Examination of Spatial Navigation Impairments in TgF344-AD Rats Through Resting-State fMRI, DTI, and Place-Action Training

FSU UNDERGRADUATE RESEARCH OPPORTUNITY PROGRAM
CENTER FOR UNDERGRADUATE RESEARCH & ACADEMIC ENGAGEMENT

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Introduction

- In early Alzheimer's disease (AD), an individual's ability for spatial navigation is impaired which serves to mark the progression of the disease (Scott D. Moffat, 2009). These impairments are seen early on in the progression of the disease when patients begin to wander and get lost in both familiar and unfamiliar environments (Yi-Chen et al. 2004).
- Spatial navigation utilizes egocentric and allocentric reference frames to maintain a path from one position to another (Gazova et al. 2012).
- Two key brain regions, the retrosplenial cortex (RSC) and parietal cortex (PC) encode for an allocentric position (world-centered) and egocentric (body-centered) reference frames (Alexander & Nitz, 2015.; Ciaramelli et al. 2010.; Cordo et al. 2009).
  - The brain regions that are affected earliest in the progression of the disease are the RSC and the PC (Tu et al. 2015).
- The RSC and PC are functionally connected to the hippocampus regions, but when there are disruptions that affect the functionality and structure of the brain from AD then spatial navigation is impaired (Wang et al. 2024).
  - Dysfunction of these key brain regions are caused by the aggregation of tau and accumulation of β-amyloid (Castanho et al. 2020).
- The parietal-hippocampal network is vital in exchanging allocentric and egocentric information for spatial navigation, but decreased functionality is found in AD (Cushing et al. 2020).

- These insights demonstrate the importance of functionality and connectivity between certain brain regions in spatial navigation and highlight how AD leads to brain network dysfunction. Consequently, impaired navigational abilities are linked to the dysfunction of the parietal-hippocampal network, disrupting the exchange of allocentric and egocentric reference frames across brain networks and impairing spatial navigation.

- Through a cross-sectional study we seek to evaluate the connectivity and structure of different brain regions, and examine allocentric and egocentric reference frames through different tasks. **We predict that TgF344-AD transgenic (expressing AD behavior and histologic results) rats will have brain network dysfunction, causing impaired egocentric and allocentric navigation. We further hypothesize that as the rats age, spatial navigation performance will decline, as well as the functionality and connectivity of brain networks examined through resting-state MRI (rsfMRI) and DTI scans.**

Methodology

Animal Model and Living Conditions:

- 18 female TgF344-AD rats live in identical environments and during behavioral testing are food deprived to 85% of their personal baseline.
- In each trial are two female littermate pairs (one control and one transgenic) are blinded to prevent bias

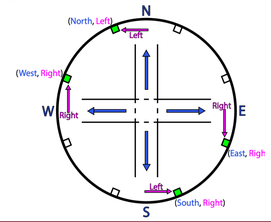
Behavioral Pre-training:

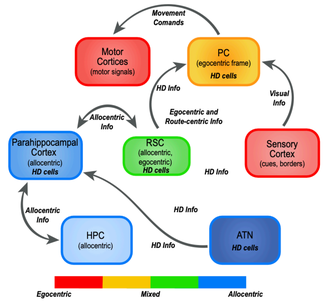
- Three pre-training tasks in the same "plus" shaped maze, with 4 weigh boats placed at the end of all arms, 20 minutes each day until completed.
- Pre-training one, rats are trained to consume two fruit loops a total of 5 times per weigh boat.
- Pre-training two, trained to take the lid off weigh boats and are hand-fed reward.
- Pre-training three, rats are trained to return back to center of the maze after removing weigh boat.
- Landmarks are on the walls for orientation

Behavioral Place-Action Task:

- 2 weigh boats are placed at the end of each arm. The experimenter is tasked with making a pseudorandomized list with the 4 zones repeated 40 times.
- The animal is placed in the center of the four doors, and the doors open according to the list. Once the rat leaves the center, the rat chooses the correct or incorrect weigh boat. Scoring of the behavior is either correct, incorrect, or no response

Design for Place-Action Task:





Parietal Cortex and Retrosplenial Cortex are anatomically and functionally well-positioned to interface between egocentric and allocentric frames of reference within a larger network.

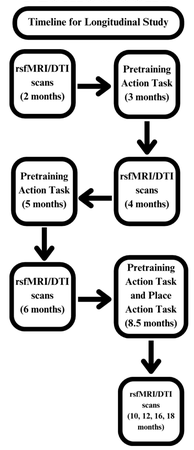
DTI:

- 25 slices (1mm) divided into 6 segments and imaged at a resolution of 200x200x1000 µm
- Images with the trigger and fat suppression on 18 diffusion directions with: TE/TR=20 ms/5s, B value=100 s/mm2, 160x120 matrix, FOV 32x44mm, and a Δ of 11ms with lowercase Δ of 3ms - 4 averages obtained. Data analyzed using DSI studios.

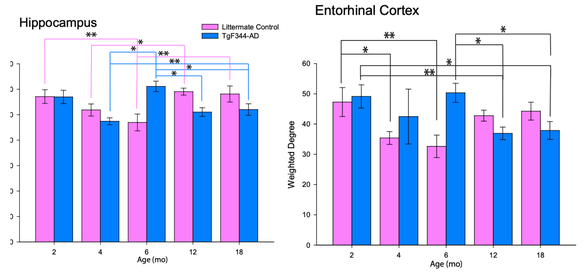
rs-fMRI:

- 25 slices (1mm) were divided into 4 segments and imaged at a resolution of 250x250x1500µm
- BOLD effect was used alongside SEP-EPI acquisition for 300 repetitions per animal at each timepoint.
- Settings were as follows: TE/TR=18,s/2.5 s, matrix size of 128x 96, and FOV of 32x24 mm.
- Data were analyzed using amira, MATLAB, BASH, GEPHI, and JMP software.

Method cont.

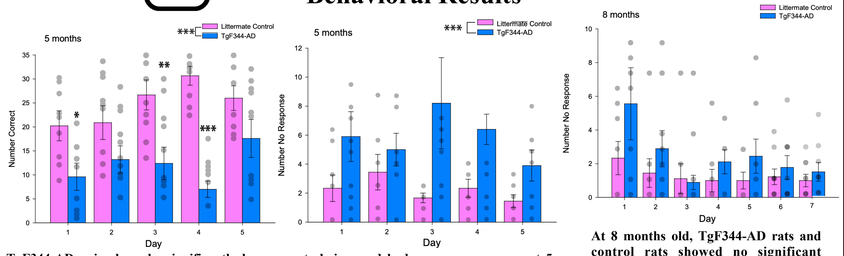


DTI Results



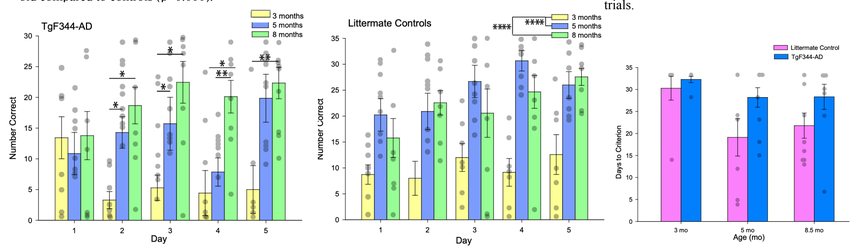
Weighted degree significantly varied with age, but there was no significant difference between the TgF334-AD group and the control group. In control rats, a reduction in weighted degree in the entorhinal cortex and hippocampus between 4-6 months of age, but in TgF344-AD rats it was delayed until 12-18 months. The only regions that had significant effects were the entorhinal cortex and the hippocampus (*p< 0.05, **p<0.01).

Behavioral Results



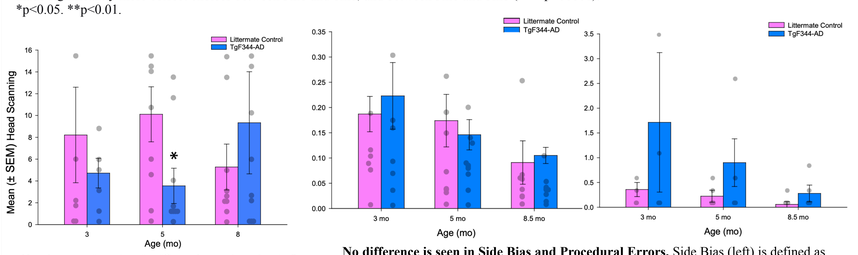
TgF344-AD animals make significantly less correct choices and had more no responses at 5 months old. *Left.* TgF344-AD rats made significantly fewer correct choices compared to controls (p<0.0117 individual comparisons following up on a day x group interaction, p<0.0003 for the main effect of group). *Right.* Transgenic animals made significantly more no response choices at 5 months old compared to controls (p<0.001).

At 8 months old, TgF344-AD rats and control rats showed no significant difference in any measure. No statistically significant difference in no response trials were seen, as well as no significant differences in correct or error trials.



TgF344-AD and Littermate Controls improved with repeated testing. TgF344-AD rats there was a significant interaction between day and age (p< 0.0046). Specifically, TgF344-AD rats made significantly more correct choices between 3mo and 8mo on days 2, 3, 4, and 5 (ps< 0.05) and between 3mo and 5mo on days 2, 3, and 4 (ps< 0.05). For Littermate controls there was a significant effect of age (p <0.0081) with made significantly more correct choices between 3mo and 8mo, and between 3mo and 5mo (****p<00001). *p<0.05. **p<0.01.

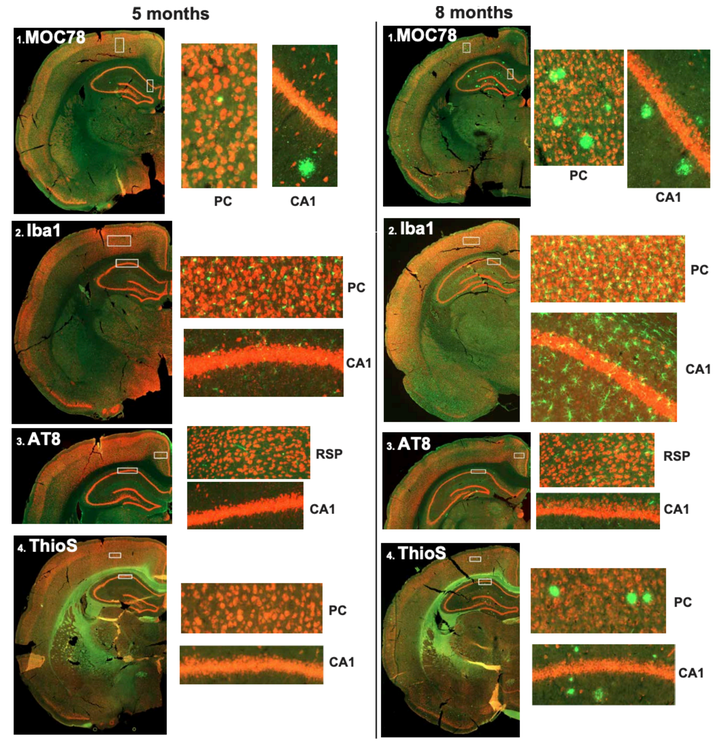
Mean ± (SEM) days to criterion including "no response" trials. No statistically significant differences were seen between groups or age.



Head scanning is significantly reduced at 5 months in TgF344AD rats. Mean (± SEM) head scans. A head scan is defined as when an animal exits the arm and looks in a particular direction (either left or right). * p<0.05.

No difference is seen in Side Bias and Procedural Errors. Side Bias (left) is defined as the ratio of preferred left or right turns toward the goal location. Procedural Errors (right) are defined as the number of times an animal traveled around the perimeter of the arena and past any of the three other arms. There was no significant side bias or procedural errors made for either group at any age.

Immunohistochemistry Results



AD pathology appears to be worsened in older TgF344-AD rats. Immunohistochemistry (MOC78, Iba1, AT8, and ThioS) was conducted at 5 and 8 months of age to compare density and number of cells per region of interests- hippocampal CA1, parietal cortex (PC), and retrosplenial cortex (RSC). Aβ aggregates appear denser in 8 month TgF344-AD rats compared to 5 months in both MOC78 and ThioS stains. Greater microglia activation is seen via Iba1 staining in the 8 month old TgF344-AD rats compared to 5 month rats. There is not much of a difference seen in phosphorylated tau accumulation between the two ages shown, as tau begins to accumulate at around 15 months of age.

Conclusion

- The current study is a cross-sectional investigation predicting that rats with AD will have brain network dysfunction that contributes to egocentric and allocentric navigation.
- Data from a prior longitudinal study have demonstrated that through place-action behavioral tasks, TgF344-AD animals make significantly fewer correct choices and have more no responses at 5 months old. However, through repeated testing, both TgF344-AD and littermate controls improve from 3 months to 5 months.
  - The deficit appearing at 5 months old could be an early emerging cognitive deficit in AD.
- Through DTI scans, the entorhinal cortex and hippocampus in control rats showed a decline in weighted degree early (4-6 months), but the TgF344-AD rats showed a decline delayed until 12-18 months. This may suggest that AD pathology is age-related since tau begins to accumulate around 15 months.
- Given these past findings, we expect our hypothesis to be supported. The cross-sectional study at 8 months could further confirm whether the improved performance is due to prior testing or other factors.

References