High-Throughput Phenotyping of Transgenic AD Models

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Introduction

The Inducible Tg4510 Transgenic Mouse Model:

- The Tg4510 tauopathy mouse model is based on the most common mutation in the tau gene, P301L mutation in exon 10, which is the cause of the frontotemporal dementia linked to chromosome 17 (FTDP-17).
- FTDP17 is an autosomal dominant neurodegenerative disorder which presents with behavioral changes, cognitive impairment, and motor symptoms, and is pathologically characterized by the presence of NFT and neuronal loss in the forebrain.

The APP Mouse Models:

- The single transgenic Tg2576 (mutant APP) and APP/PS1 double transgenic mouse models were tested.
- These mice develop a large number of fibrillar amyloid β (Aβ) deposits in the cerebral cortex and in the hippocampus as early as 13 to 16 weeks of age.
- These mice show deficits in Y maze alternation and increased activity prior to the appearance of the neuropathological changes (Holcomb et al., 1998) and this behavioral abnormality seems to persist at later ages (6 to 9 months).
- Spatial reference memory as measured by Morris water maze is altered around 5-6 months of age (Gong et al., 2004).
- Sensorimotor functions, including hearing vestibular functions and motor coordination, are intact in the APP/PS1 mice (Holcomb et al., 1999).

The purpose of this study was to evaluate behavioral deficits with novel high-throughput technology early in life to form a basis to test therapeutic interventions at early stages of developing brain pathology.

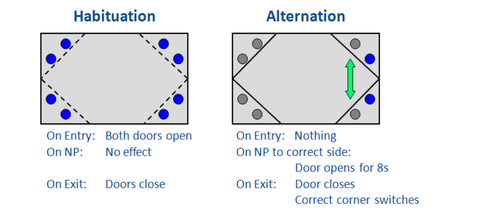
Methods

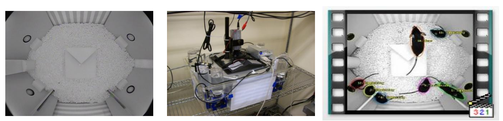
Animals:

- TG4510: The Tg4510 Tauopathy model uses an optimized promoter and capacity to suppress the transgene using the tTA system. Mice were generated crossing a transcriptional operon-Tau mouse (FVBn background) with a CamKII-transcriptional activator (tTA in a C57B6/J background) mouse. Three genotypes were tested: Tau-negative/CamK-negative (double negative; WT), Tau-negative/CamK-positive (tTA) and Tau-positive/ CamK-positive (Tg4510). Doxycycline was supplied in the chow (200 ppm - Harlan) starting at 2 m of age.
- APP: Mice were generated crossing the Tg2576 line with the mutant presenilin line at Psychogenics. Mice were initially provided by the University of South Florida through an exclusive license. Female mice were used for the studies. Two genotypes were tested: APP-negative/ PS1-negative mice (double negative; WT), and APP-positive/ PS1-positive animals (APP/PS1).

Behavioral Assessments:

- PHENOCUBE: Extensively customized Intellicage boxes (TSE) fitted with proprietary video analysis equipment were used. Animals were evaluated in 72 h test sessions and placed in the PhenoCube environment after a 16 h water deprivation period in the home cage. The cages were maintained on a 12:12 light/dark cycle, with white light during the day and red light during the night, maintaining a low subjective light level for the subjects during the night period. While inside the cage, water was only available from within the PhenoCube corners, while food was freely available on the cage floor at all times. Where possible, mice were left undisturbed during the course of experimental sessions. In both test sessions, the test animals initially received magazine training through a simple 'Habituation' protocol, allowing them to freely retrieve water from the PhenoCube corners. Prior to lights-out on day 1, after 6 h in the cage, the protocol was switched to a training protocol described as 'Alternation', requiring the animals to visit specific locations to retrieve water and to alternate between potentially reinforced locations.





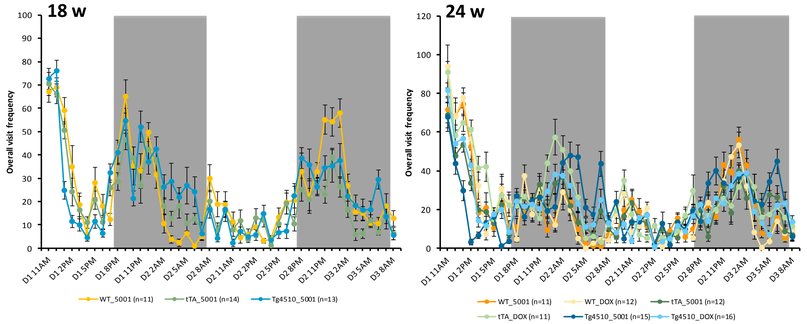
- SMARTCUBE: Mice were placed in a custom built apparatus where multiple challenges were presented over the course of each test session. Digital videos of the subjects were processed through computer segmentation algorithms to fit geometrical models to each mouse frame image. The resulting fitted parameters were then analyzed using behavioral classifier algorithms to extract behavioral states such as rearing, locomotion and immobility. The data obtained in this way were used to define a phenotypic signature.
- NEUROCUBE: Mice were allowed to acclimate in the experimental room for 1 h prior to test. Following acclimation to the test room mice were placed in the NeuroCube™ system and allowed to walk in the apparatus for a 5-min session, during which time the animals completed ~20 test runs. Digital videos of the subjects were processed through computer segmentation algorithms. The resulting fitted parameters were then analyzed to extract clips of locomotor behavior. Those clips were further analyzed to extract information about splay, gait, base, paw position, paw image intensity, limb coordination and body movement, among other features. The data obtained in this way were used to define a phenotypic signature.

Bioinformatics for SmartCube and NeuroCube: The most dominant of the features collected that define the phenotype (symptom descriptors) are identified and ranked using complex proprietary bioinformatics algorithms and an overall discrimination index is calculated for all features combined or for different subsets of features. Graphical representations of the datasets corresponding to the groups compared are derived and a p-value is calculated to assess the statistical significance of the discrimination ratios obtained.

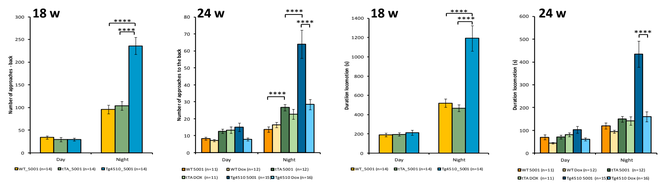
Results: Tg4510 Mouse Model

PHENOCUBE

Corner Visit Frequency: At both ages, the three genotypes were similar to each other during the day. At night, the tTA mice visited corners significantly less than the WT or Tg4510 mice at 18 weeks while the Tg4510 mice visited corners more than all other groups at 24 weeks of age.

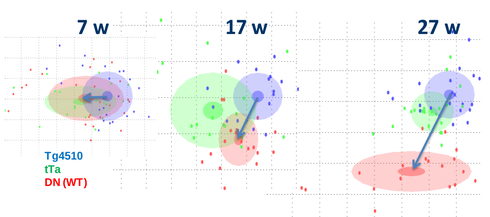


Active Social Behavior and Locomotion: Tg4510 mice showed increased active social behavior at night for both ages. This effect was attenuated by dox treatment. The pattern seen in the active social behavior is very similar to the overall locomotion.

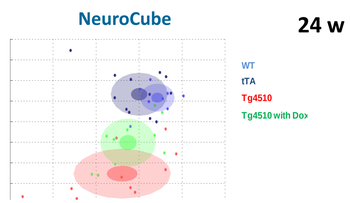


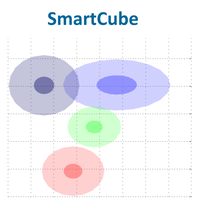
SMARTCUBE & NEUROCUBE

Age	7 w	17 w	27 w
DN v Tau	51% p > 0.63	76% p < 0.003	89% p < 0.0001



SmartCube: In a previous study Tg4510 (no Dox on 129 x FVBn background) showed that the "terminal" signature seen at 27 weeks had an onset around 17 w and was absent at 7 week. A classifier trained at each of the ages independently, however, did find significant differences suggesting that the phenotype varies qualitatively across ages. The tTA control was similar to the WT group.





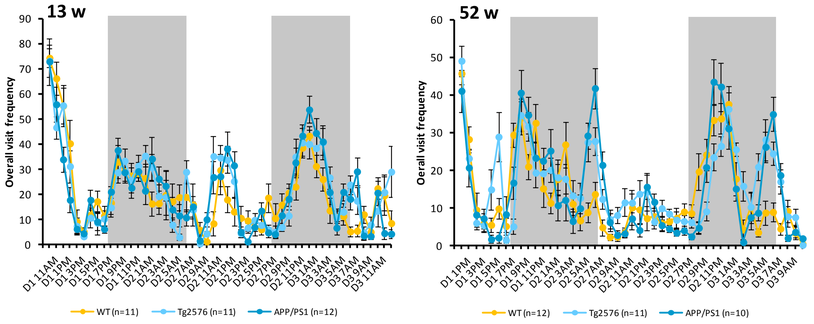
Tg4510 (no Dox) showed decreased height of the body during locomotion and its variability, reduced stride duration, decreased swing duration, increased speed. Doxycycline rescued most features to some extent and completely normalized speed. Doxycycline had no effects on the tTA and WT control groups.

Tg4510 (no Dox) showed a more elongated body, investigation of an aversive stimulus, increased mobility, decreased height of the body during locomotion and its variability, decreased rearing. The way the Tg4510 mice explore the chamber was also very different. Doxycycline partially rescued all features to some extent and had no effects on the tTA and WT control groups.

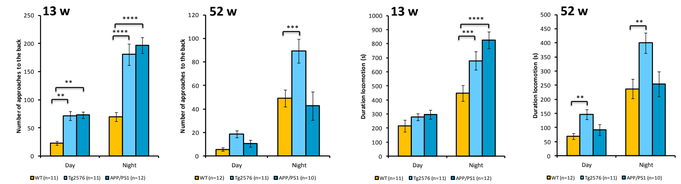
Results: APP Mouse Models

PHENOCUBE

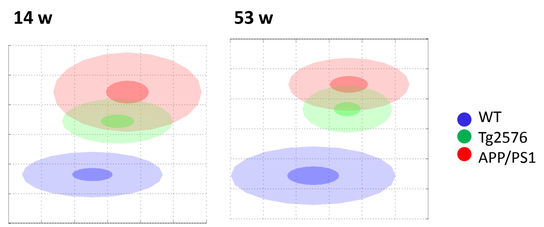
Corner Visit Frequency: The three genotype groups were similar at both ages in the number of entries to the corners, where water was available. Interestingly the two transgenic groups seem to have a delayed circadian rhythm.



Active Social Behavior and Locomotion: Both transgenic groups showed increased active social behavior, chasing, following and interacting more with a partner. At the older age this was only seen in the Tg2576, particularly at night.



SMARTCUBE



Cloud Graphical Representation: Differences between the genotypes at 14 and 53+ weeks of age. The discrimination between the WT and APP/PS1 groups reached 88%-93% (young and old, respectively). The Tg2576 was somehow intermediate but not significantly different from the APP/PS1 in this analysis. Decreased features include grooming, shape variability and rearing (n=12-14/ group). Burst of locomotion were increased although distance covered was not.

Conclusions

Tg4510

- Using a relatively novel genetic background, the FVBn x C57, we have demonstrated a robust behavioral phenotype in the Tg4510 mice in highthroughtput platforms assessing gait and responses to different anxiogenic and motor challenges.
- We have shown also that all features can be modulated by the addition of doxycycline in the chow.

APP

- We have demonstrated a behavioral phenotype in the APP/PS1 mice and Tg2576 mice comprising social, activity, and cognitive changes in a home-cage environment.