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 PS1 (M146L) mouse line (Holcomb et al 1998; 1999). These mice show increased amyloid-β 40 and 42 levels and develop early amyloid pathology in the cerebral cortex and hippocampus, accompanied by signs of neuro-inflammation (Jimenez 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., 2001), resulting in a significant memory deficit as early as 12 weeks of age in spontaneous alternation, 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:

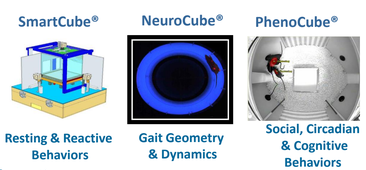
Mice were generated by crossing the Tg2576 line with the mutant presenilin line at Psychogenics. Mice were initially provided by the University of South Florida through an exclusive license. Female mice were used for the studies. Two genotypes were tested: APP-negative/ PS1-negative mice (double negative; WT), and APP-positive/ PS1-positive animals (APP/PS1). Animals were assigned unique identification numbers and group housed in OPTI mice ventilated cages for the duration of the studies. Animals were maintained on a 12 hr. /12 hr. light/dark cycle with the light on at 7:00 a.m. EST. Room temperature was maintained at 20-23˚C 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 suitability. In each test, animals were randomly assigned across groups.

Behavioral Assessments:

PhenoCube: Extensively customized Intellicage boxes (New Behavior AG) fitted with proprietary video analysis equipment. Animals were evaluated in 48 h test sessions, being 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: The NeuroCube® system is a platform that employs computer vision to detect changes in gait geometry and gait dynamics in rodent models of neurological disorders, pain & neuropathies. This platform is unique for gait testing as is completely automated and thus removes any bias or subjectivity. The sensitivity of the computer vision and bioinformatics allow PsychoGenics to capture symptoms of the disease model earlier and more accurately.



Data Analysis and 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. 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

BEHAVIOR

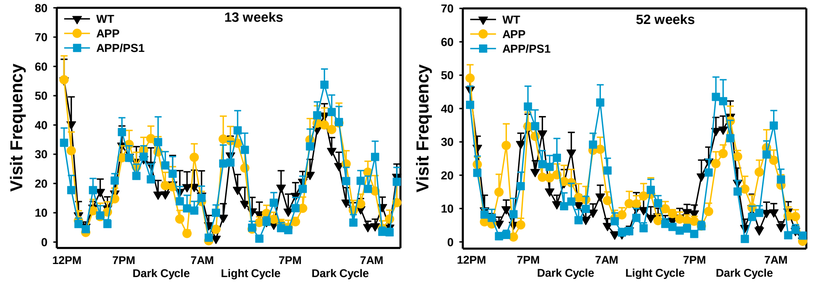


Figure 1: PhenoCube testing showed that the three genotype groups were similar at both ages in the number of entries to the corners, where water was available. Interestingly the two transgenic groups seem to have a delayed circadian rhythm

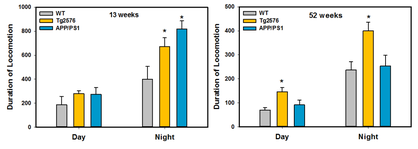


*Figure 2: PhenoCube revealed that both transgenic groups showed increased locomotor activity, particularly at night. At the older age this was only seen in the Tg2576. *p<0.05 vs WT mice*

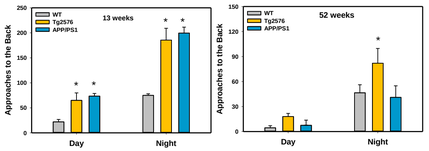


*Figure 3: PhenoCube revealed that both transgenic groups showed increased active social behavior. At the older age this was only seen in the Tg2576, particularly at night. *p<0.05 vs WT mice*

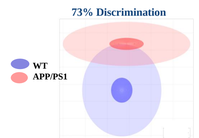


Figure 4: NeuroCube revealed significant changes in gait dynamic and gait geometry between WT and APP/PS1 mice. Cloud graphical representation found that the discrimination between the WT and APP/PS1 groups was 73%; n = 12 – 15 per group.
ABREVIATIONS: HL (Hind Limb); FL (Fore Limb), X axis represents discrimination probability as a % of WT mice

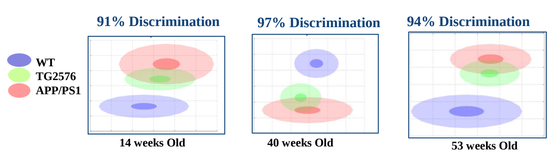
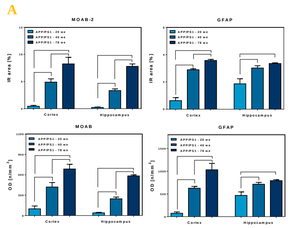


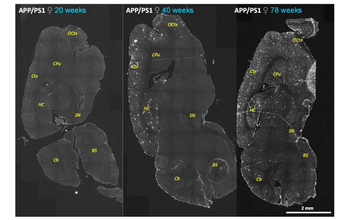
Figure 5: Cloud Graphical Representation showing Differences between the genotypes at 14, 40 and 53 weeks of age in SmartCube. The discrimination between the WT and APP/PS1 groups reached 91%-94% (young and old, respectively). The Tg2576 was somehow intermediate but not significantly different from the 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.

HISTOLOGY

METHODS

APP/PS1 brain samples derive from a separate cohort of mice, sampled at 20, 40 and 78 weeks of age. All mice were female, age- and sex-matched wildtype mice served as controls. In short mice were flushed with 0.9% saline to remove RBCs, then brains were harvested, divided at midline and the left hemisphere was post fixed for 2 hours in 4% PFA. Thereafter they were cryo-protected in 15% sucrose until sunk and frozen in tissue freezing medium within cryo-molds in dry-ice cooled liquid Isopentane. A uniform systematic random set of seven sections, ten micron thick and reaching through the whole cerebral cortex and hippocampus was immunohistochemically labeled with MOAB2 + GFAP + Iba1, counterstained with DAPI. Whole sections were imaged on an Axio.Scan Z1 slide scanner at 10x magnification. Quantifications using Image Pro Premier were automated and rater independent using constant evaluation parameters for each marker. IR was generally measured above an intensity based threshold and using size restrictions.





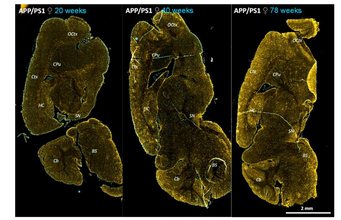


Figure 6: A: MOAB-2 IR Aβ (left) and GFAP IR astrogliosis (right) in the cerebral cortex and hippocampus of female APP/PS1 transgenic mice over age. Both Aβ load and concomitant astrogliosis rise significantly over age, whereas astrogliosis in the hippocampus does not increase anymore between 40 an 78 weeks. Data are shown for IR area percentage (upper lane) and object density (lower lane; n=4/ group). B,C: representative images of plaque load and astrogliosis over age. Note that the substantia nigra (SN) does not show plaque deposition at the age of 40 weeks.

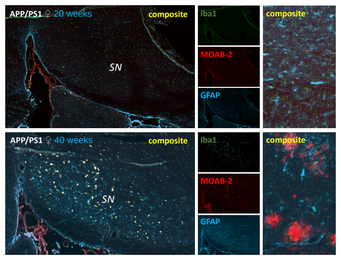


Figure 7: Development of a lysosomal storage defect in the substantia nigra (SN) of APP/PS1 transgenic mice. The large pictures left show the color composite image of the entire SN, up for a 20 weeks and down for a 40 weeks old female. In the middle the channels are shown singly, right a color composite of the hippocampal CA1 region in the same section to identify the specific labeling to plaques, astro- and microglia. Of note these mice show a very high density of autofluorescent cell debris in the reticular part of the SN, which largely excludes involvement of dopaminergic neurons. Probably affected neuron types might be cholinergics or GABAergic interneurons, yet, the true type has to be identified using specific markers.

DISCUSSION

Behavioral analysis in PGI'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 less 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 decreased swing and stride duration and higher speed compared to WT mice. Histologically plaque load and astrogliosis are commonly known to be progressive with age. However, 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.