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NEURODEGENERATION VS AGEING

Ageing is the most common risk factor for Alzheimer's disease and most other neurodegenerative disorders. Neurodegenerative disorders and natural healthy ageing show gradual deterioration in memory, cognition as well as changes in circadian rhythm. Therefore, studying features that separate the neurodegenerative phenotypes from biological ageing will allow deeper understanding of the disease pathophysiology and its potential treatments. **Here, we establish a novel method to investigate complex patterns of spontaneous behaviour to identify hallmarks of neurodegeneration, allowing its discrimination from natural healthy ageing.**

SYSTEMATIC ANALYSIS OF HOME-CAGE BEHAVIOR

We performed a systematic analysis of spontaneous home-cage behavior over a period of 2.5 days (3 dark phases, 2 light phases) in widely used mouse models for neurodegenerative diseases at different ages (models are supplemented as **Table 1**). Mice were tested for a total of 58 behavioral parameters in automated home-cages (PhenoTyper™), which were further analysed with the use of AHCODA™ analysis software (**Figure 1**). Mutant mice were compared to their wildtype litter mates (control) whereas mice treated with compounds were compared to control mice treated with vehicle. Principle component analysis (PCA) was performed on these datasets. PCA was performed using R with the PCA function from the FactoMineR package on the 58 parameters. The squared cosine ("cos²") was used for the fviz_pca_ind function from the factoextra package in order to get the importance of the contribution to the Principal Component for each individual animal on the first two PC's. The X and Y coordinates of PC1 and PC2 for each individual animal were stored for further analysis.

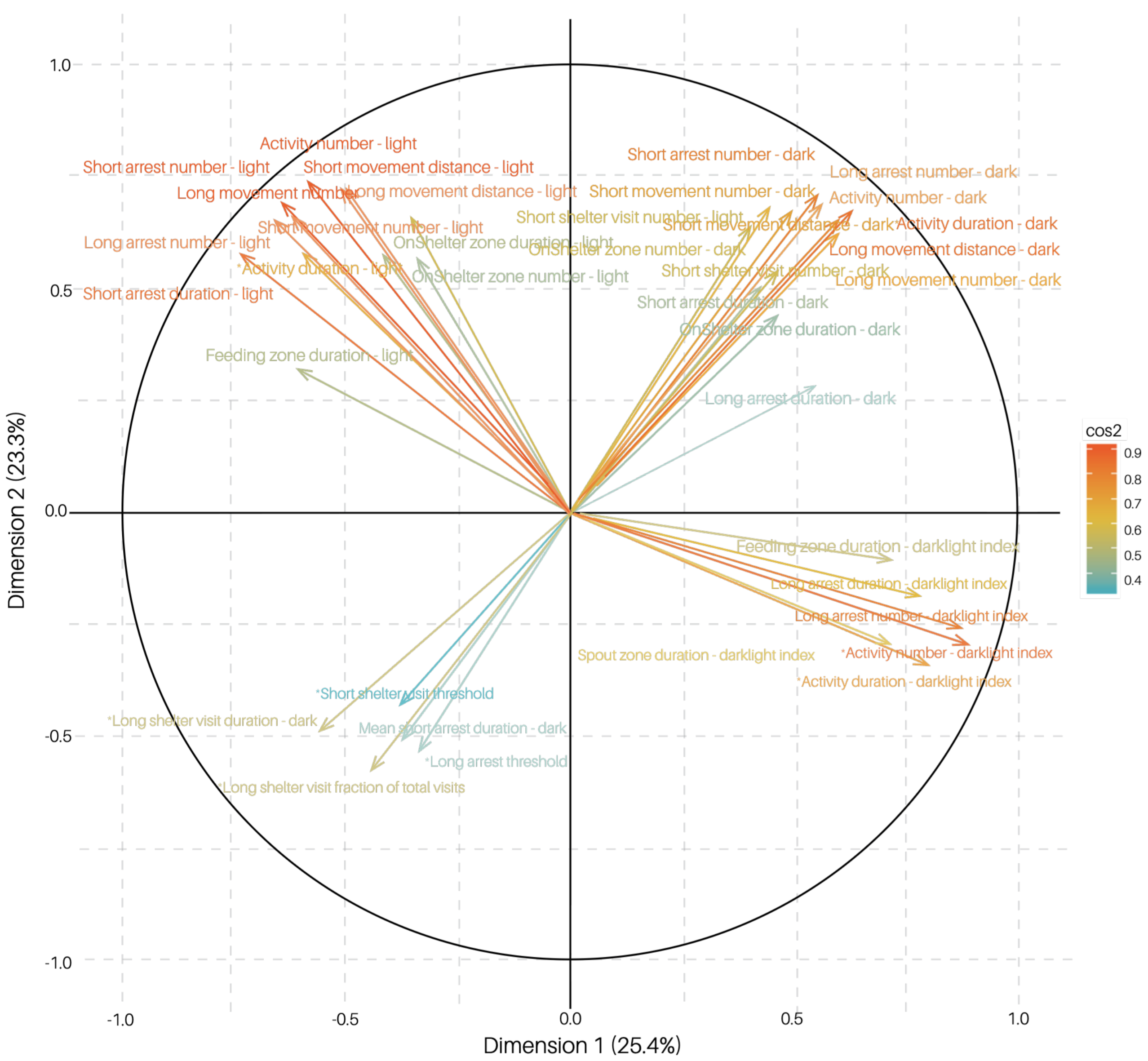


FIGURE 1. Principal Component Analysis (PCA) of 58 behavioral parameters analysed. PCA finds correlations of parameters in a large data matrix and combines the parameters in orthogonal variables called Principal Components (PCs). These correlations are controlled for quality and return a matrix of eigenvalues which represent the importance of each factor and in turn the amount of explained variation found in the dataset. The average cos² for each group used ranged between 0.21 and 0.72, meaning each group contributed in a different way to the PC1 and PC2. The contribution (cos²) for the parameters (with a high contribution >= 0.33) in the first 2 PCs were plotted (X and Y values) showing clear distinction between parameter types in different directions which can be grouped into 4 categories: 1. Activity during the light phase (negative on PC1, positive on PC2). 2. activity during the dark phase (positive on PC1, positive on PC2). 3. Dark/light behaviour change (positive on PC1, negative on PC2). 4. Long sheltering and resting (arrest) behaviour (negative on PC1, negative on PC2).

ANALYSIS OF DISTANCE MOVED

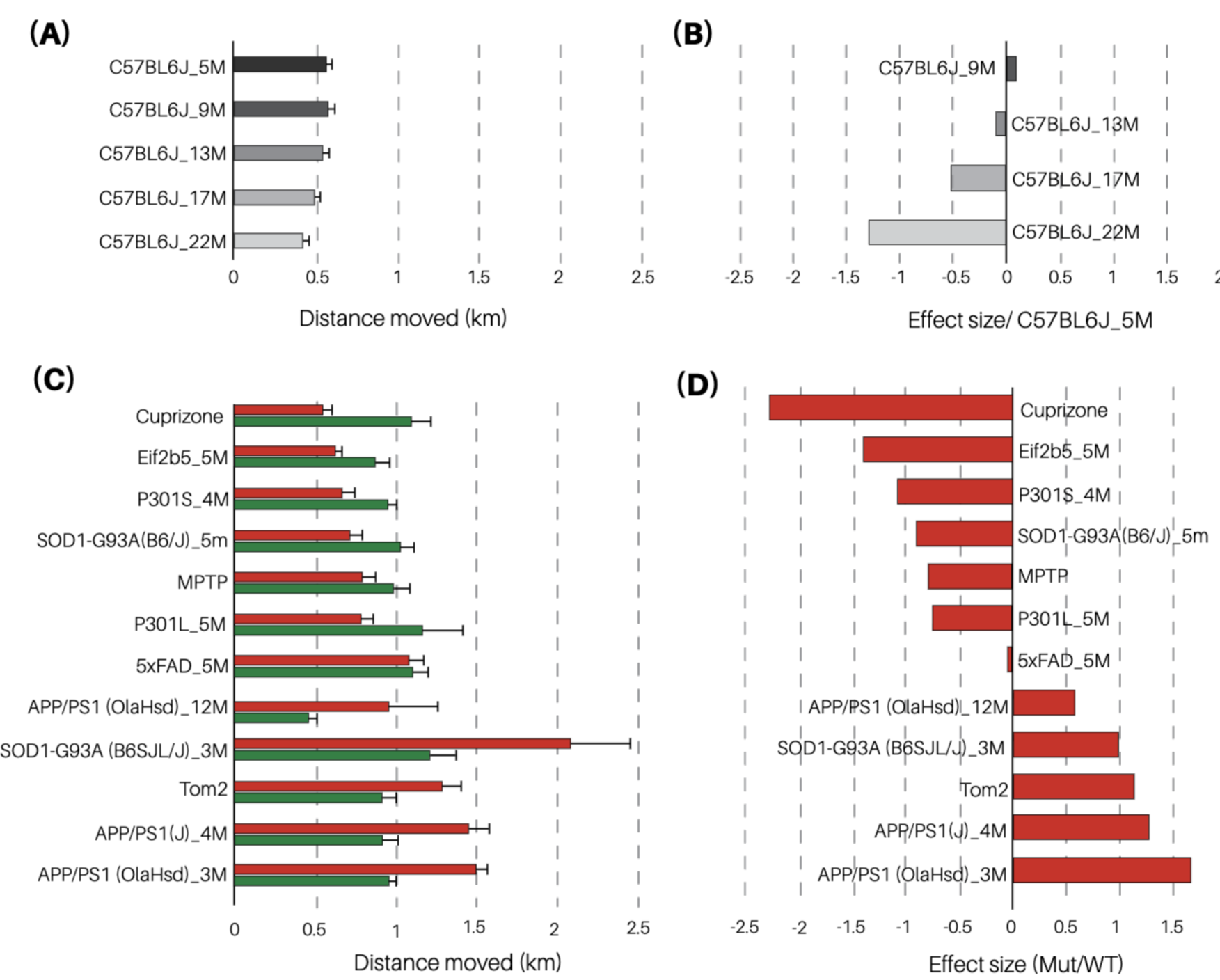


FIGURE 2. Analysis of the distance moved. (A) The distance moved was measured over a period of 2.5 days (3 dark phases, 2 light phases) and reported in kilometer (km) (shown as an illustrative example parameter). The distance moved in natural ageing mice at 5, 9, 13, 17 and 22 months of age decreases in an age-dependant way. (B) The effect size of the distance moved decreases in an age-dependant manner to reach its minimum value at 22 months of age when compared to the 5M (control). (C) Distance moved of mouse models for neurodegeneration compared to their respective control groups showed that distinct models show different trends. (D) Effect size of the differences in distance moved of mouse models for neurodegeneration in comparison with their respective control groups allowed us to cancel out the age-effect and focus on the effect of the disease-causing mutations at different ages.

UNBIASED ANALYSES OF 58 BEHAVIORAL PARAMETERS

Alzheimer's disease and tauopathy models

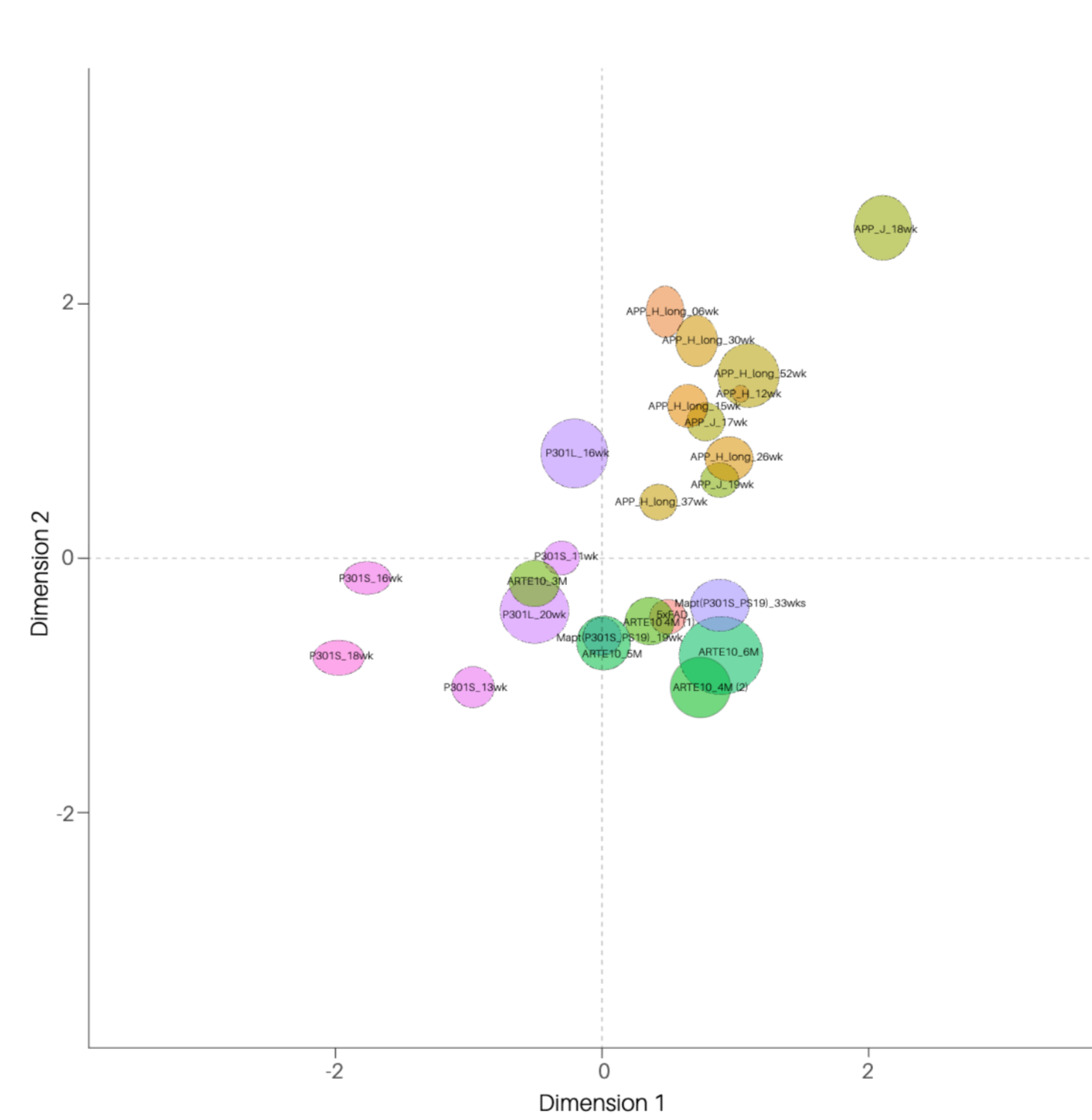


FIGURE 3. Unbiased analyses of 58 behavioural parameters shows obvious differences between different mouse models of Alzheimer's disease and other tauopathies. Effect sizes of PC1 and PC2 of the PCA on the 58 behavioural parameters. The centre of the bubble represents the effect size, while the size of the bubble represents the variance. The plot shows the effect size of the difference in scores on PC1 (x-axis) and PC2 (y-axis) between different amyloid beta (Aβ) and tau-related transgenic lines in comparison with their respective control groups. Different Aβ or tau-related transgenic lines showed distinct phenotypes in this analysis, despite mimicking the same or similar Alzheimer's disease and tauopathy-related neuropathologies. Within the same transgenic line (if tested at several timepoints), the phenotype generally became more pronounced at later timepoints.

Other neurodegenerative models and natural aging

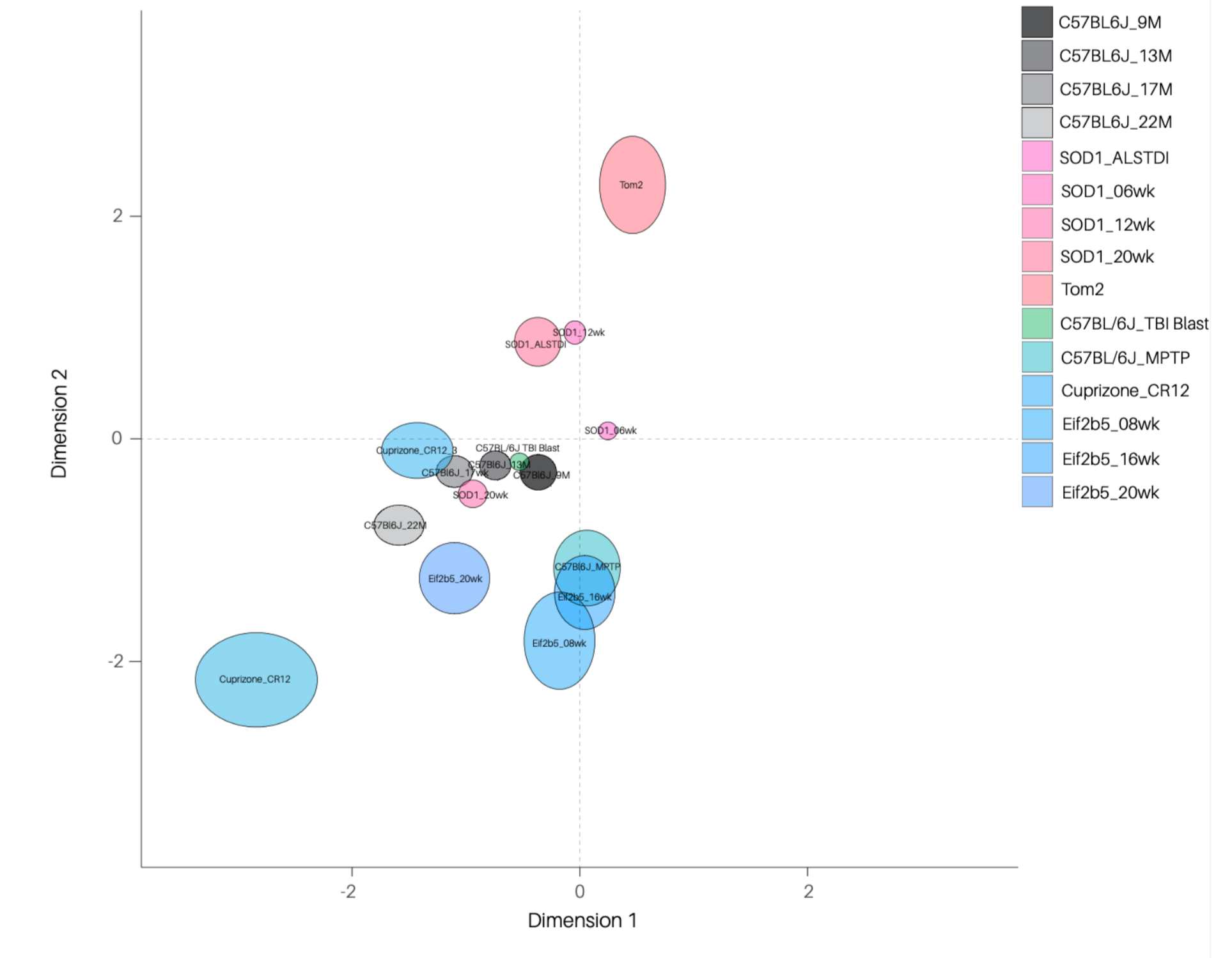


FIGURE 4. Unbiased analyses of 58 behavioural parameters shows obvious differences between natural aging and several neurodegeneration models. Effect sizes of PC1 and PC2 of the PCA on the 58 behavioural parameters. The centre of the bubble represents the effect size, while the size of the bubble represents the variance. The plot shows the effect size of the difference in scores on PC1 (x-axis) and PC2 (y-axis) between both aging mice in comparison with their 5M old control group and the models for neurodegeneration in comparison with their respective control groups. The natural aging mice (C57BL/6J) show a clear age-related progression in this plot. By plotting PC1 and PC2 we observe that the home-cage has the power to clearly discriminate the various models for neurodegeneration.

TAKE-HOME MESSAGE

The different analysed parameters of home-cage behaviour are highly sensitive among the neurodegenerative diseases tested, even before the emergence of classical disease pathophysiology, and are clearly distinguishable from normal ageing. Our approach allows us to distinguish between different disease-carrying mutants, compound induced disease models, and naturally aging mice. **Thus, this method allows for characterisation of behavioral neurodegeneration hallmarks, representing a key approach for testing novel potential treatments by providing a platform to verify the treatment efficacy in freely moving animals.**