Behavioral Manifestations, Aggression and Plaque Distribution in Transgenic Mouse Models of Alzheimer's Disease

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INTRODUCTION

Alzheimer's Disease (AD) is a chronic neurodegenerative disorder that is the most common cause of dementia in the elderly population. In rodent models, the primary measure used to examine AD is cognitive function, which declines rapidly in aged/diseased mice with the accumulation of extracellular beta amyloid and subsequent aggregation of amyloid plaques. Often ignored are the non-cognitive neuropsychiatric symptoms such as abnormal motor behavior, anxiety, aggression and irritability. Additionally, most studies in mice tend to primarily examine females as deficits in cognition seem to be gender-specific. The present study was aimed at measuring non-cognitive behaviors such as aggression using the resident intruder paradigm and relevant pathologies such as plaque load and distribution in mutant mice expressing human APP, namely the APP/PS1 (APPsw/PS1 (m146L)) and TASD41 (hAPP751 with the London V717I9/Swedish double mutation K670M/N671L). We also assessed whole animal behavior using our high-throughput, non-biased and automated screening platform, SmartCube® in order to systematically examine subtle changes in behavior that may occur early on in both models of amyloidosis.

METHODS

ANIMALS

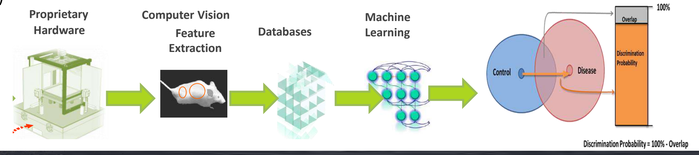
Male, APP/PS1 (APPsw/PS1 (m146L) and TASD41 (hAPP751 with the London V717I9/Swedish double mutation K670M/N671L) mice were used. Since male mice from both strains tend to show aggression early on, they were single-housed from weaning. For the resident intruder test, C57BL/6J mice were used as intruders and group housed (4 per cage). All animals were examined and weighed prior to initiation of the study. During the course of the study, 12/12 light/dark cycles were maintained. The room temperature was 20-23°C with a relative humidity maintained between 30-70%. Chow and water were provided *ad libitum* for the duration of the study

BEHAVIORAL ASSAYS

Resident Intruder: The resident-intruder (RI) paradigm was used to assess aggression in APP/PS1 and Line41 male mice. Male APP/PS1 or Line41 (Resident) mice were single-housed in standard mouse cages, without enrichment, for a minimum of 3 weeks prior to study initiation. All animals were pre-screened during a 5 minute session in order to ensure a uniform distribution of aggressive behavior across treatment groups. All testing occurred in the home cage of the resident mouse and were carried out under red-lights. During the 5 minute session, the latency time to attack , as well as the total number of attacks, were recorded manually by an experimenter blinded to the treatment groups. One week after the pre-screen RI session, resident mice (APP/PS1 or Line41) were administered vehicle or reference compound 30 minutes prior to RI test 1 and tested as described above. **Statistical Analyses:** Data obtained in resident intruder test were analyzed using multi-factorial analyses of variance (ANOVA). For each group 10-12 male mice were used.

The **SmartCube® system** is designed to and can successfully measure numerous spontaneous behaviors and response to challenges in the same testing environment. The hardware includes force sensors and a number of aversive stimuli to elicit behavior. Three high-resolution video cameras provide constant 3D view of the mouse in the SmartCube® apparatus throughout the entire testing period. During the 45 minute test session the mice are exposed to a sequence of challenges. The cubes are cleaned between each run. 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.

Feature Analysis: Data are typically presented as: Control and Disease. We first transform original feature set to the non-redundant de-correlated ranked features space and plot Control and Disease in the coordinate system formed by the two highest-ranked (best-discriminating between the two group's new features). Quality Measure of Disease Model = Overlap between the Control and Disease groups (Discrimination Probability = 100% - Overlap)



RESULTS

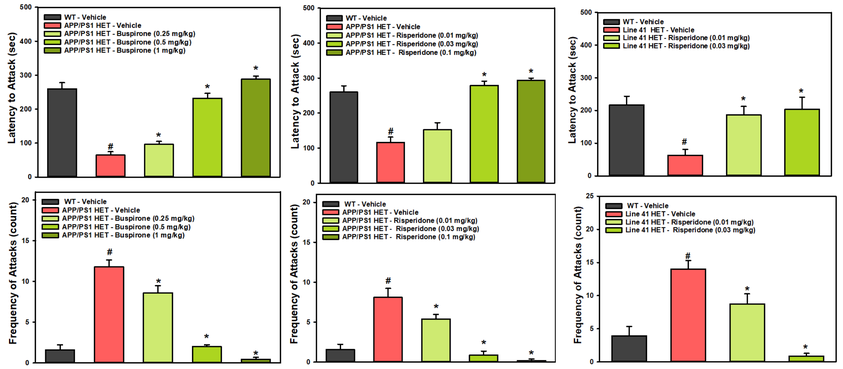
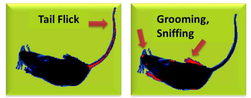


*Figure 1: Increased aggressive behavior in male APP/PS1 and Line41 mice as measured by a reduction in the latency to attack (top) and increase in frequency of attacks (bottom). Attenuation in aggression was noted following acute administration of Buspirone (0.25 – 1 mg/kg) or Risperidone (0.01 – 0.1 mg/kg). # p<0.05 compared to WT-vehicle; * p<0.05 compared to mutant-vehicle group. Mice aged 7-9 months.*



Computer vision algorithms detect various behaviors in SmartCube ®

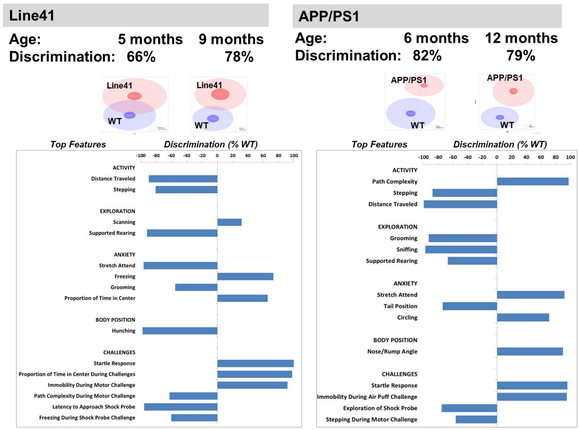


Figure 2: Top: Cloud plots to visualize Control – Mutant (APP/PS1 or Line41) groups relationship. Each cloud is a scatter plot of the mice from a particular group approximated by a 2D Gaussian (ellipse) in the 2D optimal discrimination feature space (the two coordinates being the first two Principal Components formed from the top highest ranked original features). Bottom: Top discriminating features plotted as % WT control and separated into categories pertaining to activity, exploration, anxiety, body position as well as reactions to environmental challenges.

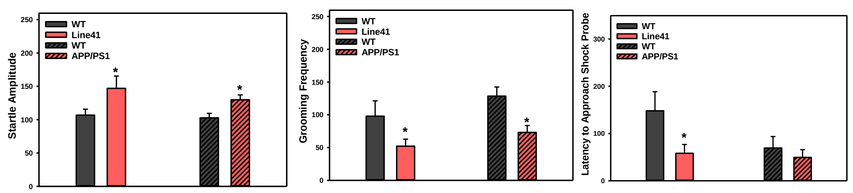


*Figure 3: Comparison of Distinct Behavioral Abnormalities in APP/PS1 and Line41 male mice as measured by SmartCube ® System. *p<0.05 compared to WT counterpart.*

Serial Two-Photon Whole Brain Imaging was performed by **TissueVision** in order to determine the distribution, size and density of Aβ-plaques in Line41 male mice using the **TissueCyte®** imaging platform. Briefly, to register the brains to a common reference atlas and quantify regional Methoxy-X04 signal density, for each brain sample, a 10% down-sampled dataset was generated from the autofluorescence channel. The Allen Institute Common Coordinate Framework (CCF) V3 was warped onto each down-sampled dataset using a course-to-fine registration consisting of translation, affine and non-linear deformations. To calculate Methoxy-X04 density per region, the number of masked (Methoxy-X04 positive) pixels were quantified per region for each full resolution coronal section in a sample. The number of region pixels for each section was then computed after scaling the warped atlas to the size of the full resolution datasets. Methoxy-X04 density is defined as the total number of Methoxy-X04 positive pixels / total number of region pixels across all sections in which a region is present.

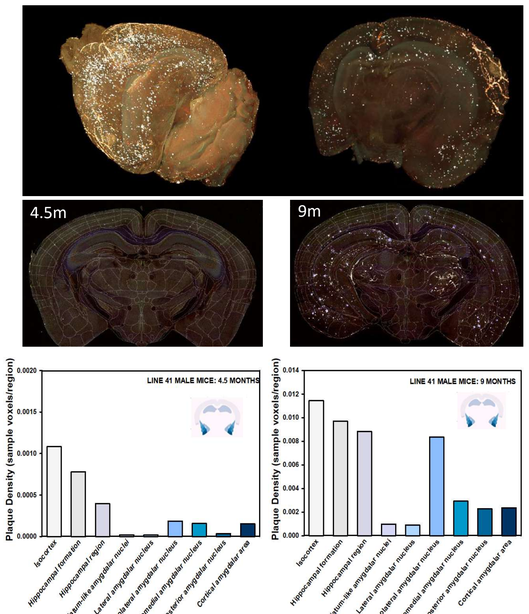


Figure 4: Top: Volumetric renderings of example Line41 whole mouse brain (left) and cross section (right) showing plaques (white) distributed across brain regions. Middle: Atlas registration to the Allen Institute CCF v3.0 allowed regional density quantification of plaque distribution in young (4.5 months and old (9 months) male Line41 mice. Bottom: Data suggests an increase in plaque load in cortical, hippocampal and amygdala regions with disease progression. More qualitative data revealed that younger mice exhibit very sparse parenchymal plaques whereas aged mice show a clear progression in plaque density that appears more pronounced in the hippocampus and basolateral amygdala.

SUMMARY

In summary, we demonstrate clear aggressive behavior and pharmacological validation with buspirone and risperidone in aged, male mouse models of amyloidosis, with more advanced computer vision systems identifying distinctive behavioral patterns and discriminating the phenotype at several disease stages. Together with the region-specific progression of plaque densities in cortical, hippocampal and amygdala sub-regions, these models present a valuable tool for early intervention and improved assessment of potential therapeutic approaches for neuropsychiatric symptoms of AD and in particular AD-induced aggression.