). Next, an unmarked reward zone was added to the task (centered 24 cm from the barrier at the end of the track) that could automatically trigger a single brain stimulation reward if the mouse remained in this zone for a sufficient period before progressing to the end of the track (**Fig. 1**). This zone is fixed in relationship to the room and the landmarks within it and was marked in camera coordinates (i.e., there were no visible markings in the room or on the track indicating the location of the reward zone). Further, since the track was moved to new positions following each trial, any olfactory or other cues on the track could not signal the location of the reward zone. If the mouse remained in the zone for the duration of a delay period (starting at 0.5 s), then a brain stimulation reward was delivered. The delay was fixed for a given day and was increased by 0.5 s (up to 2.5 s) each time the mouse met a criterion

of similar percent correct (±15%), 3 out of 4 days. Olfactory cues could not be used to solve the task, because the entire track was moved after each trial, both during alternation training and following the addition of the hidden reward zone. Thus, the reward zone occurred at a variety of physical locations on the track, but always in a fixed location within the room.

Probe Testing

In order to ensure that mice were performing the task as intended, at the conclusion of training and testing, mice underwent two probe tests. In order to ensure that memory for each starting position was not contributing to the observed effects, after reaching criteria for the last 2.5 s delay, mice underwent an additional 2.5 s reward delay session, but with 20 possible random starting positions (same range as the random 9 condition, but with smaller increments between starting locations). After mice reached asymptote on this first probe test (a drop of no more than 15% compared to asymptote from the 2.5 s reward delay), to ascertain that the mice are not using the end barrier as the sole landmark for the reward zone, a second day with 20 random starting positions was administered without the barrier at the end of the track. Mice were considered to have succeeded with the same criteria as the first probe test (no less than -15% compared to asymptote from the 2.5 s reward delay). When piloting this behavioral protocol, we found that, when the end barrier was not positioned so that it was a black barrier on a black background, performance dropped considerably during this barrier removed probe test. Therefore, care was taken to ensure the black barrier was always positioned with a black background and a barrier removed probe test was conducted for all data reported here to ensure that no mice were using the barrier as the sole landmark for the reward location. Throughout the spatial reorientation task and probe testing, the mice were video recorded and their position was tracked using video tracking software

(Neuralynx). Velocity data by position data were extracted from the tracking data and analyzed as described below using custom matlab (Matlab) scripts.

Histology

At the conclusion of the experiment, mice were given an intraperitoneal injection of Euthasol and then transcardially perfused with 0.1 *M* phosphate-buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in 0.1 *M* PBS. The whole head was post-fixed for 24 h, to allow us to identify the location of MFB electrodes, and then the brain was removed and post-fixed for another 24 h. Lastly, the brain was cryoprotected in 30% sucrose in 0.1 *M* PBS. Frozen sections were cut coronally with a sliding microtome at a thickness of 40 μm and split into 6 evenly spaced series.

Anti β-Amyloid 1-16 Immunohistochemistry. One series of sections was mounted on slides, incubated in 4% PFA for 4 min, and then rinsed with Tris-buffed saline (TBS). Next, slides were soaked in 70% formic acid for 8 – 15 min. After rinsing in TBS, slides were incubated in 0.1% Triton-X for 15 min, followed by 0.1% Triton-X and 2% bovine serum albumin (BSA) for 30 min. Sections were incubated with Anti-β-amyloid 1-16 (clone 6E10, Biolegend) 1:1000 and anti-NeuN (polyclonal, rabbit, Milipore) in 0.1% Triton-X and 2% BSA for 2 days. After rinsing with TBS, slides were soaked in 0.1% Triton-X for 15 min, followed again by 0.1% Triton-X and 2% BSA for 30 min. Staining was visualized with anti-mouse-alexa-488 (1:1000) and anti-rabbit-alexa-594 (1:500) in 0.1% Triton-X and 2% BSA for 5-6 hrs. Slides were coverslipped after being rinsed with TBS. Whole slides were imaged as described below, then the coverslip was removed and DAPI (0.01 mg/ml) was added before the slides were recoveslipped and reimaged.

Except for β-Amyloid 1-16, as noted above, histology was performed on free-floating sections. Sections are permeabilized in 0.3% Triton-X and blocked in 3% Goat Serum in TBS, then incubated in primaries antibodies.

Thioflavin S. Anti-NeuN 1:1000, overnight, was followed by anti-rabbit-alexa-594 1:500 for 5h. Sections were rinsed then immersed in a 1% Thioflavine S solution (Sigma) for 9 min, rinsed in dH2O, destained in 70% Ethanol for 5 min, rinsed in dH2O, and then transferred to TBS before mounting onto slides.

Phosphorylated tau. Anti-Phosphorylated tau 1:500 (Monoclonal, mouse, MN1020, Thermo Scientific) with Anti-NeuN 1:1000 were added overnight. Secondary antibodies were anti-mouse-alexa-488 1:1000 and anti-rabbit-alexa-594 1:500 respectively, for 6h. Section were rinsed and mounted onto slides.

M22. Sections were incubated in anti-M22 (obtained directly from Dr. Charles Glabe) 0.5ug/ml for 2 days followed by anti-rabbit-alexa-488 (IgG (H+ L), goat, A11008, Invitrogen) 1:500 overnight. Sections were rinsed several times and anti-NeuN-Cy3 (polyclonal, rabbit, ABN78C, Milipore) 1:300 was added overnight. Slides were coverslipped with Vectashield H-1000 (Vector Laboratory) and imaged.

Image Acquisition

Whole slides were scanned at 40x magnification using a scanning microscope (NanoZoomer Digital Pathology RS; Hamamatu). In addition, a subset of regions were examined in greater detail by acquiring image stacks on a confocal microscope (Olympus, FV1000).

Genotyping.

We received homozygous 3xTg-AD mice from Dr. Frank Laferla's lab. We confirmed that all mice used in the experiment contained each transgene using conventional PCR. DNA was extracted from the tails of each mouse during euthanasia. Homozygosity was confirmed by cutting the PS1 PCR fragment with the *BstEII* restriction enzyme. Only the mutated human PS1 gene contains a *BstEII* cut site and will be cut. The absence of an uncut PCR product indicated that the mouse was indeed homozygous for the human PS1. The presence of overexpressed APP and Tau were also confirmed by PCR. The following primers were used for amplifying the PS1 transgene: 5'-CAC ACG CAA CTC TGA CAT GCA CAG GC-3' (PS1 Forward) and 5'-AGG CAG GAA GAT CAC GTG TTC AAG TAC-3' (PS1 Reverse). APP and Tau primers used were: 5'- GCT TGC ACC AGT TCT GGA TGG -3' (APP Forward) and 5'- GAG GTA TTC AGT CAT GTG CT -3' (APP Reverse) and 5'- GAG GTA TTC AGT CAT GTG CT -3' (Tau Forward) and 5'- TTC AAA GTT CAC CTG ATA GT -3' (Tau Reverse) respectively.

Statistical Analyses

Data were analyzed using two-way repeated measures ANOVAs (genotype x delay, sex x delay, and age x delay) followed by planned comparisons. Planned comparisons consisted of two-group *F*-tests done within the context of the overall ANOVA (Maxwell and Delaney, 2003), comparing the non-Tg group to the 3xTg-AD group. For all statistical analyses, $p < 0.05$ was considered significant.

Results

Female 6-month 3xTg-AD mice are impaired at spatial reorientation.

As mice learn the location of the reward zone, they slow during the approach to the reward zone, then stop and remain in the zone location for the required delay to obtain a brain stimulation reward. Therefore, as a first step in assessing spatial reorientation ability in 3xTg-AD mice, we isolated velocity data for one reward zone radius just before the front edge of the reward zone (track location used for data shown in **Fig. 2A** is marked on **Fig. 2B**. with an orange bar and the reward zone location is marked on Fig. 2B. with a blue bar). This measure allows for comparison of performance across reward delays (i.e., across the range of task difficulty). In addition, measuring the velocity in front of the reward zone avoids contamination from velocity change that results from running slowly through the reward zone at easier delays (e.g., 0.5s) and stopping after accidentally obtaining the reward (as observed with easier delays). We converted these velocity

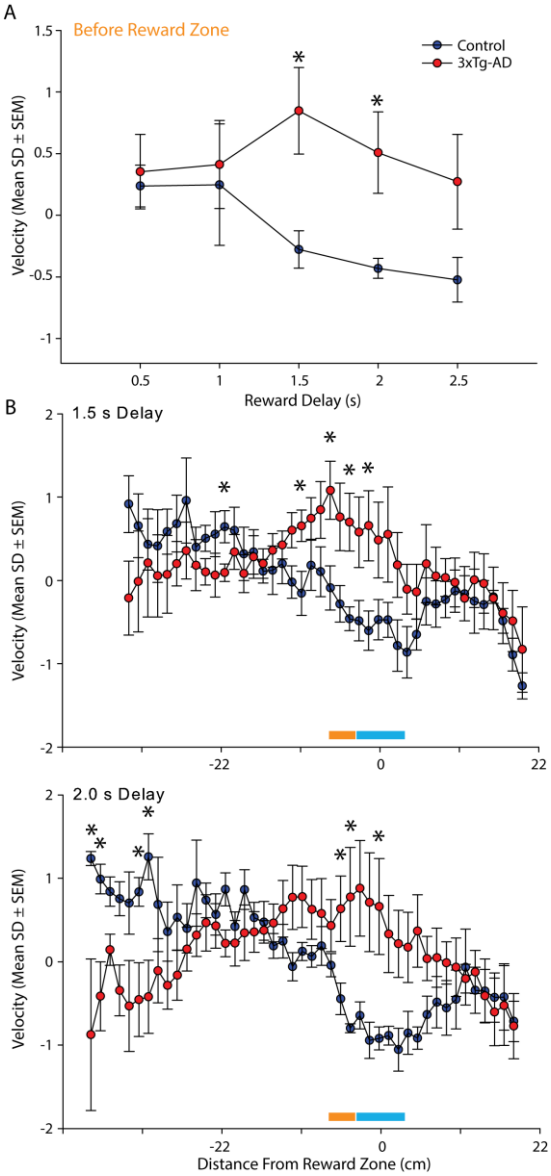

Figure 2. 3xTg-AD female mice are impaired at the spatial reorientation task A. Mean (±SEM) Z-scored velocity from a reward zone radius span of track just prior to the reward zone (orange bar in **B**; reward zone is the blue bar) for each reward delay (0.5-2 s) for 6-month female control (blue) and 3xTg-AD (red) mice. At more difficult delays, control mice slowed down more than 3xTg-AD female mice. **B**. Mean (±SEM) Z-scored velocity plotted along distance of the track during the *1.5 s reward delay* for 6-month female control and 3xTg-AD mice. Control mice slowed more for the approach and into the reward zone (blue bar) compared to 3xTg-AD mice. **C**. Same as in B, but for the *2.0 s reward delay*. Again, 3xTg-AD mice failed to slow in the reward zone compared to controls. *$p < .05$

values to z-scores in order to equalize performance across mice with different running speeds. Next, we compared these normalized velocity values during the approach to the reward zone for 3xTg-AD mice to age matched non-Tg controls. Velocity for 6-month female mice did not vary significantly across delay (**Fig. 2A**; $F_{(4, 32)} = 2.25$, $p = 0.09$), nor across genotype ($F_{(1, 32)} = 3.03$, $p = 0.12$). However, there was a significant interaction between delay and genotype ($F_{(4, 32)} = 3.00$, $p < 0.05$). We followed up on this significant interaction with planned comparisons across genotype for each delay and found that 3xTg-AD female mice performed significantly worse than non-Tg age matched controls, for the 1.5 and 2 s ($Fs_{(1, 8)} > 7.60$, $p < 0.05$), but not 0.5, 1.0 or 2.5 s ($Fs_{(1, 8)} < 3.50$, $p > 0.10$) reward delays. This suggests that non-Tg mice were identifying the location of the reward zone and slowing down in preparation for stopping in the zone, but that 3xTg-AD females were not.

Next, we examined the mean Z-scored velocities for the full length of the track individually for each reward delay where we observed a significant effect of genotype (the 1.5 and 2 s delays) (**Fig. 2B**). During the 1.5 s delay, velocity varied across the length of the track (**Fig. 2B** *top*; $F_{(41, 328)} = 2.46$, $p < 0.0001$), but did not vary across genotype ($F_{(1, 328)} = 5.01$, $p = 0.06$). However, the velocity profile varied significantly as a function of genotype and position on the track (genotype x position interaction: $F_{(41, 328)} = 1.79$, $p < 0.01$). Planned comparisons showed that 6-month female 3xTg-AD mice slowed less than non-Tg mice for multiple locations on the track just prior and into the reward zone (-9 cm, -5 cm, -2 cm, 1 cm; $Fs_{(1, 8)} > 6.17$, $p < 0.05$), but not other locations prior to the reward zone ($Fs_{(1, 8)} < 5.15$, $p > 0.05$), nor after the reward zone ($Fs_{(1, 8)} < 3.09$, $p > 0.12$). In addition, non-Tg mice ran faster than 3xTg-AD mice for 1 location near the beginning of the track (-21 cm; $F_{(1, 8)} = 6.33$, $p < 0.05$). Together this pattern of data suggest that non-Tg 6-month female

mice ran faster at early portions of the track than 3xTg-AD mice and non-Tg mice slowed more during the approach and into the reward zone than 3xTg-AD mice.

During the 2 s delay, there was not a significant main effect of genotype on velocity ($F_{(1, 328)} = 0.67$, $p = 0.44$), but there was a significant effect of position on the track (**Fig. 2B** *bottom*; $F_{(44, 328)} = 1.73$, $p < 0.01$), and a significant genotype by distance interaction ($F_{(44, 328)} = 2.58$, $p < 0.0001$). Planned comparisons showed that 6-month female 3xTg-AD mice slowed less than non-Tg for multiple locations on the track just prior and into the reward zone (-3 cm, -2 cm, 3 cm; $Fs_{(1, 8)} > 6.17$, $p < 0.05$). There were four locations present towards the beginning of the track where 3xTg-AD female mice were slower than non-Tg female mice (-42 cm, -40 cm, -34 cm, -33 cm; $Fs_{(1, 8)} > 5.58$, $p < 0.05$). No significant difference occurred at other locations prior to the reward zone ($Fs_{(1, 8)} < 4.97$, $p > 0.06$), nor after the reward zone ($Fs_{(1, 8)} < 2.41$, $p > 0.16$). Thus, at both delays (i.e., two second highest difficulty levels), control mice slowed significantly more than 3xTg-AD mice whether measures were taken just prior to entering the reward zone or across the entire span of the track.

Probe testing suggests that female mice are using distal cues to get oriented in space. Finally, we used a probe testing without the end barrier and our threshold for 'passing' this probe test was that a drop in the percentage of trials with successful rewards, could drop by no more than 15%. The reference point for measuring this drop was the asymptote of the 2.5 s reward delay. Similar results were obtained for the random 20 zone list, where again, no more than a 15% drop in correct responding was observed.

Comparing the first vs last trials at each reward delay suggests that impairments in 3xTg-AD female mice are not a consequence of forgetting from day to day. Separately for the

first ⅓, and again for the last ⅓ of the trials for each daily session, we calculated the mean Z-scored velocity for the portion of the track just in front of the reward zone. Then, we subtracted the first ⅓ and last ⅓ velocity data to calculate a change in velocity from the beginning to the end of each session. If the impaired performance in 3xTg-AD mice was a consequence of impaired learning across days, but intact learning within the daily session as was previously reported for the Morris water maze (Billings et al 2005), then for this 'change score' there should be an interaction between reward delay and genotype. Velocity change from the first ⅓ of trials to the last ⅓ of trials for 6-month female mice did not vary significantly across delay ($F_{(4, 32)} = 0.39$, $p = 0.81$), nor across genotype ($F_{(1, 32)} = 4.32$, $p = 0.07$). Further, there was not a significant interaction between delay and genotype ($F_{(4, 32)} = 0.52$, $p = 0.72$). Thus, forgetting across delays was not likely to explain the impaired spatial reorientation we observed in 6-month 3xTg-AD female mice.

Male 6-month 3xTg-AD mice are not impaired at spatial reorientation.

Next, we examined performance of 6-month male mice. As with female mice, the male mice Z-scored velocity from the portion of the track just in front of the reward zone again did not vary across delay ($F_{(4, 32)} = 1.12$, $p = 0.37$), nor across genotype ($F_{(1, 32)} = 2.34$, $p = 0.16$). However, unlike females of the same age, there was not an interaction between delay and genotype ($F_{(4, 32)} = 0.52$, $p = 0.72$). Thus, 6-month female but not male 3xTg-AD mice are impaired at the spatial orientation task.

Differences in markers of pathology but not average velocity corresponds to the sex difference in 6-month 3xTg-AD mice. First, we checked to see if a sex difference in non-Tg male and female mice might contribute to an apparent sex difference in 3xTg-AD mice of the same age.

No significant effect of sex on velocity was present ($F_{(1, 32)} = 3.89$, $p = 0.08$), or a significant effect of reward delay ($F_{(4, 32)} = 1.84$, $p = 0.15$). In addition, there was not a significant interaction between sex and delay ($F_{(4, 32)} = 1.43$, $p = 0.25$). Second, to ensure that differences in running speed between the 6-month male and female mice were not contributing to the observed effects, we assessed mean raw velocities. Velocities were only included from the portion of the track where differences in Z-scored velocities were not observed (i.e., from 104 cm to 39 cm in front of the reward zone). In other words, we looked at average velocity from the portion of the track where running speed was not expected to vary in order to perform the task (i.e., baseline velocity). Baseline velocity did not vary between sex ($F_{(1, 16)} = 0.70$, $p = 0.41$), or genotype ($F_{(1, 16)} = 0.30$, $p = 0.59$) and there was not a significant interaction between genotype and sex ($F_{(1, 16)} = 0.56$, $p = 0.46$; Mean ±SEM velocity for non-Tg females: 10.03 ±1.95; non-Tg males: 10.16 ±0.39; 3xTg-AD females: 9.75 ±0.87; 3xTg-AD males: 11.92 ±1.65).

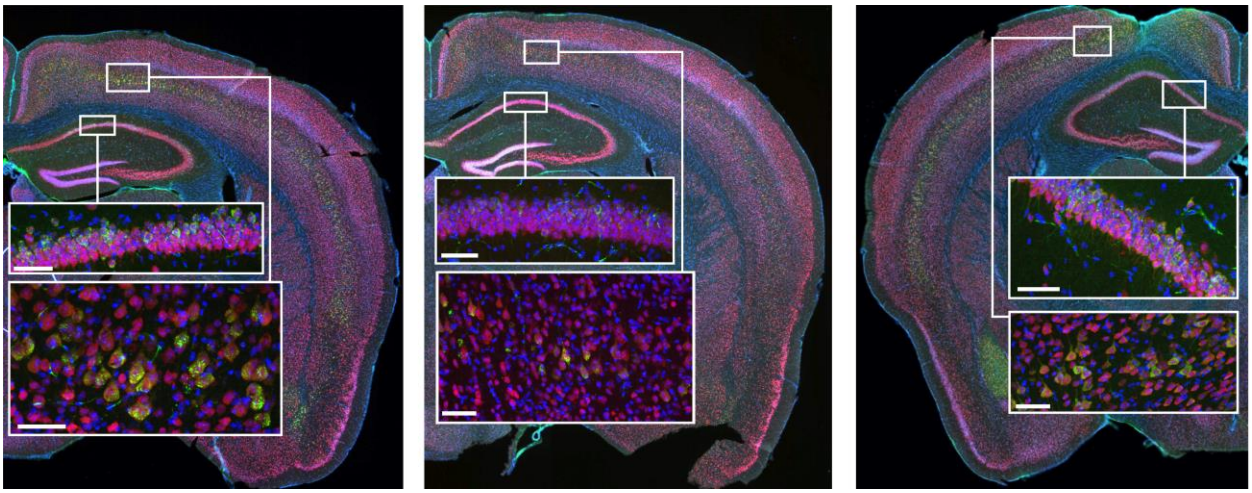

Figure 3. Female 6-month 3xTg-AD mice have more disease related pathology than 6-month males. Sections were triple labeled with DAPI (blue), NeuN (red) and using β-Amyloid 1-16 (green; $A\beta_{1-17}$ clone). Whole section images were acquired and high resolution using a slide scanner, then selected regions of interest were imaged using a confocal microscope (insets). Representative staining is shown for for a 6 month 3xTg-AD female mouse (*left*) a 6-month male mouse (*right*) and a 3-month female mouse (*below*). At 6-months female and male mice have only intraneuronal Aβ and no extracellular pathology is observed. Both male mice and 3-month female mice appear have less intracellular Aβ in hippocampus and cortex. Representative confocal images are shown in the insets for cortex (*top*) and CA1 of hippocampus (*bottom*) of for each hemi-section. Scale bars are 60 μm.

Finally, when we examined the histology from these male and female mice it appeared that amyloid beta (Aβ) pathology, as measured by the proportion the subjective number of β-Amyloid 1-16 (Aβ1-16 specific antibody) positive cells in 6-month female 3xTg-AD mice, appears to be greater than in males (**Fig. 3**). The level of other markers of pathology were very low at this age (thioflavin S, phosphorylated tau, M22; i.e., floor effect) and thus were not compared between males and females (not shown). This suggests that a sex difference in pathology, but not sex differences in non-Tg animals or differences in running speed, may correspond to the sex difference in impaired spatial orientation in 6-month 3xTg-AD mice.

Female 3-month 3xTg-AD mice are not impaired at spatial reorientation.

Since impairment in spatial reorientation appeared in female 3xTg-AD mice at 6-months, we assessed 3-month females on this task to determine if this impairment occurred earlier on in disease progression. Mean Z-scored velocity just in front of the reward zone at this age did not vary across delay ($F_{(4, 32)} = 0.68$, $p = 0.61$) or genotype ($F_{(1, 32)} = 0.43$, $p = 0.53$) and there was not a significant interaction between delay and genotype ($F_{(4, 32)} = 0.98$, $p = 0.43$). Next, we examined the possibility that an age difference in the ability of control mice to perform the task might contribute to the lack of impairment in 3-month 3xTg-AD female mice. That is, the lack of impairment observed in 3-month old mice might actually be an inability to perform the task. However, when 3-month and 6-month non-Tg female mice were compared, velocity did not vary with age ($F_{(1, 32)} = 0.22$, $p = 0.65$), or reward delay ($F_{(4, 32)} = 1.75$, $p = 0.16$), and there was not a significant interaction between age and delay ($F_{(4, 32)} = 0.79$, $p = 0.54$). This suggests that performance of 3-month control mice was not significantly worse than 6-month controls and thus provides further evidence that 3-month 3xTg-AD female mice do not have impaired spatial orientation. Next, to ensure that developmental differences in running speed were not contributing

to the observed effects, we assessed mean raw velocities between 3- and 6-month female mice. Velocity did not vary across age ($F_{(1, 16)} = 0.04$, $p = 0.85$), nor across genotype ($F_{(1, 16)} = 0.18$, $p = 0.68$) and there was not a significant interaction between genotype and sex ($F_{(1, 16)} = 0.45$, $p = 0.51$; Mean ±SEM velocity for non-Tg 3-month females: 9.08 ± 0.26; 3-month 3xTg-AD females: 10.30 ± 0.61; non-Tg 6-month females: 10.03 ± 1.95; 6 month 3xTg-AD females: 9.75 ± 0.87). Finally, we examined Aβ in 3-month 3xTg-AD mice and found less Aβ pathology compared to 6-month 3xTg-AD female mice. (**Fig 3**). Pathology in 3-month 3xTg-AD mice appears to be less than 6-month mice, but greater than that observed in 6-month 3xTg-AD male mice.

Discussion

We found that 6-month female 3xTg-AD mice were impaired at spatial reorientation when challenged with a task requiring them to learn to utilize distal cues to become reoriented, compared to non-Tg mice. In contrast, younger female mice (3-months of age) and 6-month male mice were not impaired at the same spatial reorientation task. The impaired spatial reorientation in female mice was observed early in disease progression, when *intracellular* accumulation of Aβ was apparent, but *prior to the appearance of extracellular pathology*. The lack of impairments in younger female mice, and male mice of the same age, corresponded to an earlier point in the disease progression when the intracellular pathology was less apparent, compared to 6-month female mice. Together our data suggest that impairments in spatial reorientation emerge very early in disease progression and that the ability to get reoriented in space may be sensitive to subtle differences in disease progression in early AD.

The impaired spatial reorientation we observed in 3xTg-AD mice is consistent with impairments in spatial memory dependent tasks such as the Morris Water maze, Barnes maze and

T-maze in 3xTg-AD mice (Davis et al 2017, Stover et al 2015). Interestingly, the deficit in the Morris Water maze was also shown to emerge early in disease progression, before extracellular pathology was apparent; however, the spatial navigation impairment in the water maze appeared to be driven, at least largely, by forgetting from day to day with apparently normal learning across the sessions within a day. However, we did not see evidence for such an effect in our data when we compared early and late training trials, suggesting a disassociation at a similar point in disease progression (intracellular pathology) between memory and navigation impairments. Together, this suggests that it might be possible to assess deficits in either memory or navigation in AD and assess the relative effects of treatments on these potentially dissociable abilities.

In humans, AD has been shown to be more prevalent in females, including early stages of disease progression (Vina & Lloret 2010). Similarly, we observed that 6-month female, but not male mice, were impaired at spatial reorientation. Consistent with the lack of impairment with males, less pathology was observed in male mice. Thus, while the precise mechanism of this sex difference is unknown, the underlying cause may be related to a sex difference in pathology. Similarly, female 3xTg-AD mice also perform worse than 3xTg-AD male mice on the Morris water maze (Cañete et al 2015). However, in contrast to the results for spatial reorientation and water maze tasks where females performed worse, males were impaired on the Barnes maze spatial navigation task but females were not (Stover et al 2015). The relationship between the sex difference and brain pathology was not described for that report, so it is unclear if variability in brain pathology, or other factors contributed to this contradictory result.

The ability to successfully reorient during the spatial reorientation task is correlated with the realignment of the hippocampal place field map from start box centered coordinates to room centered coordinates and aged rats have both poorer realignment to room centered coordinates and

also impaired spatial reorientation (Rosenzweig et al 2003). Together, this suggests that a similar dysfunction in hippocampal place fields realignment to distal cues may be the cause of the behavioral impairment observed in female 3xTg-AD mice. Interestingly, the entorhinal cortex is also dysfunctional in AD models that mimic tauopathy, suggesting a larger brain network might be involved in spatial navigation impairments (Fu et al 2017). Future studies could directly examine the possibility that impaired hippocampal place field map alignment is ultimately responsible for the deficits we observed by recording from the hippocampus of 3xTg-AD mice while performing this task.

Navigation deficits are also a key feature of humans with AD, emerging early in disease progression (Henderson et al 1989, Weintraub et al 2012), especially when navigating in new surroundings (Allison et al 2016). The recent finding of impaired navigation in new surroundings is especially relevant for the present paper, because this is a key feature of the spatial reorientation task. This task requires the mouse to use features of the environment to determine the new starting location, and thus might be related to the impaired navigation in new environments observed in humans with AD. Interestingly, most human and animal studies have focused on allocentric (map-like) aspects of spatial navigation; however, a recent report showed that a distinguishing feature of human AD is the presence of both egocentric (viewer-dependent) and allocentric navigational impairments (Tu et al 2017). In fact, the spatial reorientation task requires translating between egocentric (viewer dependent) information about the landmarks and allocentric (map-like) representations of space (Byrne et al 2007, Clark et al 2018, McNaughton et al 1994, Oess et al 2017, Peyrache et al 2017, Whitlock et al 2012, Wilber et al 2014, Wilber et al 2017). This suggests that hippocampal and even entorhinal cortex changes may not be sufficient to explain the navigational deficits we and others have observed, and that future studies should look at

hippocampus and a larger brain network for spatial navigation in order to understand the changes underlying navigational impairments in AD.

In summary, we have shown a sex-dependent impairment in spatial reorientation in 3xTg-AD mice, early in disease progression. The spatial reorientation task we utilized requires translation between viewer dependent and allocentric (map-like) reference frames, thus setting the stage for future studies examining the brain systems that underlie this ability in AD.

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