The Dissociation of Normal Aging from Alzheimer's Disease in Mouse Models of Tauopathy and Amyloidosis

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INTRODUCTION

Aging is the leading risk factor for chronic diseases that account for the bulk of morbidity, mortality, and health costs. Although there's been tremendous progress in understanding the underlying molecular processes important in the aging cascade, much remains unknown specifically with regard to the relationship of normal aging to more advanced pathological states such as Alzheimer's disease (AD). In order to better understand this critical relationship and provide more robust pre-clinical models for drug development, the goal of the current study was two-fold; 1) to identify the prominent behavioral features related to standard aging in C57BL/6 mice using both standard and computational analyses; 2) to use computational tools in order to dissociate the effects of aging from two mouse models of AD, namely rTg4510 (Tauopathy) and APP/PS1 (Amyloidosis).

METHODS

ANIMALS: Male and Female C57Bl6J mice were used for phenotyping of aged mice. The Tg4510 mice were generated crossing a transcriptional operon-Tau mouse (FVBn background) with a CamKII-transcriptional activator (tTA in a C57B6/J background) mouse. The APP/PS1 mice were generated crossing the Tg2576 line with the mutant presenilin line. Female mice were used for both Alzheimer's Disease (AD) lines as they demonstrate a robust behavioral profile resembling cognitive deficits in AD. Animals were group housed for the duration of the study and 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%.. For each group 12-15 mice were tested.

Y-maze: The Y-maze is an acute, rapid test that provides a measure of working memory and exploratory behavior. It is based on the innate tendency of a mouse to explore novel rather than familiar environments: thus, when allowed to explore a 3-armed maze ('Y-maze') the subjects alternate their arm visits so that they avoid re-visiting the most recently explored arm. The percentage of alternations performed, relative to the total number possible given an animals' overall number of visits, provides a useful measure of working memory.

Prepulse Inhibition: Mice were placed in the PPI chambers (Med Associates) for a 5 min session of white noise (70 dB) habituation. The session started with a habituation block of 6 presentations of the startle stimulus alone, followed by 10 PPI blocks of 6 different types of trials. Trial types are: null (no stimuli), startle (120 dB), startle plus prepulse (4, 8 and 12 dB over background noise i.e. 74, 78 or 82 dB) and prepulse alone (82 dB). Trial types were presented at random within each block.

Social Interaction (SI): SI testing was performed in a custom open field arena (43.2 X 43.2 X 30.5cm) with an automated video tracking program (View Point, Life Sciences) Briefly, social approach behavior was evaluated in the presence of a novel C57Bl6J mouse. The time spent within a pre-specified interaction zone was recorded.

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)

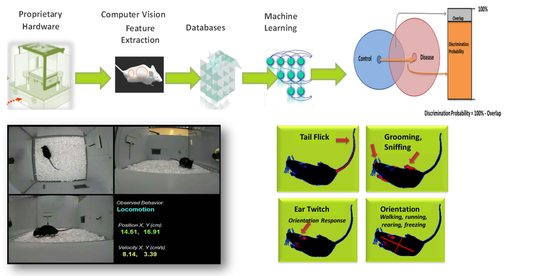

Figure 1: Computer vision algorithms detect various behaviors in SmartCube ®

RESULTS

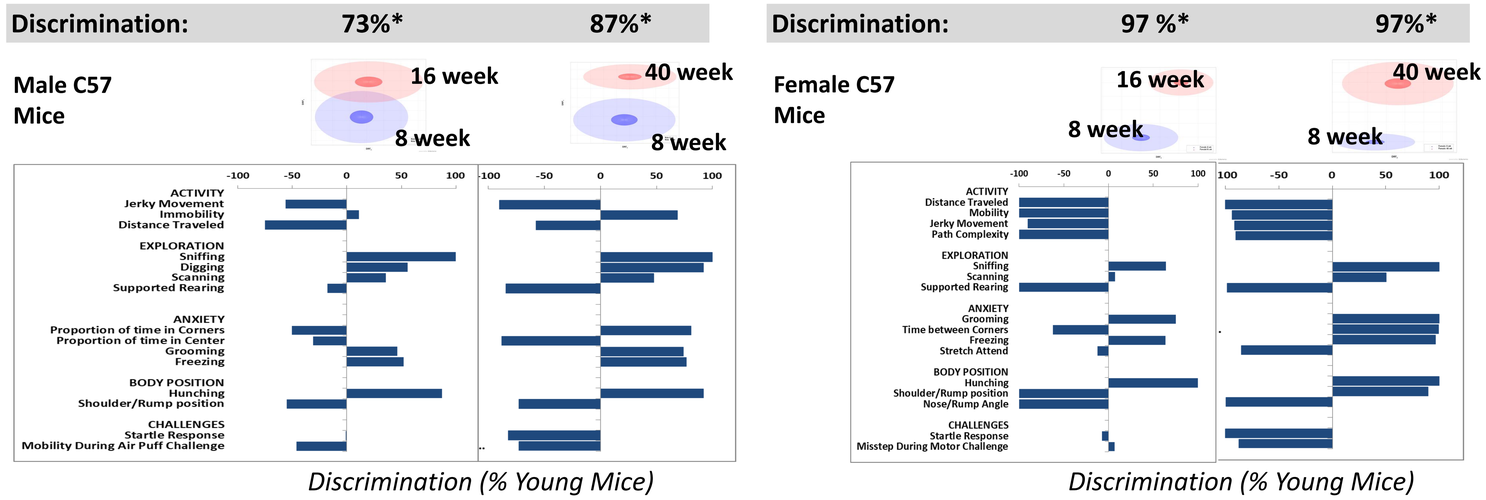

Figure 2: Top: Cloud plots to visualize young – old (C57Bl6) group relationship. Each cloud is a scatter plot of the mice from a particular group approximated by a 2D Gaussian (ellipse) in the 2D optimal discrimination feature space (the two coordinates being the first two Principal Components formed from the top highest ranked original features). Bottom: Top discriminating features plotted as % young control and separated into categories pertaining to activity, exploration, anxiety, body position as well as reactions to environmental challenges.

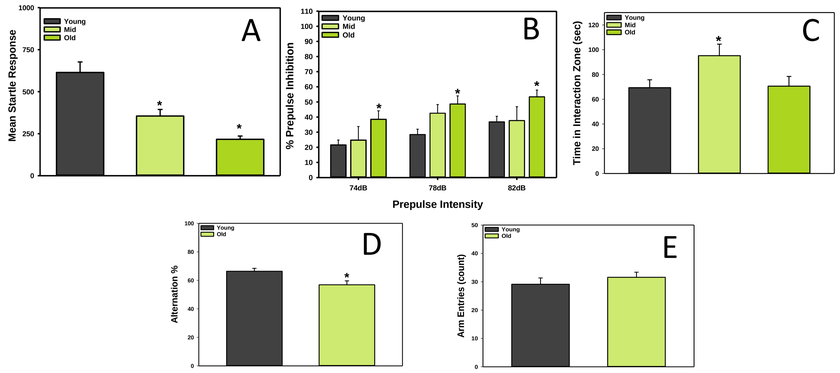

Figure 3: Behavioral Assessment of Aging C57Bl6 mice. Reduced startle response in mid and old-aged mice compared to young mice; Increased % PPI in old compared to young mice (B); Increased interaction time in mid-aged mice compared to young mice (C), Reduced alternations in the Y-maze in old versus young mice (D) with no change in arm entries (E).
* p<0.05 compared to Young Mice. Young (8-12 weeks); Mid (16-20 weeks); Old (37-42 weeks).

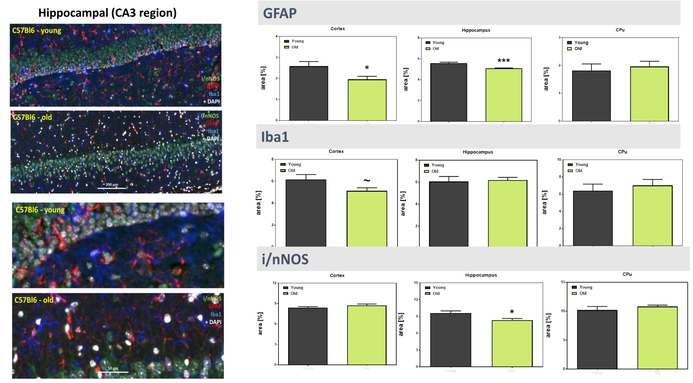

Figure 4: C57Bl6 mice show several age-dependent alterations in overall measurements of glia and i/nNOS. Specifically, reduced GFAP in the cortex and hippocampus, trend of reduced Iba1 labeling in the cortex and reduced i/nNOS in the hippocampus of older animals (~12 months). This is suggestive of lower complexity of glial cells, indicating a loss of function that may negatively alter neuronal function. Further in depth analyses are ongoing. * p<0.05 compared to Young Mice, ~ p=0.1 compared to Young Mice.

Dissociation of Aging in Mouse Models of Alzheimer's Disease

Group	Discrimination Prior to Removal of Aging Features	Discrimination After Removal of Aging Features
WT_WT (3.5 mo vs 10 mo)	85%*	59%
tTA (4.5 mo vs 6 mo)	90%*	58%
APP/PS1 (3.5 mo vs 10 mo)	77%*	74%*
Tg4510 (4.5 mo vs 6 mo)	92%*	90%*

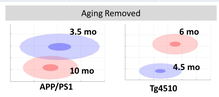

Figure 5 Group discrimination and cloud plots demonstrating the relationship between young and old animals for each particular model after computational removal for age-related behavioral features. Aging features were identified by comparing control animals at two different time points.

Disease Progression in APP/PS1 Mouse Model of Amyloidosis (Aging Removed)

Discrimination: 70% 78%
Age: 3.5 mo 10 mo

APP_PS1
WT

Change in Behavior	Top Discriminating Behaviors	
Increased	Path complexity, portion of time in the center, hunching	Free rearing, freezing, Immobility
Decreased	Startle Response, miss-steps during motor challenge, supported rearing	Body movement, miss-steps during motor challenge, path complexity

Disease Progression in Tg4510 Mouse Model of Tauopathy (Aging Removed)

Discrimination: 90%* 89%*
Age: 4.5 mo 6 mo

Tg4510
tTA

Change in Behavior	Top Discriminating Behaviors	
Increased	Body movement, mobility during motor challenge, activity	Mobility, hunching, Time between corners
Decreased	Digging, rearing, time in the corners, miss-steps during motor challenge	Digging, path complexity, miss-steps during motor challenge

Figure 6: Top: Cloud plots to visualize control (WT or tTA) – AD models (APP/PS1 or Tg4510) group relationships. Computational analyses were performed to account for the effects of aging for each model above. The remaining non-aging features are summarized in table form (bottom). Data is shown compared to age-matched WT or tTA controls.

CONCLUSION

In summary, we demonstrate here the behavioral features related to aging in C57Bl6 mice using both standard and algorithm-based behavioral assays. Specifically, using SmartCube® analyses we are able to identify very subtle behaviors related to aging in both male and female C57Bl6 mice. Standard behavioral tests also demonstrate a deficit in startle response and working memory in older animals. More importantly, we are able to utilize computational analyses in order to dissociate aging-specific behavioral features from mutant lines of Tauopathy and Amyloidosis and identify, with more precision, AD-specific features using advanced and un-biased computer vision systems. Such an approach would be extremely valuable when assessing novel potential therapeutic approaches for AD.