Comprehensive Behavioral and Pathological Profiling of 5xFAD Mice: Gender Differences and Sleep Dysregulation in Alzheimer's Disease.

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by progressive cognitive decline and behavioral disturbances. To better understand the interplay between neuropathological progression, cognitive impairment, and behavioral dysregulation, we conducted a comprehensive study using the 5xFAD transgenic mouse model. This integrated investigation examined both gender-specific differences in disease progression and sleep-wake cycle alterations using automated behavioral monitoring.

We first assessed sex-based differences in amyloid-beta (Aβ) deposition, neuroinflammation, and cognitive function. Female 5xFAD mice exhibited significantly higher Aβ accumulation and inflammatory markers in the cortex and hippocampus compared to males. These pathological changes were accompanied by earlier and more severe cognitive deficits, as measured by Morris Water Maze, Y-maze, and novel object recognition tests. Neurofilament light (NF-L) levels in cerebrospinal fluid (CSF) further indicated accelerated neuronal damage in females, with differences emerging as early as 3 months of age.

In parallel, we employed Home Cage Analysis (HCA), a non-invasive, high-throughput behavioral monitoring system, to evaluate circadian and sleep-related phenotypes in the same cohort. 5xFAD mice demonstrated increased wakefulness, particularly during the daytime rest phase, with disruptions becoming more pronounced at 9 and 12 months of age. These alterations persisted despite age-related declines in locomotor activity, suggesting a decoupling of sleep regulation from general activity levels. Pharmacological validation in wild-type mice confirmed the sensitivity of HCA to detect drug-induced behavioral changes.

Together, these findings highlight the importance of incorporating both sex as a biological variable and naturalistic behavioral monitoring in preclinical AD research. The integration of neuropathological, cognitive, and behavioral data provides a more holistic understanding of AD progression and enhances the translational value of the 5xFAD model for therapeutic development.

METHODS

Animals

- Altogether 5xFAD mice and age-matched wildtype (WT) littermate C57BL/6 congenic mice bred from male 5xFAD [B6.Cg-Tg (APPSwFlLon, PSEN1*M146L*L286V) 6799Vas/Mmjax, Jackson Laboratories, USA] and C57BL/6 (Koatech, Korea) were used for experiments.

CSF and plasma collection

- The mice were euthanized with terminal dose of pentobarbital, and CSF was collected through the cisterna magna into Eppendorf microtubes. These were frozen on dry ice and stored at -80°C.
- Blood samples were collected by cardiac puncture. Whole blood was collected into lithium-heparin tubes, and plasma was separated by centrifugation (3000 rpm for 15 min) at 4°C. Separated plasma was collected in Eppendorf microtubes, frozen on dry ice and stored at -80°C.

Nf-L measurement in both CSF and plasma

- The level of neurofilament light chain (Nf-L) in mouse CSF was determined using the Simoa® NF-light Advantage Kit (Quanterix Corp, Boston, MA; item 103400).

Home cage analysis

- Home Cage Analysis continuously tracks spontaneous behaviors(27/7); locomotion, sleep/wake cycles, and social interactions in group-housed mice with HD video and RFID.

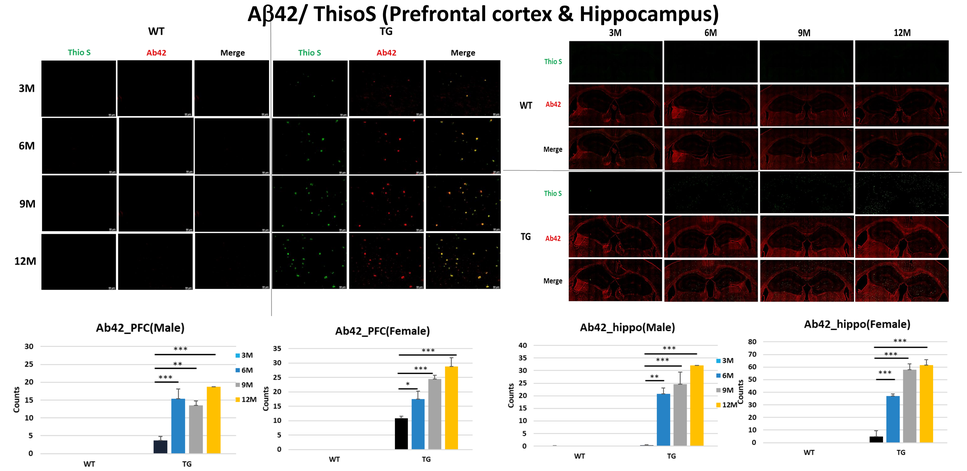
Histology

- 1st Abs
- Aβ42 (Millipore, cat#AB5078) ,
  Thioflavin S (Sigma Aldrich, cat#T1892)
- Sample : Whole brain (PFC, Hippo)
- Imaging: Axio Scan 7 (Zeiss)

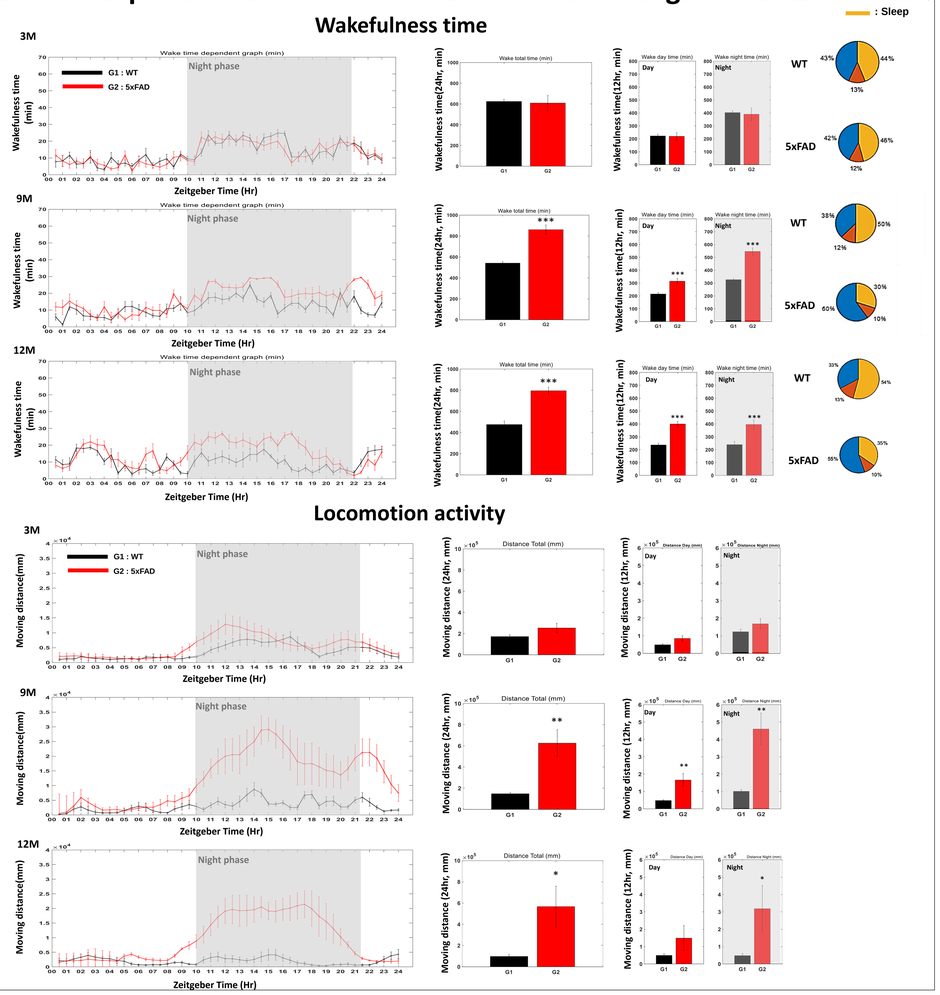
<Home cage analysis™ >

RESULTS

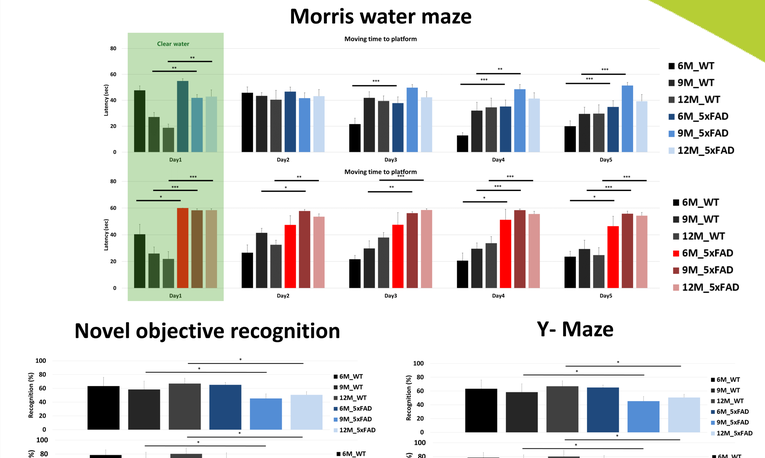
1. Time dependent histological biomarkers change in 5xFAD



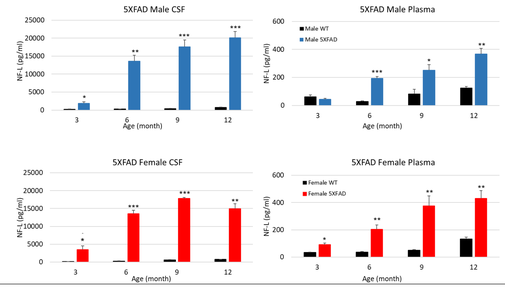
2. Time dependent Wakefulness & Locomotion change in 5xFAD



3. Time dependent cognition impairment in 5xFAD mouse.



4. Time dependent Nf-L changes in 5xFAD mouse



CONCLUSIONS

1. This study investigates gender-specific differences in Alzheimer's disease progression using the 5xFAD mouse model, which expresses human APP and PSEN1 mutations linked to AD.
2. These findings show that female mice exhibit elevated amyloid-beta (Aβ) accumulation and earlier cognitive decline compared to males.
3. Cognitive impairments in females were more pronounced between 9 and 12 months, while males showed later onset.
4. Neurofilament light (NF-L) variations in cerebrospinal fluid(CSF) & Plasma were detectable from 3 months, with both male and female exhibiting similar progression.
5. 5xFAD mice exhibited increased daytime wakefulness and age-related changes in locomotion patterns, as detected by Home Cage Analysis.
6. The study emphasizes the importance of considering gender differences in preclinical AD research and therapeutic evaluation.