Differences between Tg2576 and APP/PS1 mice in high throughput behavioral screening correlates with differences in brain pathology

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INTRODUCTION

The APP/PS1 double transgenic mice were created by cross between Tg2576 (APP695sw) and a mutant P1 (M146L) mouse line (Holcomb et al. 1998, 1999). These mice showed increased amyloid β 40 and 42 levels and develop early amyloid pathology in the cerebral cortex and hippocampus, accompanied by signs of neuro-inflammation (Fieni et al., 2008), characterized by significant microglia activation and significant astrogliosis in cortex and hippocampus. The mice also show increased brain and peripheral inflammatory markers. Behavioral deficits correlate to brain pathology (Gordon et al., 2003). The results also show significant memory defect as early as 12 weeks of age in spatial working memory, fear conditioning and spatial learning. 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). Also, sensorimotor functions, including hearing vestibular functions and motor coordination, are intact in the APP/PS1 mice (Holcomb et al., 1999).

METHODS

Animals

Mice were generated by crossing the Tg2576 line with the mutant presenilin line in Psychogenics. Mice were initially provided by the University of South Florida for a cerebral cortex analysis. Male mice were used for the studies. Two genotypes were tested: APP- negative PS1-negative (double negative, WT), and APP-positive PS1-positive (APP/PS1), because there was a significant range in phenotype between male and female animals. Each male was mated to 4 females with the APP/PS1 genotype. APP-positive PS1-positive animals were generated and identified by genotype, numbers groups and housed in OPTI mice ventilated cages for the duration of the studies. Animals were maintained on a 12 h light/dark cycle: the lights on at 7:00 AM EST. Room temperature remained constant with relative humidity maintained at 30-70%. Prior to testing, all animals were examined on a regular basis, handled, and weighed to assure adequate health and survivability. In such tests, animals were randomly assigned across groups.

Behavioral assay

PhenoCube: Extensively customized Intellcube boxes (New Behavior AG) fitted with proprioceptive video analysis equipment. Animals were evaluated 48 h test sessions, being placed in the PhenoCube environment after a 15 h water deprivation period in the home cage. The cages were maintained at a 12:12 light 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 centers, while food was freely available on the cage floor at all times. 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 ‘Halitersm’ protocol, allowing them to freely receive water from the PhenoCube center. Prior to light-on, a day 1 after it, in the cage, the protocol was switched to a training protocol described as ‘Alerting’, requiring the animals to visit specific locations to receive water and to alternate between motorically confined locations.

SmartCube: Mice were placed in a custom built apparatus where multiple challenges were presented over the course of each such test session. Digital videos of the subjects were processed through computer segmentation algorithms to fit geometrical models to each mouse home image. The resulting fitted parameters were then analyzed using behavioral 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: The NeuroCube system is a platform that employs computer vision to detect changes in gait geometry and gait dynamics in rodents specific to neurological disorders, pain & neuropathy. This platform is unique in that gait analysis is completely automated and thus removing any bias or subjectivity. The validity of the computer vision and bioinformatics allow PsychoCube to capture signatures of the disease model earlier and more accurately.

Behavior

Data Analysis and bioinformatics

For SmartCube and NeuroCube, the most dominant of the features collected that define the phenotypes (symptom descriptors) are identified and ranked using complex proprietary bioinformatics algorithms and an overall discrimination index is calculated for all 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. PhenoCube data are presented as mean SEM. ANOVA or t-test were used for analysis.

DISCUSSION

Behavioral analysis in PhenoCube's proprietary technologies shown that APP/PS1 and Tg2576 mice showed robust and surprising changes in social behavior and increased locomotor activity particularly during night time. SmartCube showed that APP PS1 mice had lower grooming and rearing and confirmed the hyperactivity seen in PhenoCube. Gait differences were also seen between WT and APP PS1 mice where Tg mice showed increased swing and stride duration, and higher speed compared to WT mice. Histologically plaque load and astrogliosis are commonly known to be progressive with age, but brain regions related to gait disturbances, such as the substantia nigra and the cerebellum, are vastly free from plaque deposition at the age of appearance. Pathological alterations such as the lysosomal storage defect in the nigra might contribute to the altered motor phenotype measured at 40 weeks of age.

HISTOLOGY

APP/PS1 mice samples derive from a separate cohort of mice, sampled at 28, 40 and 78 weeks of age. All mice were female, age- and sex-matched with mice served as controls. In short mice were flushed with 1% saline to remove MoS2, then brains were harvested, divided at midline and the left hemisphere was por bored for 2 hours in 4% PFA. Thereafter they were cryo-processed in 15% sucrose until坚 and frozen in frozen trimming medium within styro-cooled liquid nitrogen. A uniform sequence random of set of sections, ten microscan at thickness through the whole cerebral cortex and hippocampus was immunofluorescently labeled with antibodies (anti-Iba1, anti-Zo-1, anti-ZO-1) and thermally processed. Both sections were stained in an Aqua Free 2® liquid scanner at 10x magnification. Quantifications using Image Pro-Plus were automated and rate independent using constant evaluation parameters for each marker. IR was generally measured as average intensity labeled and using size restriction.

Figure 1: PhenoCube

Figure 2: APP/PS1 mice showed increased locomotor activity, particularly at night. At the older age this was only seen in the Tg2576, particularly at night. ‘9% to 14% to WT mice.

Figure 3: PhenoCube revealed that both transgenic groups showed increased active social behavior. At the older age this was only seen in the Tg2576, particularly at night. ‘9% to 14% to WT mice.

Figure 4: SmartCube revealed significant changes in gait dynamic and gait geometry between WT and APP/PS1 mice. Cloud graphical representation found that the discrimination between the WT and APP/PS1 mice is 9-12% - significant. 

Figure 5: Clud Graphical Representation showing Difference between the younger at 14, 40 and 53 weeks of age. APP/PS1. The discrimination between the WT and APP/PS1 groups reached 9%-9% (young) and all respectively. The Tg2576 was found no significant differences from the APP/PS1 in this analysis. Decreased features include grooming, shape variability and turning (x±5±53). Rate of locomotion were increased although Distance covered was not.

Figure 6: A: HISTOLOGY: IR (left) and GFAP IR astrogliosis (right) in the cerebral cortex and hippocampus of female APP/PS1 transgenic mice over time. Both cortical and hippocampal astrogliosis rise significantly over age, whereas astrogliosis in the hippocampus does not increase over time. B: Representative image of plaques load and cerebellum cortex. Note that the substantia nigra (SN) does not show plaque disposition at the age of 40 weeks.

Figure 7: Development of a lysosomal storage defect in the substantia nigra (SN) of APP/PS1 transgenic mice. The large purple left show the whole composite image of the entire SN, as a 40-week old and for a 46-week old female. In the middle the channel is shown single, right a composite brain of the APP/PS1 and left the SN plaques, microglia plaques, and microglia. IR of these mice show significantly increased levels of this protein in the substantia nigra, after comparing both genotypes on the right side.