Phenotyping Age-Dependent Changes in the 5xFAD Alzheimer's Disease Model Mice

ChemPartner — Dedicated to LifeScience

Charles R. Yang, Ruiben Feng, Ling Yang, Shujuan Jiang, Na Liu, Li Yan, Yi Liu, Lizhao Wang, Cecilia S.Yang, and KeWei Wang[1],
ChemPartner Co Ltd., Shanghai, and [1]Peking University School of Pharmaceutical Sciences, China

Program #: LB556

Abstract (11970)

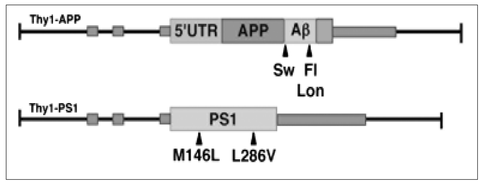
5XFAD transgenic (Tg) mice express 5 human familial Alzheimer's Disease (AD) gene mutations --- Amyloid Precursor Protein mutations from the Swedish, Florida and London families, along with 2 mutations in presenilin-1. Their early onset brain pathology, accelerated $A\beta_{42}$ amyloid deposition, age-dependent neuro-degeneration and cognitive decline, make these mice an attractive translatable AD model.

We used behavioral, biochemical and electrophysiological analyses to track age-dependent changes in 5XFAD mice, compared with their age-matched wild-type mice. With sensitive NanoPro Western, 3 month old 5XFAD mice showed a significant increase of p-^{T181}-Tau in the cerebral spinal fluid. Their hippocampal ELISA $A\beta_{40}$ and $_{42}$ levels increased progressively from 2,3,6 months. Under confocal microscopy, the secondary and tertiary dendritic spine counts in Golgi-stained 9 month prefrontal and hippocampal pyramidal neurons showed a significant loss. Hippocampal slice electrophysiology using the multielectrode MED64 system is used to assess synaptic plasticity, i.e. long-term potentiation. Using the Laboras vibration-sensitive platforms, we discovered that there was a significant dark phase hyperactivity and bi-directional circling that resembled striatal degeneration, and hyperlocomotion at 9, 12, but not 2 month, matching the chronic EEG recordings of a significant reduction in NREM and REM sleep at 9 month.

The onset of cognitive decline started at 4 months (working memory, Y-maze spontaneous alteration), 5 month (episodic memory, Novel Object Recognition), and 6 month (spatial memory, Morris Water Maze). Sub-chronic treatment in 4 months old 5XFAD mice by: a) Donepezil + Memantine (2 weeks), or b) Traditional Chinese herbal formula (1 month), led to a significantly improvement in Y-maze spontaneous alternation performance. Findings from these studies guide us to select the right ages of 5XFAD mice to test for pharmacological changes in AD biomarkers and behaviors in AD research.

INTRODUCTION

The 5XFAD transgenic mice express 5 human familial Alzheimer's Disease (AD) gene mutations --- Amyloid Precursor Protein mutations from the Swedish, Florida and London families, along with 2 mutations in presenilin-1. Their early onset brain pathology, accelerated Aβ42 amyloid deposition, age-dependent neurodegeneration and cognitive decline, make these mice an attractive translatable AD model.



To utilize these 5XFAD mice (C67/BL6 background) for testing potential therapeutic agents that will be used for the treatment of Alzheimer's Disease (AD), it is important to first determine what age-dependent biomarkers, behavioral and electrophysiological readouts are changing at specific ages so that meaningful readouts at the proper age of animals can be determined. In the following studies, we used behavioral, biochemical and electrophysiological analyses to track age-dependent changes in 5XFAD mice, compared with their age-matched wild-type mice.

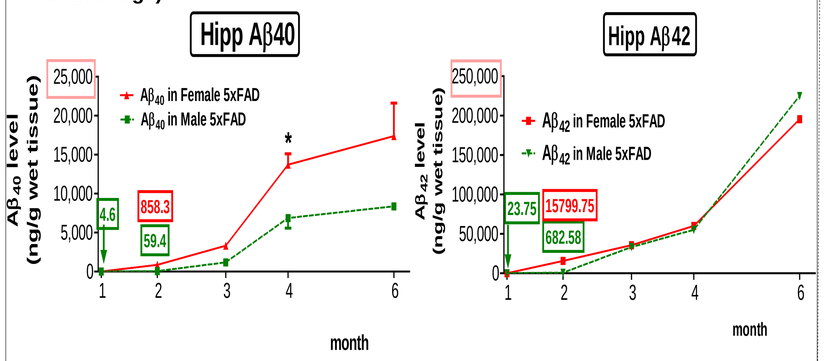
METHODS

1) Biomarkers $A\beta_{42}$ and $A\beta_{40}$ were measured by ELISA and Western Blot.
2) Dendritic Spines after Golgi stained (FD Neurotechnologies) were analyzed under confocal microscopy and spine dennsity of secondary and tertiary dendritic branches were quantifield using ImageProPlus software.
3) Hipppocampal CA1 LTP was induced and recorded using MED64 multielectrode planar chip placed under perfused hippocampus slices that were specially prepared using N-methyl-D-glucamine recovery solution (15mins).
4) Sleep/wake EEG recordings were made using DSI telemetry system and EEG data measured using SleepSign software.
5) Behavioral data (locomotion, circling, rearing, climbing, grooming) were recorded using the Laboras vibration-sensitive platforms.
6) Nest building behaviors were recorded by video and scored and analyzed offline.

RESULTS

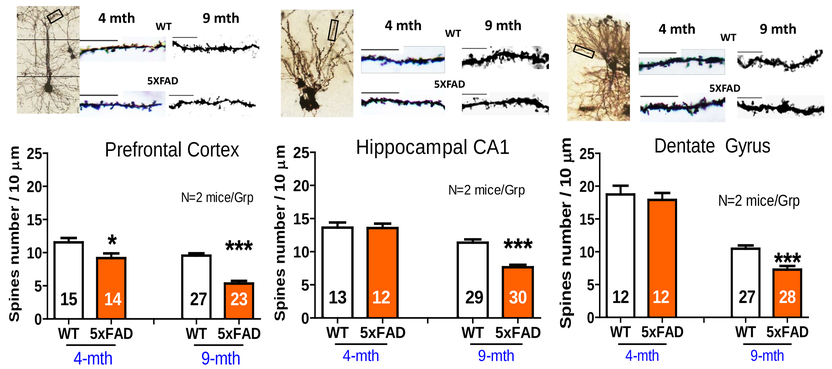
1 Hippocampal $A\beta_{40}$ & $A\beta_{42}$ Changes with Age in 5XFAD Mice

(Hippocampal $A\beta_{40}$ & $A\beta_{42}$ levels were below 5 ng/g in wild type mice from 2-6 month of age)



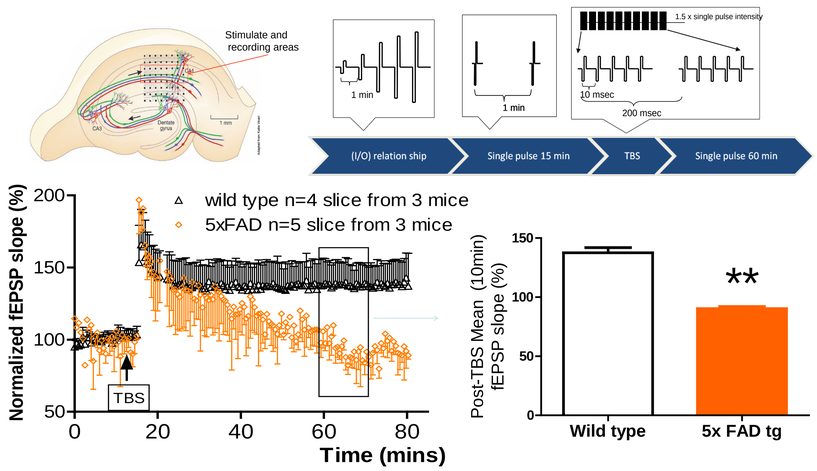
Female 5xFAD mice showed a significantly greater accumulation of $A\beta_{40}$, but not the $A\beta_{42}$, at a younger age than in the males,.

2 Dendritic Spine Density in Prefrontal and Hippocampal Neurons



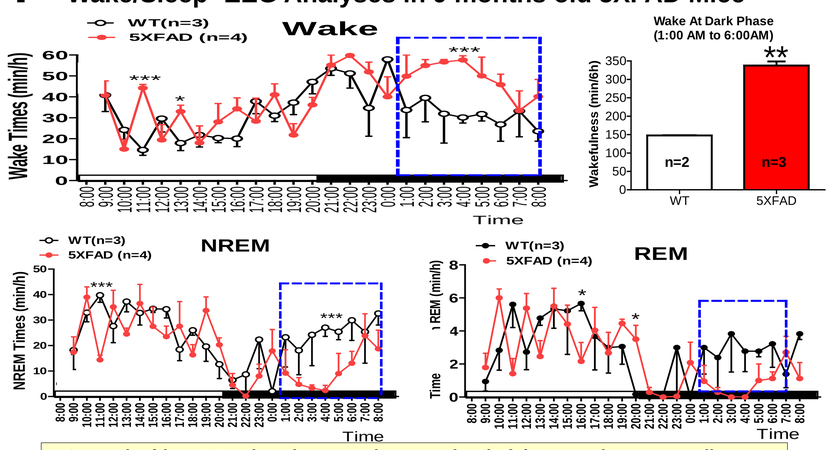
Significant reduction of spine density on the secondary and tertiary dendritic branches of : A) apical dendrtite of medial prefrontal Layer II/III pyramidal neurons, B) Hippocampal CA pyramidal neurons and Dnentate Gyrus granule cells in 5xFAD mice both at 4-month and 9-month old.

3 Hippocampal LTP – in 10 month old 5XFAD vs WT Mice



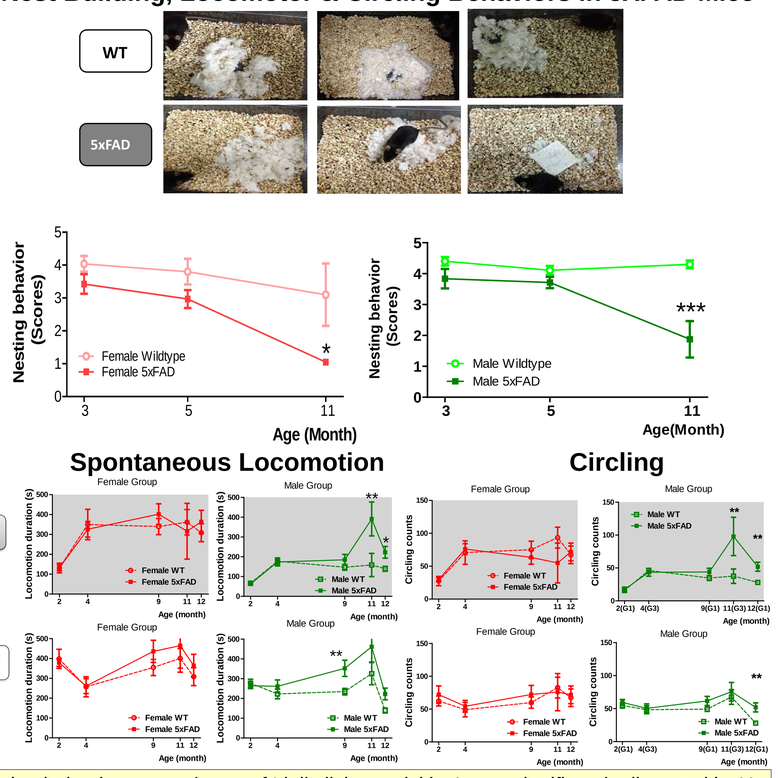
In 10 month old 5XFAD mice, the post-TBS synaptic response Schaffer Collateral - CA1 decayed with time while the WT showed a full sustained LTP.

4 Wake/Sleep EEG Analyses in 9 months old 5XFAD Mice



In 9 month old 5XFAD mice, there was increased wakefulness and corresponding reduction of REM and NREM sleep during the dark (active) phase.

5 Nest Building, Locomotor & Circling Behaviors in 5XFAD Mice



Nesting behavior, an analogue of 'daily living activities', was significantly disrupted in 11 month old 5XFAD mice. At dark phase, there was also a significant increase in spontaneous locomotor activities and bi-directional circling behavior.

Summary and Conclusion

5X FAD Mice ages	2 mth	3 mth	4 mth	6 mth	9 mth	10-11 mth	12 mth
Cortical Aβ (ELISA)	Increase↑	Increase↑	Increase ↑	Increase ↑			
Hippocampus Aβ(ELISA)	Increase↑	Increase↑	Increase ↑	Increase ↑			
Dendritic Spine Density of Prefrontal Neurons			Decrease↓		Decrease↓		
Dendritic Spine Density of Hippocampal Neurons			NC		Decrease↓		
Wake EEG					Increase ↑		
NREM EEG					Decrease ↓		
Spontaneous Locomotor Activity					Male (light phase) Increase ↑	Male (dark phase) Increase ↑	Male (dark phase) Increase ↑
Circling	NC		NC		NC	Male (dark phase) Increase ↑	Male (dark & Light phases) Increase ↑
Nest Building		NC			NC	Decrease ↓(male and female)	
Hippocampal LTP						Lack of LTP	

NC = No Change

The onset of age-dependent changes in the biomarkers and phenotypes in 5 X FAD mice enable us to initiate drug testing at specific ages that pairs with specific meaningful readouts for detection of pharmacological effects.