Evaluating Cognitive Deficits in 3xTg-AD Mice Using a Linear Reorientation Task

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Introduction

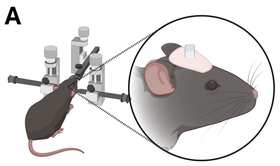
- Alzheimer's disease is a progressive neurodegenerative disorder characterized by cognitive decline, memory loss, and impaired spatial navigation.
- Our lab has demonstrated impaired spatial orientation in 6-month-old 3xTg-AD mice on a hybrid (129x1/C57BL6) background.
- For this study, we used 3xTg-AD mice bred onto a pure C57BL/6 background, as this strain exhibits more stable circadian rhythms
- We previously demonstrated impaired spatial orientation in 6-month-old 3xTg-AD mice on a hybrid (129x1/C57BL6) background (Stimmell et al. 2019).
- We designed a behavioral paradigm to assess spatial learning and memory by requiring mice to navigate a linear track using distal environmental cues and learn to stop at a certain zone.
- We used a genetically engineered model that expresses amyloid-beta plaques and tau pathology, recapitulating key features of AD.
- We utilized a brain stimulation reward system, specifically in the Medial Forebrain Bundle to reinforce correct stopping behavior within an unmarked reward zone.
- Findings contribute to evidence supporting spatial navigation tasks as a robust measure of cognitive decline in AD models, providing insights into early intervention strategies.
- **We hypothesized that 6-month female 3xTg-AD C57BL/6 mice would have impaired task performance compared to controls, indicating impaired spatial reorientation.**

General Methods

Subjects & Design: Six-month-old female 3xTg-AD mice on C57BL/6 background and wild-type (WT) controls were used to assess spatial learning and memory during the dark cycle.

Surgery: Two bipolar stimulating electrodes were implanted bilaterally in the medial forebrain bundle.

A



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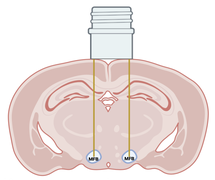


Task Overview: Mice were trained to navigate a linear track to an unmarked goal location in two distinct phases:

- **Alternating Phase:** Mice traveled back and forth between two endpoints for a water reward consumed in enclosed start box.
- **Goal-Learning Phase:** Mice had to stop within unmarked reward zone during the outbound trajectory to receive a brain stimulation reward.

Histological Validation:

•Following behavioral testing, mice were euthanized for brain tissue collection.

•Using a sliding microtome, frozen tissue was coronally sectioned and collected at 40 um and evenly placed in a series of six wells.

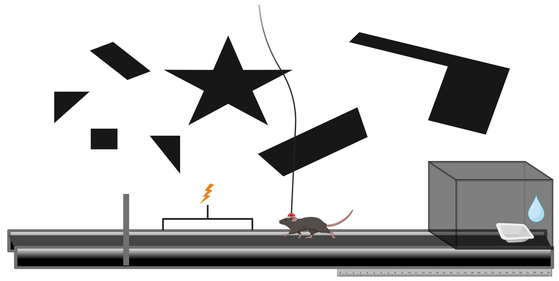
- Six types of immunohistochemical staining confirmed AD pathology and medial forebrain bundle (MFB) electrode placement (see Table).

Stain	Target
MOC22	Amyloid deposits
MOC78	Plaques and unique populations of intraneuronal and intranuclear amyloid; center of nascent neuritic plaques.
IBA1	Microglia and macrophage markers.
Ptau	Accumulation of phosphorylated tau in the brain
6E10	All forms of amyloid beta and the precursor APP
NeuN	Visualize neurons in brain tissue
GFAP	Visualize astrocytes in brain tissue

Experimental Methods

Experimental Procedure:

- Mice were individually housed in a 12-hour light/dark cycle with ad libitum access to food and were water-restricted to maintain motivation for the task.
- An overhead camera system tracked the mice's position in real time.
- Custom software automated reward delivery upon correct stopping behavior within the unmarked zone.
- The linear track was repositioned randomly between trials, requiring mice to rely on distal environmental cues for spatial reorientation rather than egocentric strategies.
- Training continued until stable task performance was observed, specifically 80% pass rate 3 out of 4 days in a row.
- Performance was measured by the number of correct stops within the reward zone.

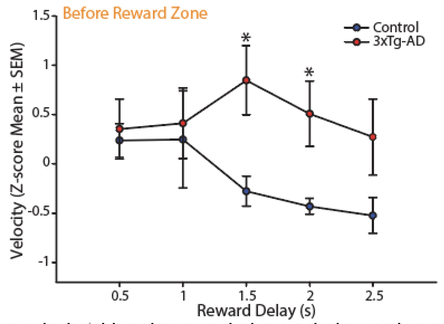


Data Collection & Analysis:

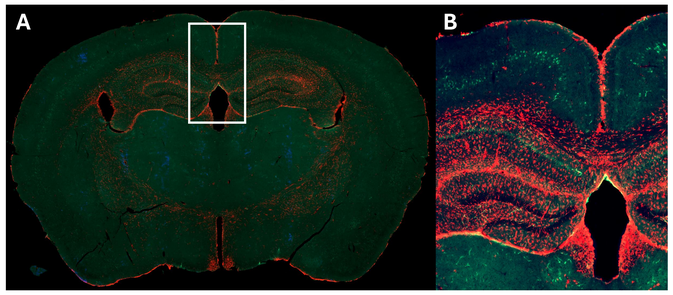
- Statistical comparisons between 3xTg-AD and WT mice were conducted using MATLAB.

Results

Behavioral Performance on Reorientation Task



- 3xTg-AD mice on a hybrid background showed slower learning rates compared to controls.
- Differences in stopping behavior at the reward zone suggest possible impairments in spatial learning/motivation.



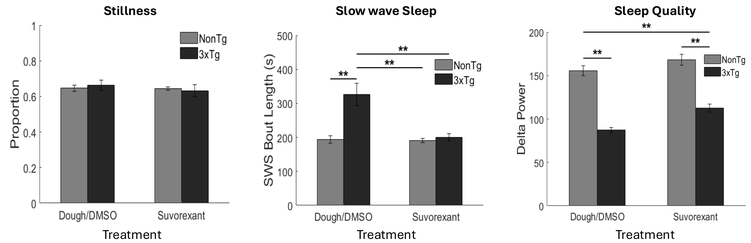
Representative figure showing 6-month 3xTg-AD female mouse brain (10X) stained for 6e10 (green), GFAP (Red), NeuN (blue) (A).

Figure showing 20X scan of the approximate location marked in A.

Results

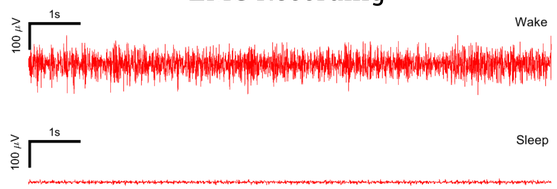
Currently being processed and will be updated as soon as possible.

Dora Intervention in 3xTg-AD Mice



- Potential rescue sleep deficits in early stages of disease progression.

EMG Recording



Conclusions

- There were observed impairments in reorientation task in 3xtg mice
- Spatial awareness, navigation, and orientation task issues emerged early in disease progression, with six-month old females showing the highest AD pathology.
- These findings contribute to evidence supporting the use of spatial navigation tasks as a robust measure of cognitive decline in AD models, offering insights into potential therapeutic targets for early intervention.

References

- Cushing, S. D., Skelin, I., Moseley, S. C., Stimmell, A. C., Dixon, J. R., Melilli, A. S., Molina, L., McNaughton, B. L., & Wilber, A. A. (2020). Impaired Hippocampal-Cortical Interactions during Sleep in a Mouse Model of Alzheimer's Disease. Current Biology, 30(13), 2588-2601.e5. doi: 10.1016/j.cub.2020.04.087.
- Stimmell, A.C., Baglietto-Vargas, D., Moseley, S.C. *et al.* Impaired Spatial Reorientation in the 3xTg-AD Mouse Model of Alzheimer's Disease. *Sci Rep* **9**, 1311 (2019). https://doi.org/10.1038/s41598-018-37151-z

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