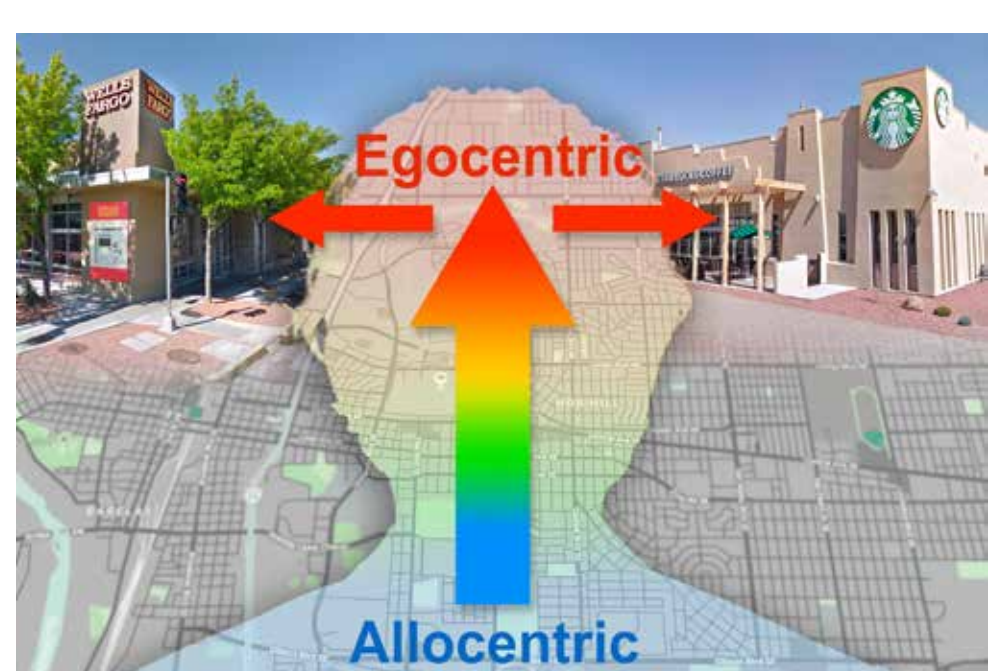


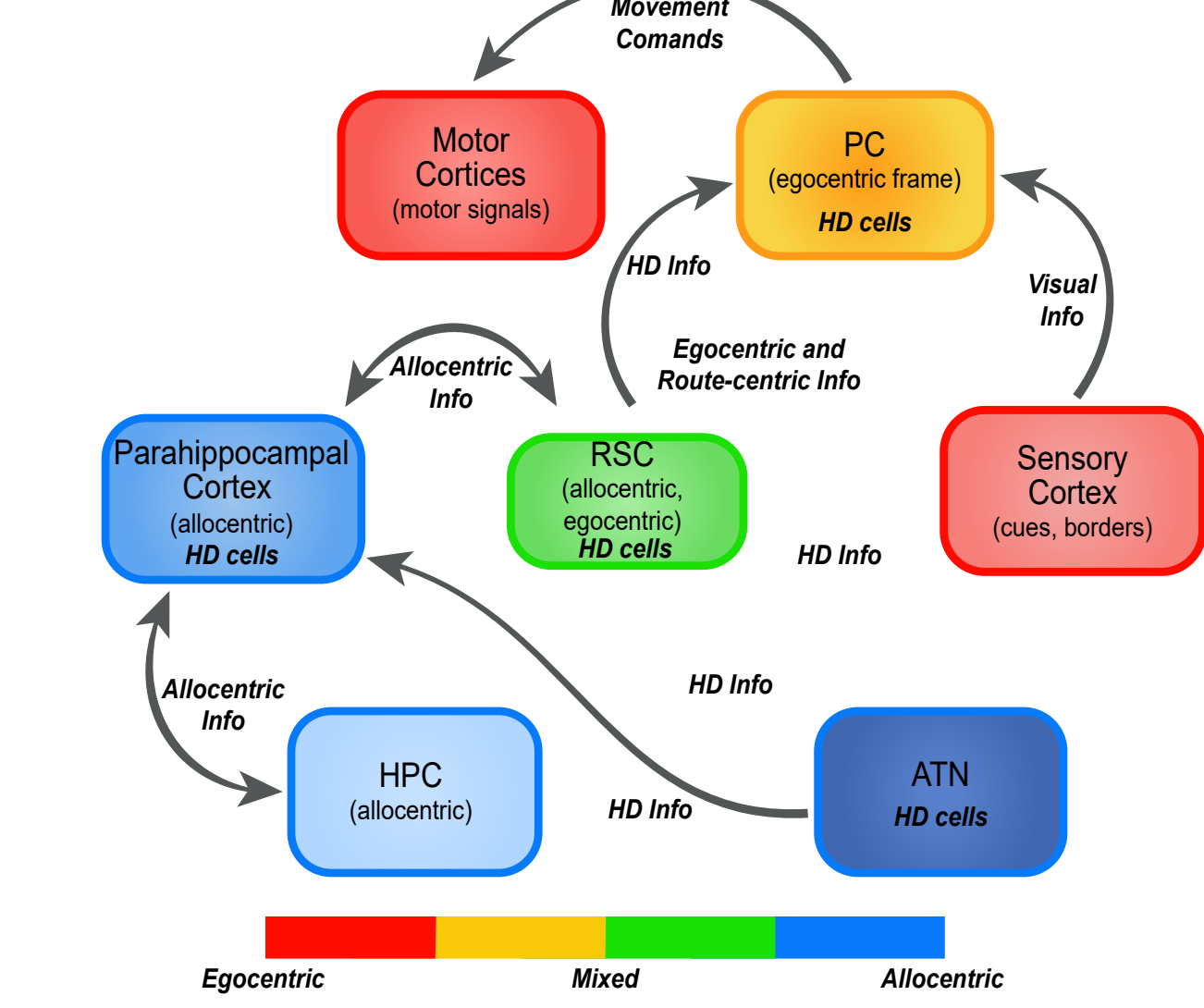
Introduction

- 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 (Bentham et al., 2020; Manno et al., 2019; Pengas et al., 2012).
- These findings highlight that both pathology deposition, 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.



Coordination between allocentric (map-like) and body-centered (egocentric) frames of reference. 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).



rsfMRI, DTI & sodium 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.

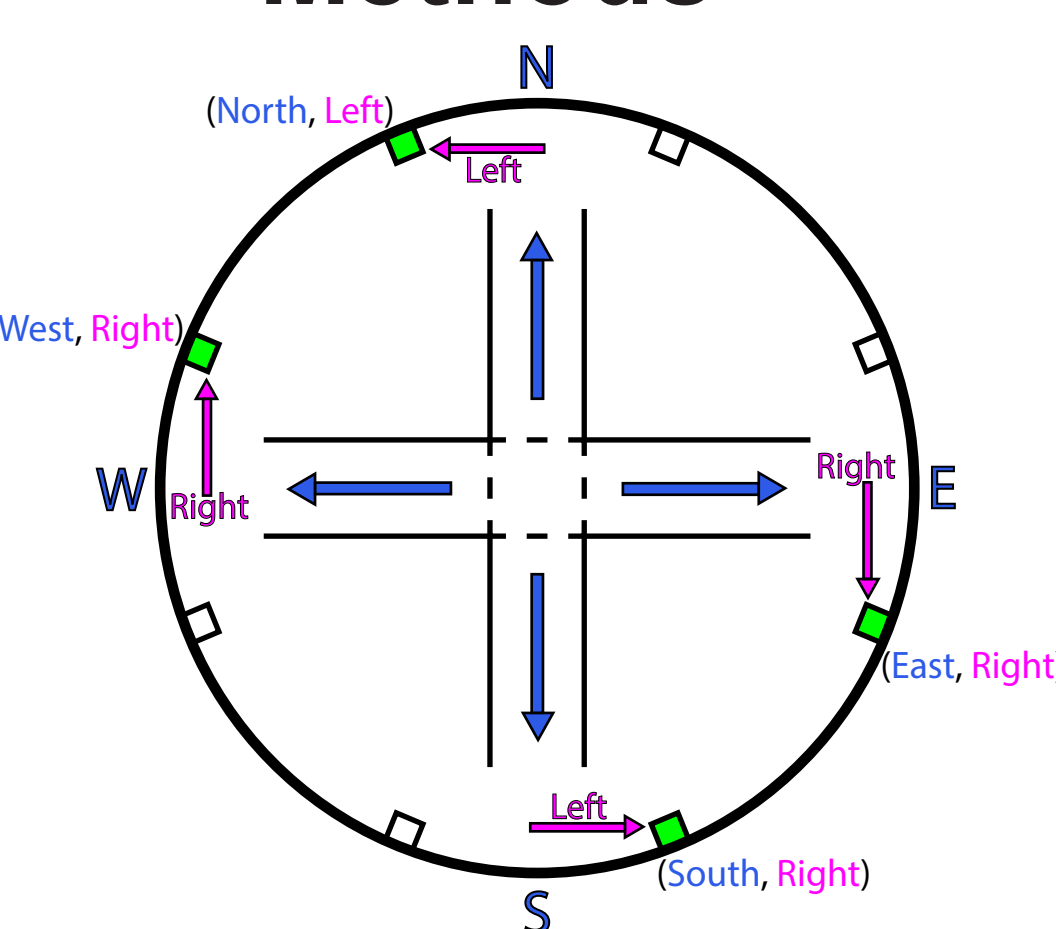
rs-fMRI:

- 25 slices (1mm) were divided into 4 segments and imaged at a resolution of 250x250x1500 μm
- BOLD effect was used alongside SEP-EPI acquisition for 300 repetitions per animal at each timepoint.
- Settings were as follows: TE/TR=18s/2.5 s, matrix size of 128x96, and FOV of 32x24 mm.
- Data were analyzed using amira, MATLAB, BASH, GEPHI, and JMP software.

Na CSI:

- 3D, whole head imaging
- 1 mm³ isotropic resolution
- Upsampled to 0.123 mm³ isotropic resolution
- 32x32x32 mm FOV
- 32min acquisition

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

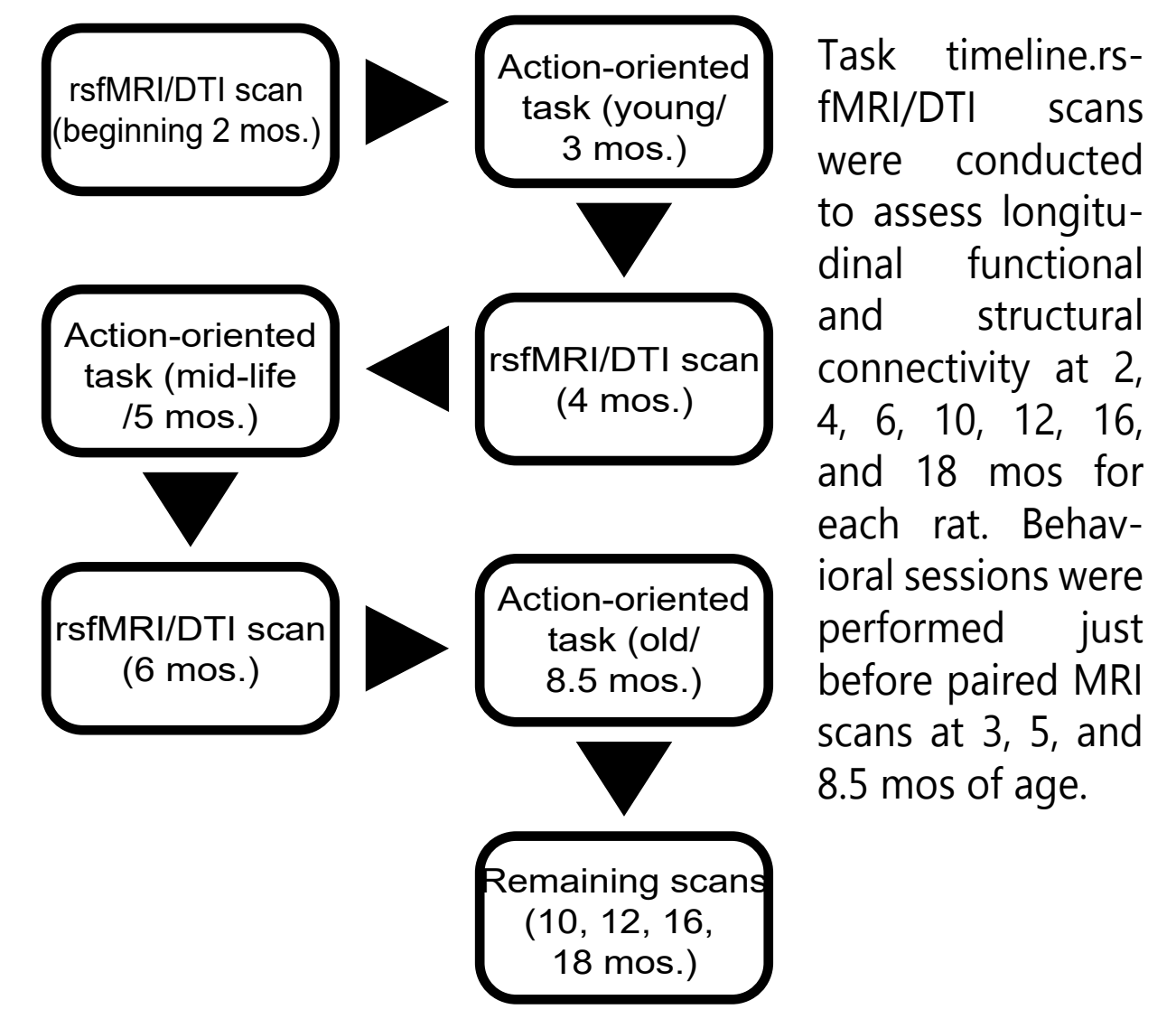
- 18 TgF344-AD rats housed in a 12:12 hour light/dark cycle & food deprived to 85% of baseline (behavioral testing only)
- Littermate pairs of rats (1 hAPP(-/-)/hPS1(-/-) & 1 hAPP(+/-)/hPS1(+/-))

Pre-training

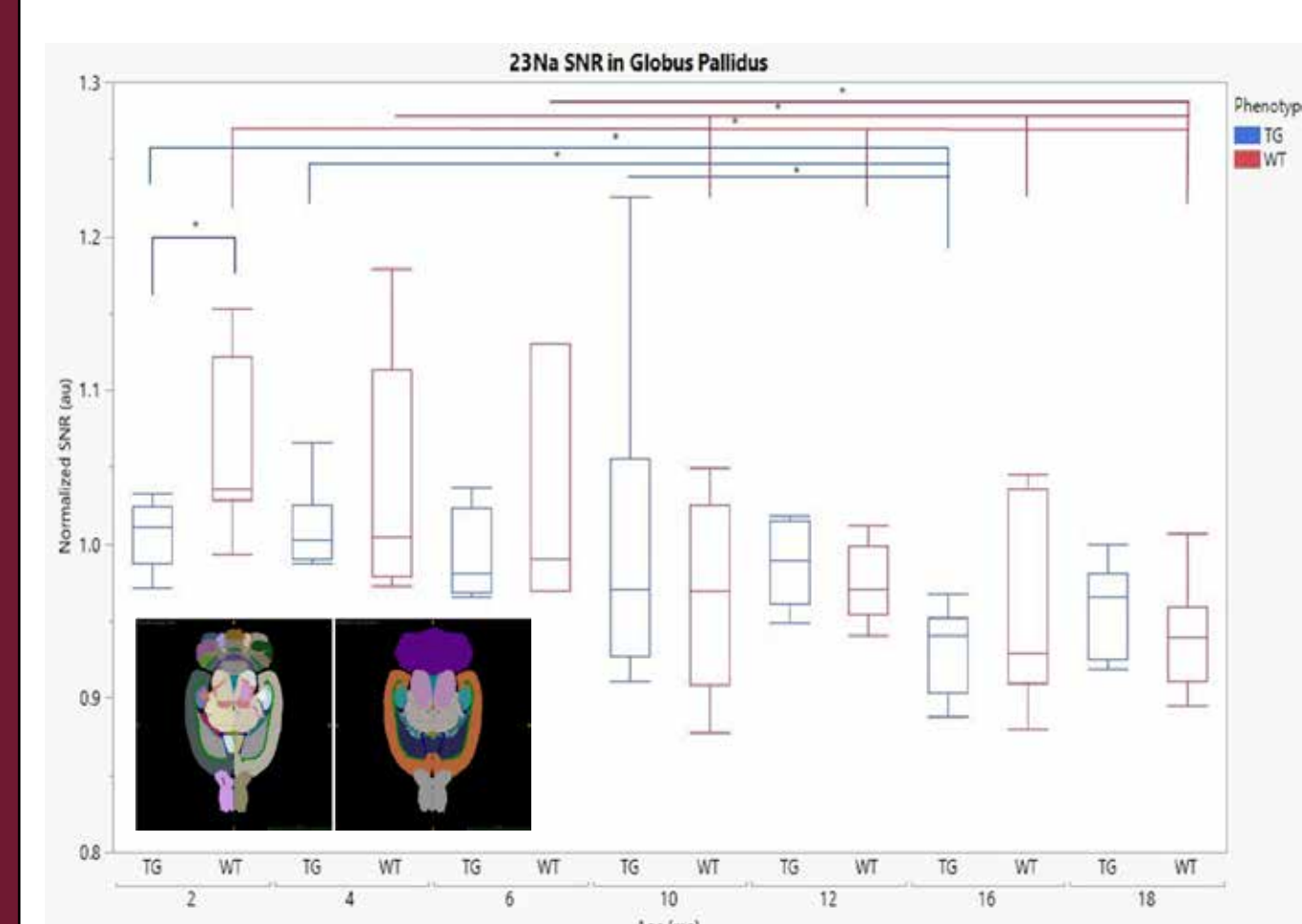
- 4 weight boats covered with identical objects are placed in front of each lane (20 min each day until criterion)
- Rats were trained to remove objects for a food reward
- Landmarks on walls are for maintaining orientation in space

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

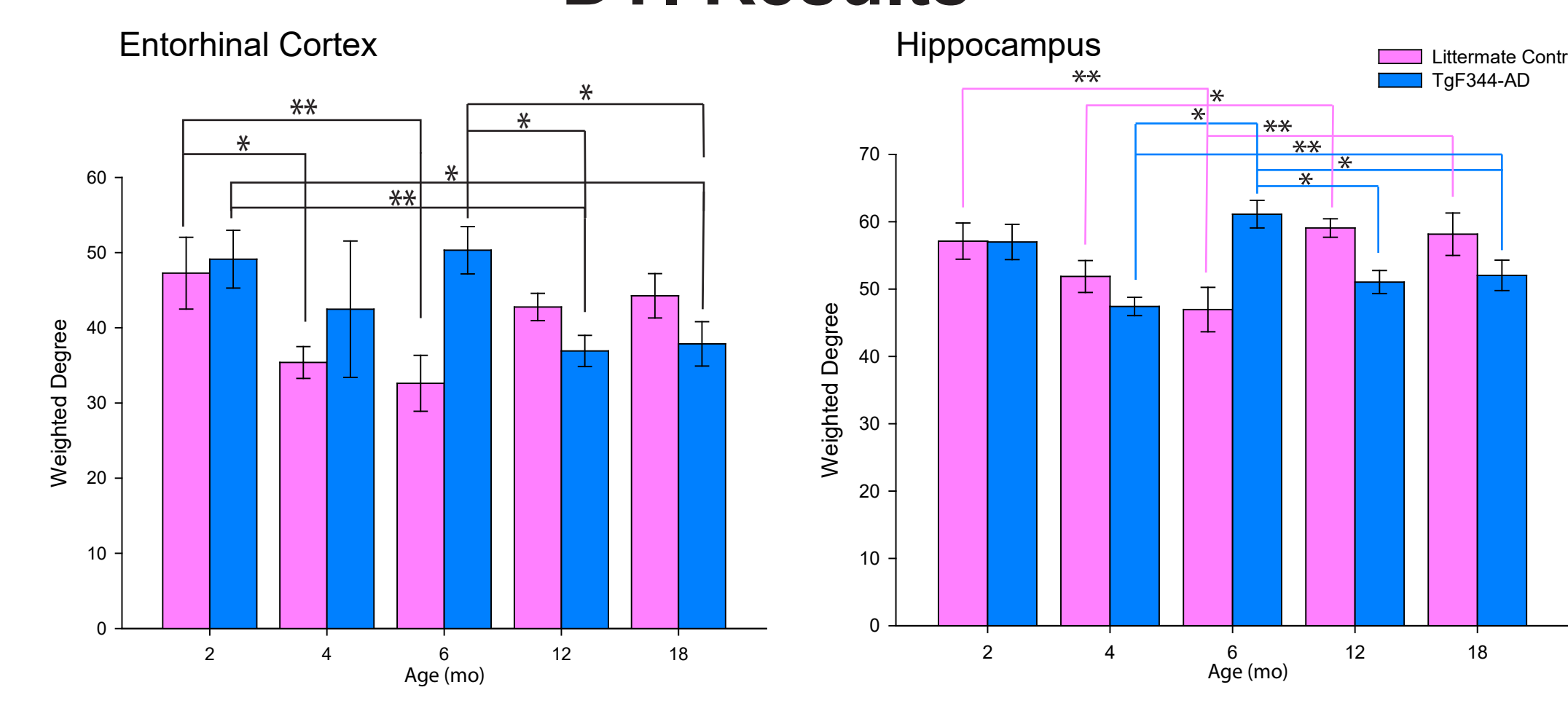


Sodium MRI Results



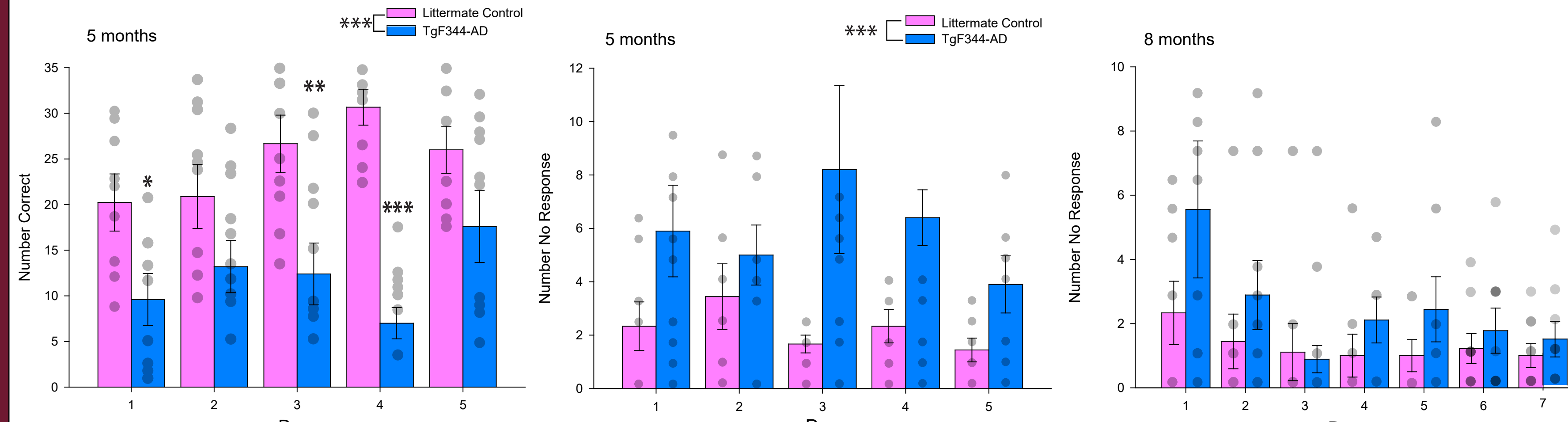
TgF344-AD rats have less variability in sodium and less sodium than NonTg littermates at 2-months that dissipates with age. There were several brain regions with similar trends which are best illustrated in the globus pallidus where both TgF344-AD rats (blue) and littermate controls TgF344-AD rats had greater variance in sodium levels than the nonTg littermate controls. TgF344-AD rats had less sodium from 2 to 6 months, but this difference was only significant at 2-months. Brackets indicate significance (p<0.05) based on post-hoc Student's T test.

DTI Results



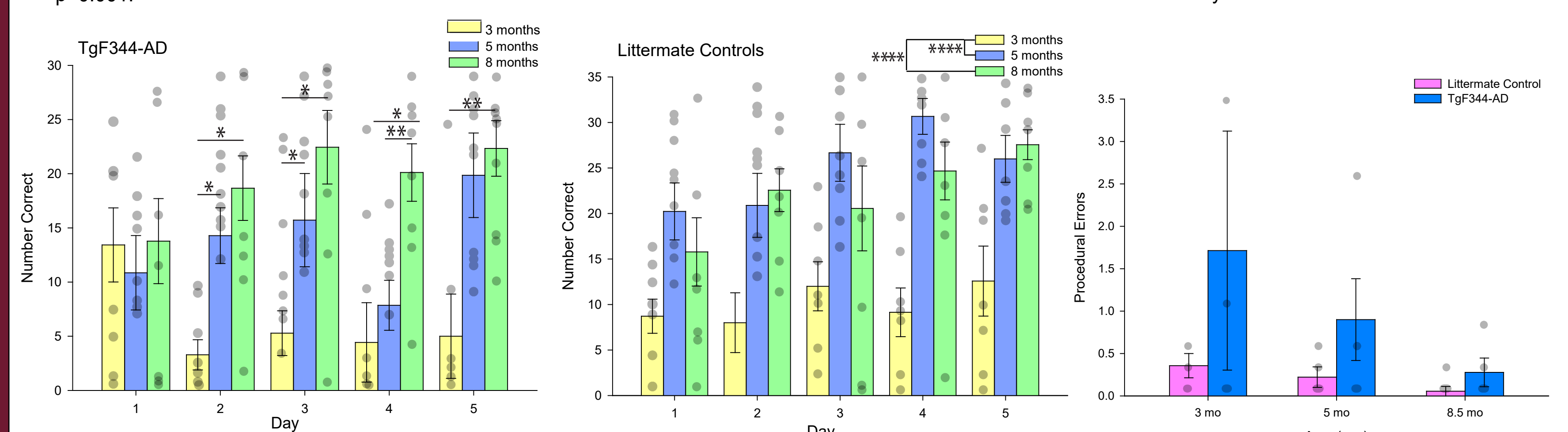
Weighted degree significantly varied with age but not group in the Entorhinal Cortex and Hippocampus. Left: The weighted degree (Mean ± SEM) is shown for the entorhinal cortex (Left) and hippocampus (Right) for TgF344-AD rats and littermate controls. DTI data was assessed for a subset of 286 brain regions (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



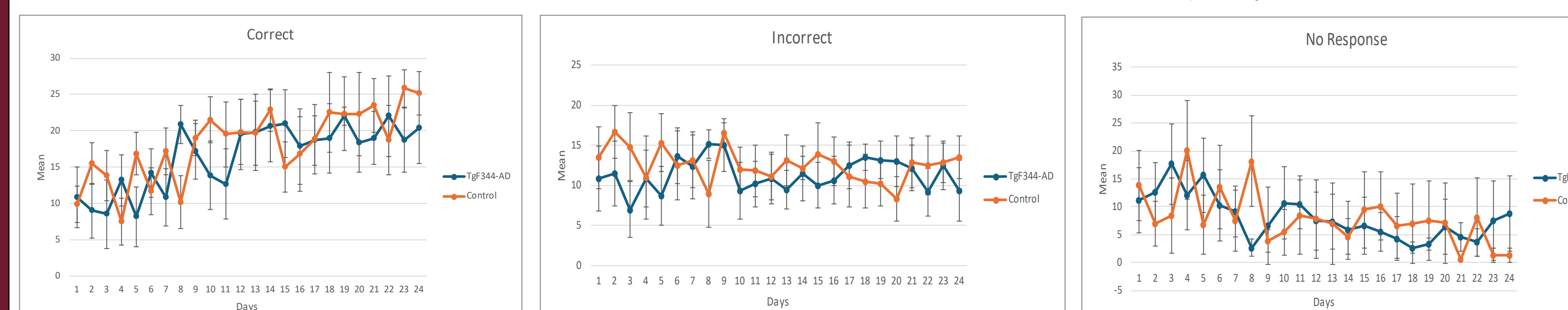
TgF344-AD animals make significantly less correct choices and had more no responses at 5 months old. Left: Choices were quantified as correct if they knocked over the rewarded object. Transgenic animals made significantly less correct object choices at 5 months old compared to controls (individual comparisons 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 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 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.



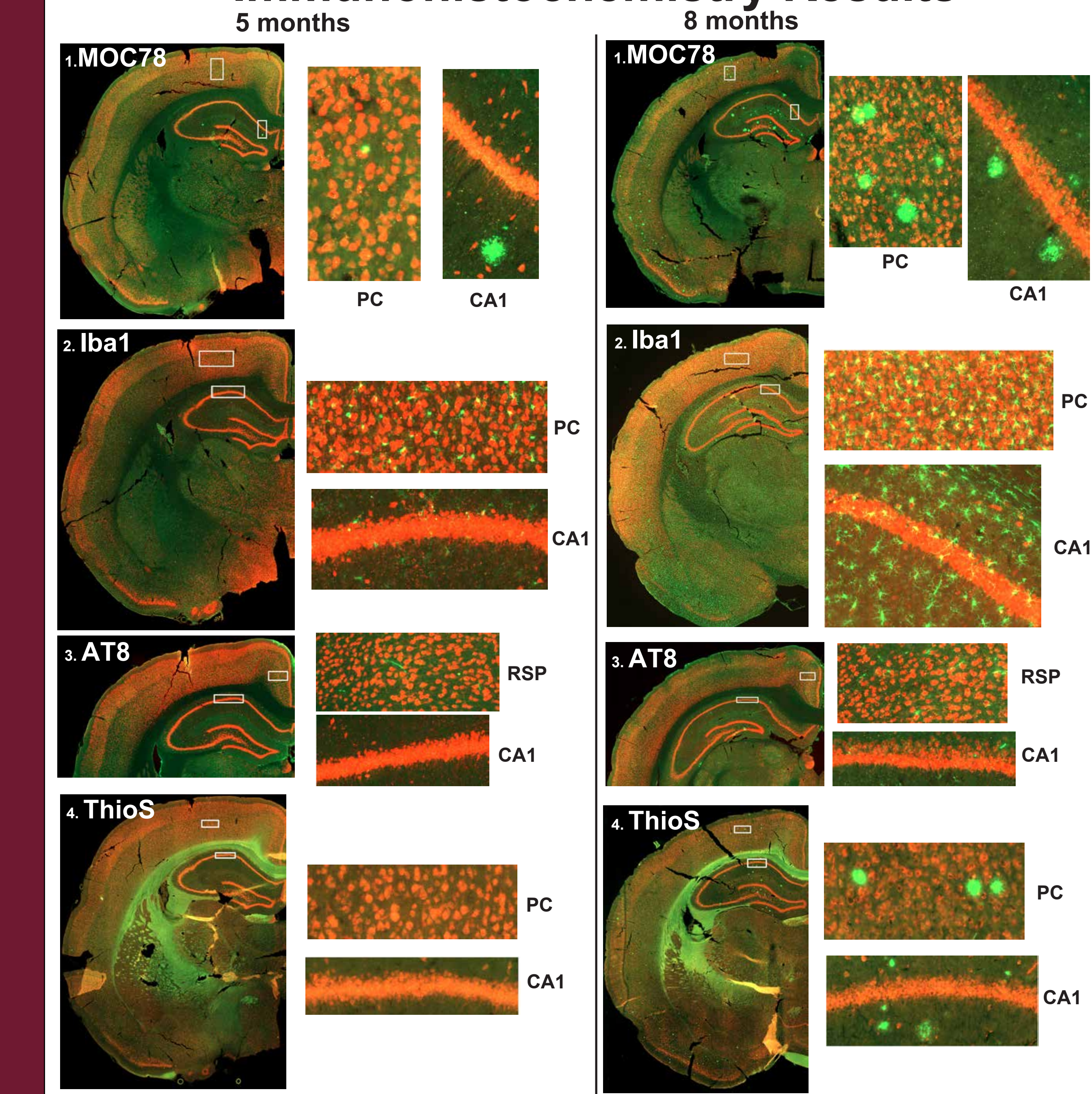
TgF344-AD and Littermate Controls improved with repeated testing. Number Correct (± SEM). The first 5 days of the task was set as a learning curve based on the cutoff from the 5-month group where one animal reached criterion in 5 days. For TgF344-AD rats there was a significant interaction between day 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 between 3mo and 5mo (****p<0.00001). *p<0.05, **p<0.01.

No difference is seen in Procedural Errors. 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 procedural errors made for either group at any age. No difference in head scanning, side bias, and criterion at the same age (not shown). Side Bias is defined as the ratio of preferred left or right turns toward the goal location. Procedural Errors are defined as the number of times an animal traveled around the perimeter of the arena and past any of the three other arms.



Control group cross sectional rats at 8.5 months cannot learn task criterion and do not differ from TgF344-AD rats. Only one control group rat and no TgF344-AD rats were able to learn and complete the task. Furthermore, there was no statistically significant results from correct, incorrect, and no response choices. The decrease in no response trials, unchanged incorrect trials and increasing correct trials suggests that rats are able to learn the task potentially as a result of decreasing anxiety.

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 accumulate 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 Future Directions

- 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 based on cross-sectional study data in 8 month rats.
- 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.
- Use of a cross-sectional design at 8-months did not reveal an impairment at this age, but does suggest that rats need to experience the full task at two timepoints to reduce anxiety with the task so the second timepoint can detect differences across genotype.
- In future studies, we propose using a different background strain to potentially eliminate anxiety from both animal groups.