Longitudinal characterization of resting state fMRI, DTI, and action-place spatial learning in the TgF344-AD rat reveals impaired action-place learning emerging at 5-months

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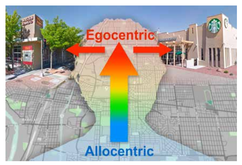
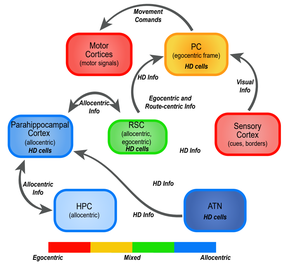
Introduction

- Alzheimer's disease (AD), patients experience impaired spatial navigation early in disease progression (Pai & Jacobs, 2004). In AD, this manifests in various ways, such as wandering and disorientation, and is one of the earliest markers of disease development (Yatawara et al., 2017).
- The regions responsible for the maintenance of proper spatial navigation prove to be some of the earliest sites of amyloid beta and tau neuropathology development, as well as changes to structural and functional connectivity (Park et al., 2004).
  - The retrosplenial cortex (RSC) and the parietal cortex (PC) show some of the most prominent changes in preclinical AD.
    - Both encode allocentric (world-centered) and egocentric (body-centered) reference frames within the parietal-hippocampal network (Clark et al., 2018; Whitlock et al., 2008).
  - Decreased functional connectivity between hippocampal regions, and impaired hippocampal-cortical interactions are found in AD (Benthem et al., 2020; Manno et al., 2019; Pengas et al., 2012).
- These findings highlight that both pathology deposition and structural and functional connectivity changes contribute to impaired navigation in AD. Thus, navigational dysfunction in AD may be a consequence of impaired reference frame coordination as a consequence of dysfunction in a parietal-hippocampal brain network.

- We are longitudinally assessing structural and functional connectivity as well as a novel navigation task designed to tax coordination between world-centered and body-centered reference frames.
- We hypothesized that the Tg-F344-AD rat (expressing age-dependent amyloid deposition) would demonstrate impaired coordination between reference frames with more pronounced impairment at older timepoints. We further hypothesized that, behavioral changes, will be paralleled by functional and later structural network changes assessed with DTI and resting state (rs) fMRI.
- Understanding of navigational deficits, particularly in the preclinical stage in humans, in parallel

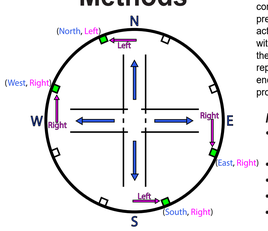


Coordination between allocentric (map-like) and body-centered (egocentric) frames of reference *left*. Our brain maps our position in allocentric coordinates; however, our interactions with the world are body-centered or egocentric by nature (e.g., we turn right at a particular intersection). A fundamental problem is how these frames of reference interact. For example, the action taken at a common city intersection (turn left vs. turn right) is dependent on knowledge of a distant goal location and one's allocentric location in an environment (approaching the intersection from the north).



Parietal Cortex and Retrosplenial Cortex are anatomically and functionally well-positioned to interface between egocentric and allocentric frames of reference within a larger network *right*. Illustration of anatomical and functional connectivity of reference frame processing by brain regions that comprise the extended PC-HPC network. HPC and para-hippocampal regions (entorhinal cortex, postsubiculum, and parasubiculum) encode an animal's position in space predominantly in allocentric coordinates. The PC interfaces between egocentric actions, and allocentric spatial and HD information. The PC is centrally positioned within a larger brain network that includes medial temporal lobe regions such as the HPC, as well as motor cortex, sensory cortices, and the RSC. The box colors represent a colormap denoting the relative density of egocentric vs. allocentric encoding for each region. Inset. This proposal is focused on the HPC-PC circuit providing allocentric context for egocentric action.

Methods



Place Action Task Design. Four arms of equal length each bisect a pair of feeding stations - one rewarded, 'correct' and one non-rewarded, 'incorrect' location. Each reward station is associated with a different combination of allocentric place and egocentric action.

Animal Model and Housing Conditions

- 18 TgF344-AD rats housed in a 12:12 hour light/dark cycle & food deprived to 85% of baseline (behavioral testing only)
- Litermate pairs of rats (1 hAPP(-/-)/hPS1(-/-) & 1 hAPP(+/-)/ hPS1(+/-)

Pre-training

- 4 weight boats covered with identical objects are placed in front of each lane (20 min each day until criterion)
- Rats were trained to remove objects for a food reward
- Landmarks on walls are for maintaining orientation in space

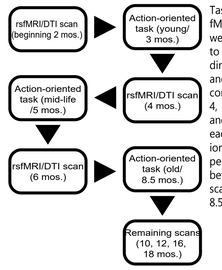
Place-Action Training

- 8 stations are covered with objects and designated as "correct" or "incorrect" for each arm (see Figure)
- Trial begins in center and ends when an object is removed
- Pseudorandom selection for lane/trial order for 40 trials
- Rat leaves lane, turns and removes one of two object options
- Removal of an object was scored as 'correct' or 'incorrect' or If none removed or rat remained in middle 'no response'

rsFMRI/DTI scanning

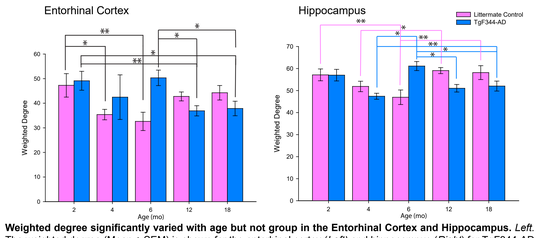
- Rats were imaged at the National High Magnetic Field Laboratory for 21.1 Tesla imaging
- **DTI**:
  - 25 slices (1mm) divided into 6 segments and imaged at a resolution of 200x200x1000 μm
  - Images with the trigger and fat suppression on 18 diffusion directions with: TE/TR=20 ms/5s, B value=100 s/mm², 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 resolutioon 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.

Methods Cont.



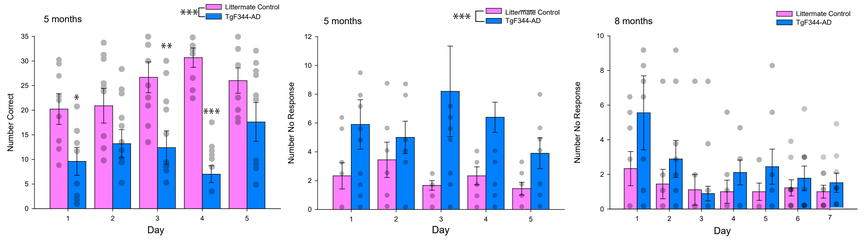
Task timeline.rs-fMRI/DTI scans were conducted to assess longitudinal functional and structural connectivity at 2, 4, 6, 10, 12, 16, and 18 mos for each rat. Behavioral sessions were performed just before paired MRI scans at 3, 5, and 8.5 mos of age.

DTI Results



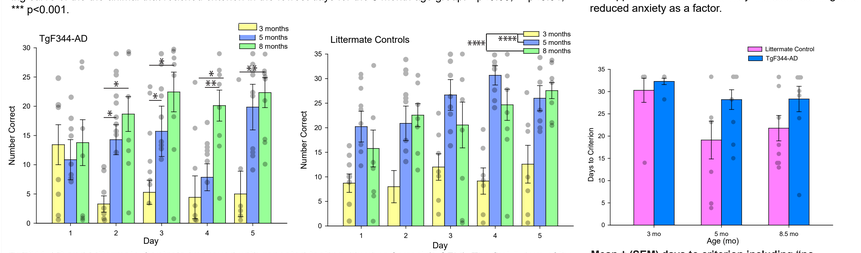
Weighted degree significantly varied with age but not group in the Entorhinal Cortex and Hippocampus. *Left*. The weighted degree (Mean ± SEM) is shown for the entorhinal cortex (*Left)* and hippocampus (*Right*) for TgF344-AD rats and littermate controls. DTI data was assessed for a subset of 286 brain regions (hippocampus, entorhinal cortex, piriform cortex, parietal cortex, somatosensory cortex, retrosplenial cortex & corpus callosum) for a subset of rats enrolled in the study (TgF344-AD n=3, Littermate controls n=4). There were no significant results except for hippocampus and entorhinal cortex. For the entorhinal cortex and hippocampus, there was a reduction in weighted degree in controls at 4-6 months of age and this reduction was delayed until 12-18 months of age in Tg rats. * p<0.05, ** p<0.01.

Behavioral Results

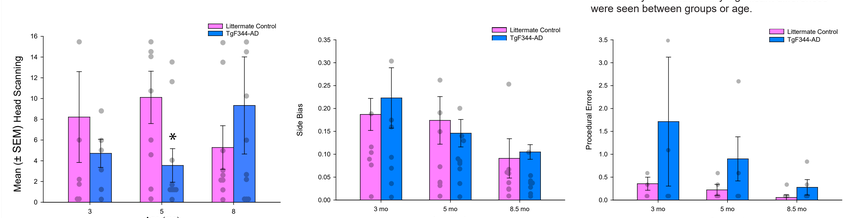


TgF344-AD animals make significantly less correct choices and had more no responses at 5 months old. *Left.* Choices were quantified as correct if they knocked over the rewarded object. Transgenic animals made significantly less correct object choices at 5 months old compared to controls (Individual comparisons following up on a day x group interaction (p<0.0117). There was also a main effect of group (p<0.0003). Right. Choices were quantified as no response if the animal stayed in the middle or did not knock over any object after 1 minute. Transgenic animals made significantly more no response choices at 5 months old compared to controls (p<0.001), indicating a behavioral impairment at this age. Analyses were quantified individually during the first 5 days of the task, which marks the learning curve of the the animal that reached criterion in the fewest days for the 5 month age group. * p<0.05, ** p<0.01, *** p<0.001.

No difference is in any measure at 8 months. Choices were quantified as no response by either staying in the middle or not knocking over any object after 1 minute. No statistically significant difference in no response trials were seen at this age. Similarly, there were no significant differences in correct or error trials. In comparison to their impaired performance at 5 months, the apparent lack of deficit may indicate learning and reduced anxiety as a factor.



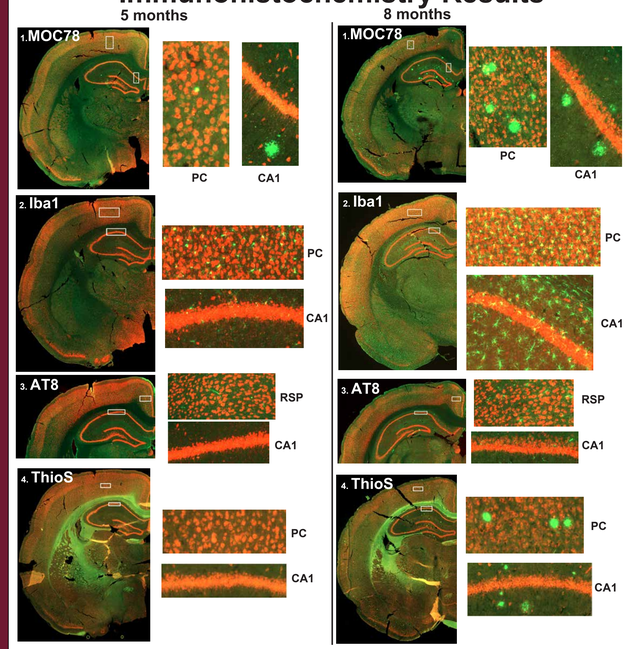
TgF344-AD and Littermate Controls improved with repeated testing. Number Correct (± SEM). The first 5 days of the task was set as a learning curve based on the cutoff from the 5-month group where one animal reached criterion in 5 days. For TgF344-AD rats there was a significant interaction between day 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 beetween 3mo and 5mo (***p<00001). *p<0.05. **p<0.01.



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 erros made for either group at any age.

Immunohistochemistry Results



AD pathology appears to be worsened in older TgF344-AD rats. IHC (MOC78, Iba1, AT8, and ThioS) was conducted at both 5 and 8 months of age to compare density and number of cells per region of interests, primarily focused on hippocampal CA1, parietal cortex (PC) and retrosplenial cortex (RSP). Amyloid beta aggregates appear denser in the 8 month old TgF344-AD rats compared to 5 month olds 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 accululate at around 15 months of age.

1. MOC78. Example staining of MOC78 (green), NeuN (red) for CA1 (right) and PC (left). 2. Iba1. Example staining of Iba1 (green), NeuN (red) for CA1 (bottom) and PC (top). 3. AT8 Example staining of AT8 (green), NeuN (red) for CA1 (bottom) and RSP (top). 4.ThioS. Example staining of ThioS (green), NeuN (red) for CA1 (bottom) and PC (top).

Summary and Conclusions

- Impairments in place-action learning emerge in 5-month old TgF344-AD rats and coincide with a reduction in weighted degree in the hippocampus and the entorhinal cortex that occurs in control but not Tg rats.
- Behavioral impairments are not sustained at 8 months
  - Performance improves from 3 months to 5-months in Tg and Control rats
  - Immunohistochemistry shows more pathology in 8 month old TgF344-AD rats.
  - The 8-month performance in Tg rats may reflect a repeated testing effect.
  - We are performing a cross-sectional study in 8 month rats to test this hypothesis.
- The deficits appearing at 5 months old, despite potential repeated testing effects from 3 to 5-months suggests reference frame coordination may be an early emerging deficit in rodent models and potentially also humans with AD.

Acknowledgements
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