Amyloid Pathology Profile Across a Parietal-Hippocampal Brain Network Associated With Spatial Reorientation Learning and Memory Performance in the 3xTg-AD Mouse

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Background:

One of the earliest symptoms of Alzheimer's disease is impaired spatial navigation. This ability is impaired in AD mice (Stimmell et al, 2019). We previously demonstrated that the 3xTg-AD mice, specifically 6-month females, show impairments on spatial reorientation tasks. This mouse model has tau and amyloid beta aggregation. We previously analyzed tau and amyloid beta accumulation patterns relationship with reorientation deficits (Stimmell et al, 2021). At 6-months, female 3xTg-AD mice showed tau accumulation in spatial navigation networks that predicted spatial reorientation impairments as identified by independent components analysis. Amyloid beta accumulation did not predict performance. However, we assessed amyloid accumulation with non-specific amyloid beta 1-16 (6e10) and conformation specific amyloid beta 1-42 (moc78) stains. This current project assess if MOC22, another conformation specific amyloid beta stain that is also specific to amyloid beta 1-42, is a better predictor of spatial navigation performance than MOC78.

Methods:

Animals. Male and female 3xTg-AD mice and age matched controls were group house in a 12:12h light to dark cycle. Water restriction was administered to no less than 80% of starting body weight so water could be used to motivate mice to run back and forth on the track.

Surgical Procedures. After pretraining mice to run back and forth on a track for a water reward, two bipolar stimulating electrodes were surgically implanted to activate the medial forebrain bundle and allow use of MFB stimulation as an additional reward (Benthem et al., 2020).

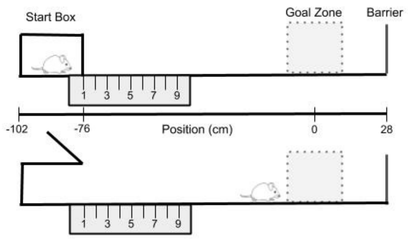

Figure 1: Spatial reorientation task. The goal zone (rewarded location; grey box) is always fixed within the room; however, the start box moves between trials. The sequence of events for each trial are illustrated (*top to bottom*). Each trial ends (*bottom*) with movement of the track to a new randomly selected start location while the mouse is consuming a water reward. Thus, each trial begins (*top*) 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 self-motion (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.

Behavioral Procedure. The mice run along a linear track and can slow in an unmarked reward zone for a brain stimulation reward. The unmarked reward location is fixed in the room; however, the start position varies between trials. If only ran in the long track (**Fig. 1** *top*), the mouse could get position estimates from self-motion and distal cues; however, since there are randomly selected track lengths, only surrounding room cues give accurate position information. Note, barrier is not used as a cue if the black barrier is placed on a black background. Probe trials at end of training ensure the mouse has not used barrier as cue for finding reward zone. The time the mouse must remain in the reward zone varies over the course of training from 0.5-2.5s with increases by 0.5s each time asymptote is achieved.

Staining Protocol. After mice have completed the behavior task, they are perfused, and brains are extracted and sectioned. Tissue sections are rinsed and then blocked in TBS-Triton + 3% goat serum. Tissue is then incubated with *Anti-MOC22*and anti-NueN overnight. after rinsing again, tissue incubates with Anti-rabbit-alexa-488 and anti-chicken-alexa-594 for 6hrs to overnight. Finally, slices are mounted slides and coverslipped using antifade mounting media with DAPI. Cell counting was done manually, and z-scored across raters to account for any differences in vision. Cells positive for amyloid beta as measured by MOC22 were compared to behavioral performance in the 3xTg-AD mice using independent component analysis to identify staining patterns followed by performing correlations (corrected for repeated comparisons) between each independent component and behavioral data.

Impaired spatial reorientation in 6-month female 3xTg-AD mice

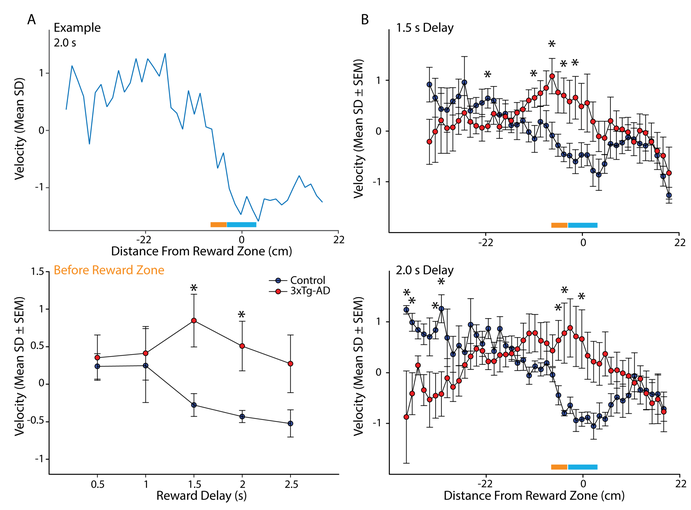

Figure 2: 6-month 3xTg-AD female mice are impaired at the spatial reorientation task. (**A**) *Top*. Example mean Z-scored velocity for a single 6-month female non-Tg mouse for the 2.0 s reward delay. This example shows that for a longer reward delay this mouse had learned to slow as it approached the reward zone. *Bottom*. 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 (0.5–2.5 s) for 6-month female non-Tg (blue) and 3xTg-AD (red) mice. At more difficult delays, non-Tg mice slowed down more than 3xTg-AD female mice. (**B**) *Top*. Mean (±SEM) Z-scored velocity plotted along distance of the track during the *1.5 s reward delay* for 6-month female non-Tg and 3xTg-AD mice. Non-Tg mice slowed more for the approach and into the reward zone (blue bar) compared to 3xTg-AD mice. *Bottom*. Same as *Top* but for the *2.0 s reward delay*. Again, 3xTg-AD mice failed to slow in the reward zone compared to controls. $*p < 0.05$.

Results:

The 6-month male and female mice only had intracellular pathology and male mice has less pathology, specifically in the dorsal hippocampus compared to the female.

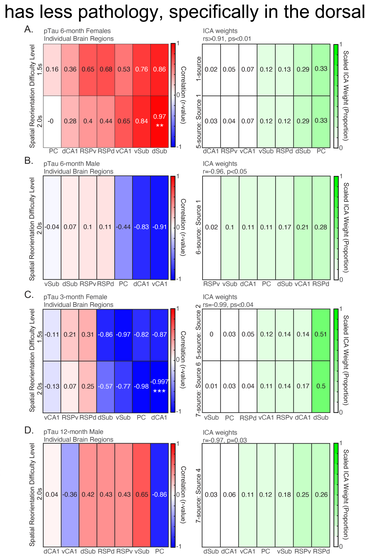

Figure 3: More pTau pathology is predictive of spatial reorientation performance, but only for 6-month female mice. *Left*. Correlations between pTau raw density for individual brain regions and spatial reorientation performance for the 1.5 and 2.0 s difficulty levels for 6-month female (**A**) and male (**B**) mice. *Right*. Brain region weights (expressed as a proportion) identified by Independent Components Analysis (ICA) for the linear combination that was significantly correlated with spatial reorientation performance for the 1.5 and 2.0 s difficulty levels for 6-month female (**A**) and male (**B**) mice. $**p < 0.01$, $***p < 0.001$.

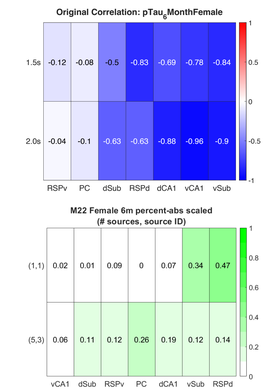

Figure 4: *Top*. Correlations between M22 raw density for individual brain regions and spatial reorientation performance for the 1.5 and 2.0 s difficulty levels for 6-month female There were no significant correlations between individual brain regions and behavioral performance. *Bottom*. Brain region weights (expressed as a proportion) identified by Independent Components Analysis (ICA) for the linear combination that was significantly correlated with spatial reorientation performance for the 1.5 and 2.0 s difficulty levels for 6-month female mice. There were two ICA identified sources that led to a significant correlation between a weighted combination of brain region data and spatial reorientation performance. Source 1 from the 1-source iteration was negatively correlated with spatial reorientation performance for 1.5s difficulty level (rs=-0.93, p=0.02). In addition, source 3 from the 5-source iteration was negatively correlated with the 2s difficulty level (r = -0.966, p = 0.007).

Conclusions:

- AD may cause spatial disorientation as a result of impaired use of landmarks.
- ICA with M22 predicts spatial learning and memory as was found previously for pTau but the direction is opposite for M22 with more M22 staining related to better performance.
- This suggests that AD may cause spatial disorientation from an accumulation of tau but not amyloid beta in the parietal hippocampal network.
- Future work will assess a non-confirmation specific but amyloid beta 1-42 specific stain.

References:

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