Determining age of onset of cognitive impairment in TgF344-AD male and female rats

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Abstract

Alzheimer's Disease (AD) is characterized by age-dependent cognitive declineand neurodegeneration and is the most common form of dementia in the 65+ aging population in the United States. The pathological hallmarks of AD include the formation and aggregation of amyloid beta plaques and hyperphosphorylated tau proteins leading to failure of critical brain circuit function. Increasing evidence shows that the dorsal hippocampus and medial prefrontal cortex (mPFC) are among the brain regions that are most susceptible to AD pathology. These regions are crucial for learning, memory and spatial navigation and show significant impairment during progression of AD. A novel model of AD was developed by Cohen et al. in 2013 in Fischer 344 rats that express the familial AD human mutant genes: Swedish amyloid cursor protein (APPsw) and presenilin-1 delta E9 (PS1ΔE9). The TgF344-AD model results in a comprehensive set of AD-like phenotypes including: 1) progressive amyloid plaque aggregation and formation, 2) endogenous rather than engineered tauopathy leading to the formation of neurofibrillary tangles (NFTs), 3) cognitive decline and 4) gliosis and neuronal loss. While there has been some characterization of the behavioral status of the TgF344-AD rats, the onset of the behavioral deficit has been roughly determined to be around 9 months in both cross sectional (Cohen et al 2013) and in longitudinal (Berkowitz et al 2018) studies. A more fine-grained month by month analysis of when the behavior begins to change in the TgF344-AD male and female rats has yet to be determined. The purpose of this study was to identify this transition across several behavioral domains including the hippocampus-dependent spatial version of the Morris watermaze task and the amygdala-midbrain-dependent elevated zero (EZ) maze task. Six groups (n=64) of male and female TgF344-AD and wildtype (WT) rats at ages 4 months, 5 months, 6 months, 9 months, 10 months, and 11 months of age were tested on the tasks discussed above. Both male and female TgF344-AD rats were comparable in performance to their age-matched WT controls at 4 months, 5 months, 6 months of age on the spatial version of the Morris watermaze, and on the EZ maze task. Ongoing testing of male and female TgF344-AD rats at 7 months, 8 months, 9 months, 10 months, 11 months and 12 months will determine the precise age-of-onset of impairment due to AD pathology across the behavioral domains of this study.

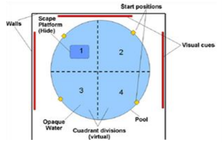
Methods

Experimental Procedure

Six age groups of male and female transgenic Fischer-344-AD (TgF344-AD) rats at 4-, 5-, 6- month-old (n=34), specifically AD (n=16) 8 males & 8 females and WT controls (n=18) 9 males & 9 females. At 9-, 10- and 11-months-old (n=30), specifically AD (n=19) 6 males & 13 females and control WT (n=11) 4 males & 7 females. These rats were tested on the Morris Water Maze illustrated in **Figure 1.** assessing spatial learning and memory. Over a course of 4 days, rats were placed at various locations around the pool to determine how well they used spatial and visual cues to determine the location of the hidden platform.

Figure 1: Morris Water Maze

Schematic image of water maze external cue placement, internal platform location and rat drop locations.



These rats were also tested on the Elevated Zero Maze (EZ maze), as illustrated in **Figure 2.** This behavioral task was used to determine if neuropsychiatric disorders such as anxiety like behavior in AD pathology can be characterized in these transgenic rats.

Figure 2: Elevated Zero Maze

Schematic image of the Elevated zero maze, open areas and hidden arms.

Results

Spatial Water Maze

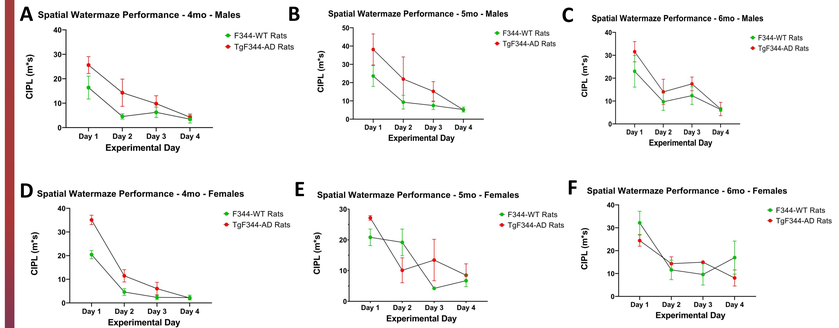


Figure 3. Spatial water maze performance of male TgF344-AD and wildtype (WT) rats at 4-, 5- and 6-months-old.
A-C) A 2-way ANOVA test showed a statistically significant improvement in performance of both TgF344-AD and WT rats across experimental days (p=.0001) at the three age points.

Figure 4. Spatial water maze performance of female TgF344-AD and wildtype (WT) rats at 4-, 5- and 6-months-old.
D-F) A 2-way ANOVA showed a significant improvement in performance of both TgF344-AD and WT rats across experimental days (p<.0001).

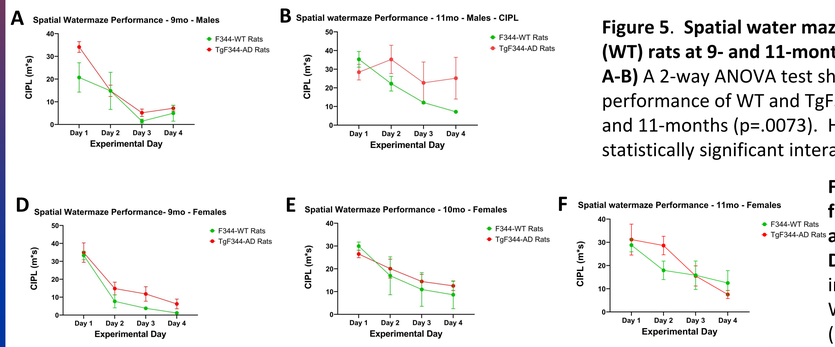


Figure 5. Spatial water maze performance of male TgF344-AD and wildtype (WT) rats at 9- and 11-months-old.
A-B) A 2-way ANOVA test showed a statistically significant improvement in performance of WT and TgF344-AD rats across experimental days at 9 (p<.0001) and 11-months (p=.0073). However, only 11-month-old male rats showed a statistically significant interaction of day and genotype (p=.0415).

Figure 6. Spatial water maze performance of females TgF344-AD and wildtype (WT) rats at 9-, 10- and 11-months-old.
D-F). A 2-way ANOVA test showed a significant improvement in performance of both TgF344-AD and WT rats across experimental days at 9- (p<.0001), 10- (p=.0005) and 11-months old (p<.0001) respectively.

Elevated Zero Maze

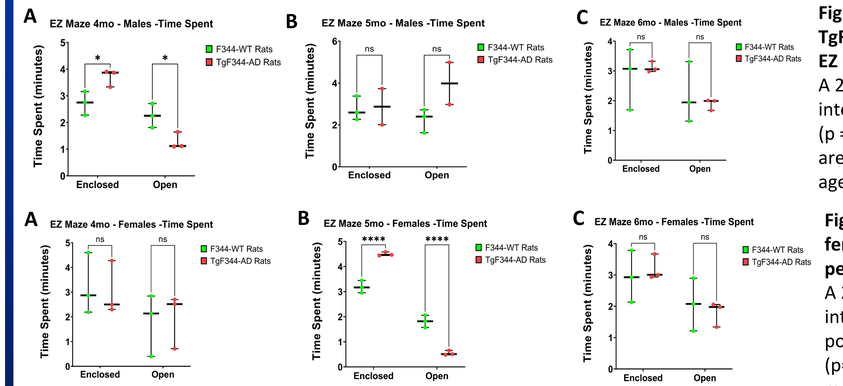


Figure 7. Assessment of the 4-, 5- and 6-month-old male TgF344-AD and wildtype (WT) rats' performance on the EZ Maze task.
A 2-way ANOVA test showed a statistically significant interaction in the time spent inside the enclosed portion (p =.0292) of the maze and out in the open (p =.0297) areas between TgF344-AD and WT rats at the **A)** 4-month age point but not at **B)** 5 months or **C)** 6 months old.

Figure 8. Assessment of the 4-, 5- and 6-month-old female TgF344-AD and wildtype (WT) rats' performance on the EZ Maze task.
A 2-way ANOVA test showed a statistically significant interaction in the time spent inside the enclosed portion (p=.0009) of the maze and out in the open areas (p=.0011)between TgF344-AD and WT rats at the **B)** 5-months-old but not at **A)**4- or **C)** 6-months-old.

Results Cont.

Elevated Zero Maze

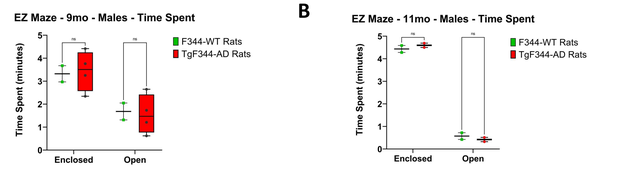


Figure 9. Assessment of the 9- and 11-month-old male TgF344-AD and wildtype (WT) rats' performance on the EZ Maze task.
A 2-way ANOVA test showed no statistically significant difference in the time spent inside the enclosed portion of the maze and out in the open areas between male TgF344-AD and WT rats at the **A**) 9- and **B)** 11-months-old.

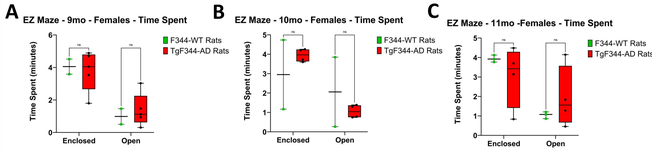


Figure 10. Assessment of the 9- ,10- and 11-month-old female TgF344-AD and wildtype (WT) rats' performance on the EZ Maze task.
A 2-way ANOVA test showed no statistically significant difference in the time spent inside the enclosed portion of the maze and out in the open areas between female TgF344-AD and WT rats at the **A**) 9- and **B)** 10- and **C)** 11-months-old.

Discussion

- Overall, no significant differences in spatial learning performance on the Morris watermaze between male and female TgF344-AD and WT rats at the relatively early ages of 4, 5, 6 months old
- Female TgF344-AD and WT rats also showed no significant differences in spatial learning performance on the Morris watermaze at 9-,10- and 11.
- Male TgF344-AD and WT rats showed no significant difference in spatial learning performance on the Morris watermaze at 9-month-old ,while TgF344-AD males at 11 months showed a trend toward impaired spatial learning compared to WT controls.
- On the EZ maze task, the female TgF344-AD rats at 5 months and male TgF344-AD rats at 4 months showed performance consistent with higher anxiety than did the wildtype control animals.
- Ongoing experiments will continue to examine 7-,8-,9-,10-,11- and 12-month-old male and female AD and WT rats to determine if a different pattern of behavior emerges in these animals, more consistent with their neural AD pathology.

References

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Acknowledgements

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