NATURALLY AGED C57BL/6J MICE AS A TRANSLATIONAL MODEL OF BRAIN AGING

Priyanka Chanana, Stefanie Siebert-Krassnig, Roland Rabl, Stefan Ebner-Benke, Tobias Pendl, Livia Breznik, Magdalena Daurer, Laurenz Jauk, Paula Jauregi, Irati Castañares-Hermoso, Aaron Fantina-Woblistin, Manuela Prokesch

Scantox Neuro GmbH, Grambach, Austria

For further information and inquiries contact office-austria@scantox.com

INTRODUCTION

Aging is a dynamic, time-dependent process characterized by the progressive decline of physiological functions. In the central nervous system, this systemic decline leads to profound structural, vascular, and proteostatic changes in the brain, often accompanied by behavioral impairments. Naturally aged wild-type mice represent an important model in biological research on brain senescence. To examine how age-related changes in emotion, motor performance, and cognition interact, we established a comprehensive behavioral test battery at 21 months of age, followed by a detailed histological characterization of the age-associated neuropathology at 24 months in separate, independent cohorts of wild-type C57BL/6J mice.

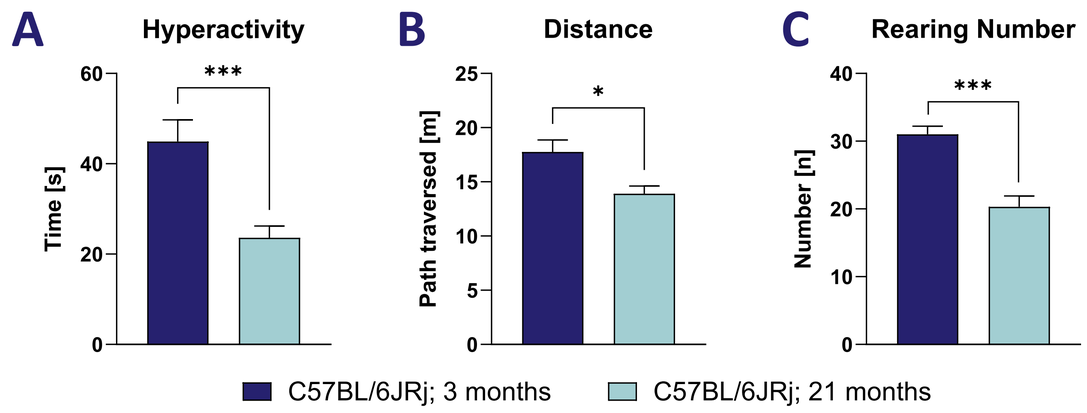
METHODS

▲ **Figure 1: Schematic design and overview of cohorts.** Naturally aged mice were compared with young adult (3-month-old) controls. At 21 months, aged mice underwent behavioral testing, including Open Field test, a modified Irwin test battery, Barnes Maze, T-Maze, and Passive Avoidance test. A separate cohort of aged mice (24 months) was used for terminal histological evaluation of brain tissue.

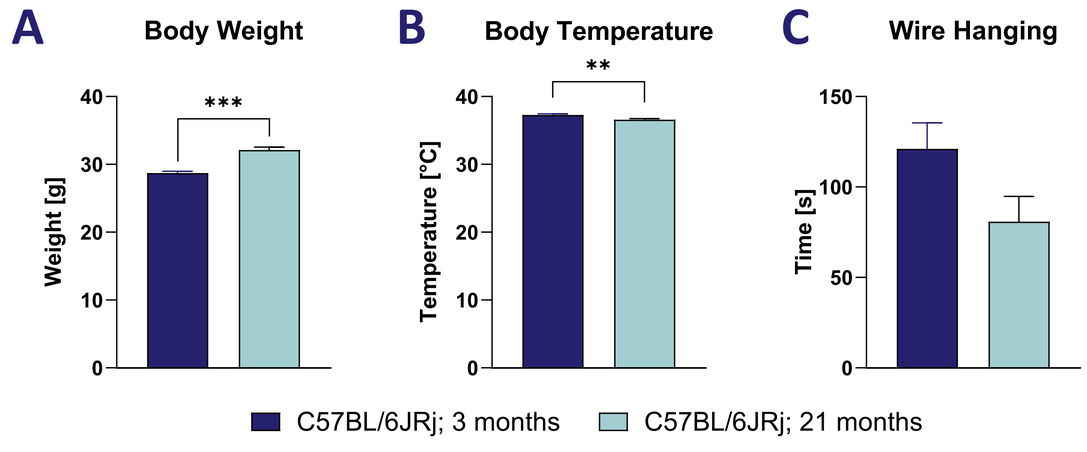
RESULTS

Behavioral phase

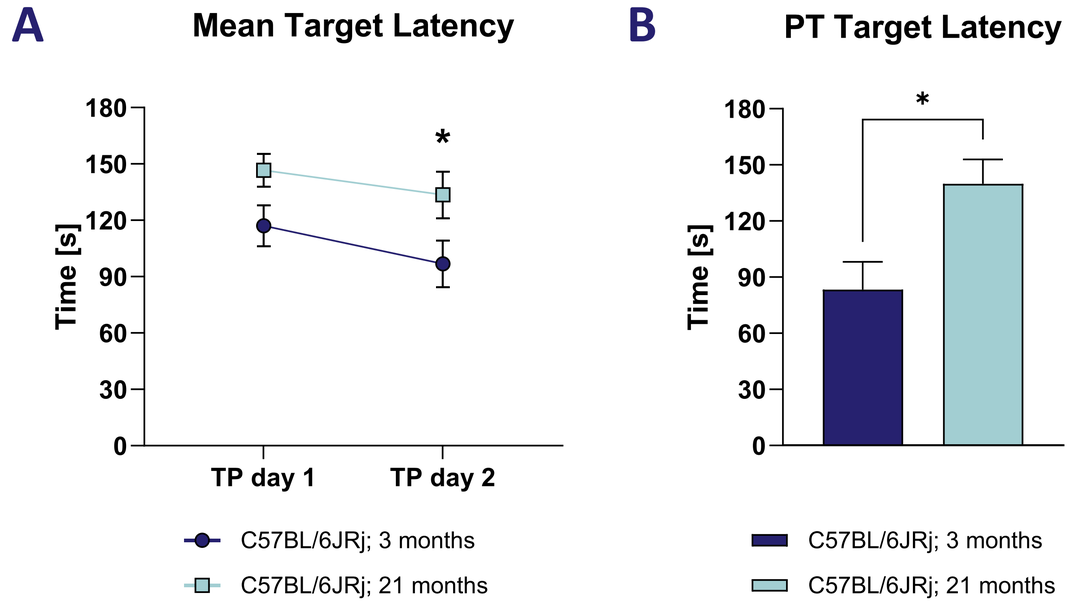
Aged mice exhibited marked exploratory reductions, showing decreased hyperactivity, total distance traveled, and rearing frequency (**Fig.1**). Clear physiological changes were evident in aged mice, including significantly increased body weight and decreased body temperature (**Fig. 2**). Young control animals demonstrated a significantly reduced latency to enter the target escape hole compared to aged mice during training (**Fig.3**).



▲ **Figure 2: Open field test.** Graphs represent hyperactivity (**A**), the total pathlength (**B**), and the number of rearings (**C**). Unpaired t-test with Welch's correction (hyperactivity, number of rearings) or Mann Whitney's test (distance). Mean + SEM; n = 20 per group; *p <0.05; ***p <0.001.

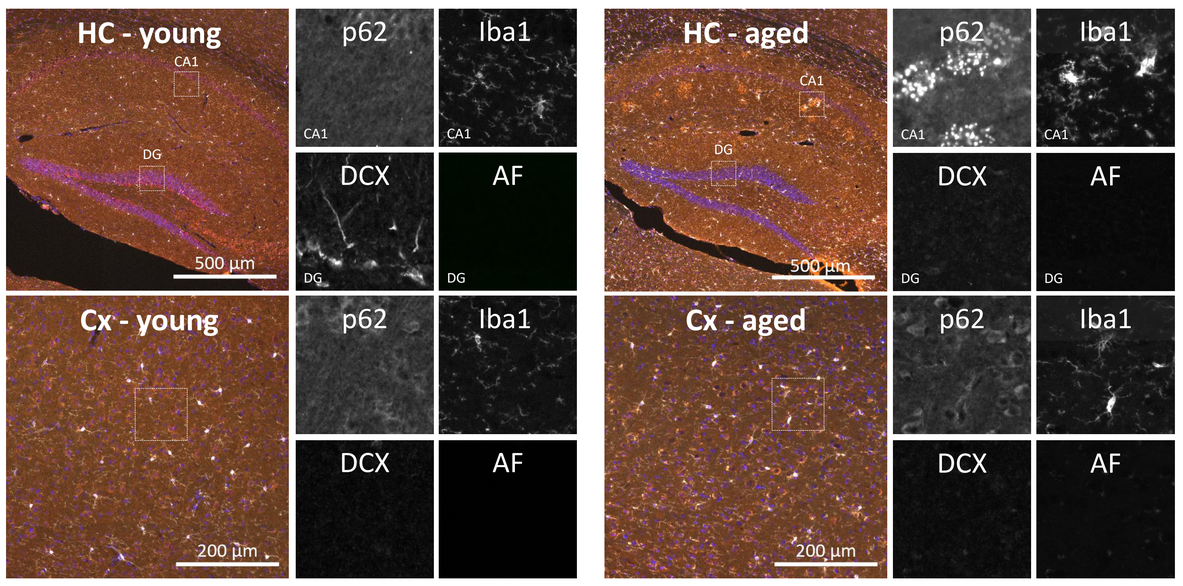


▲ **Figure 3: Modified Irwin test.** Graphs represent body weight (**A**), body temperature (**B**) and the latency to fall in the wire hanging test (**C**). Unpaired t-test with Welch's correction. Mean + SEM; n = 20 per group; **p <0.01; ****p <0.001.

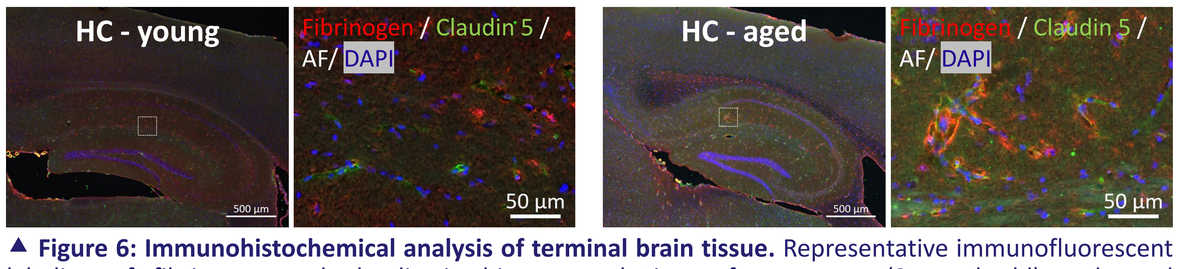


▲ **Figure 4: Barnes maze – longitudinal analysis.** Graphs represent the latency to enter the target hole during the training phase (TP) (**A**) and the latency to find the target hole during the probe trial (PT) (**B**). Two-way ANOVA followed by Bonferroni's *post hoc* test (**A**) and Mann Whitney's test (**B**). Mean ± SEM (**A**); Mean + SEM (**B**); n = 20 per group; *p <0.05.

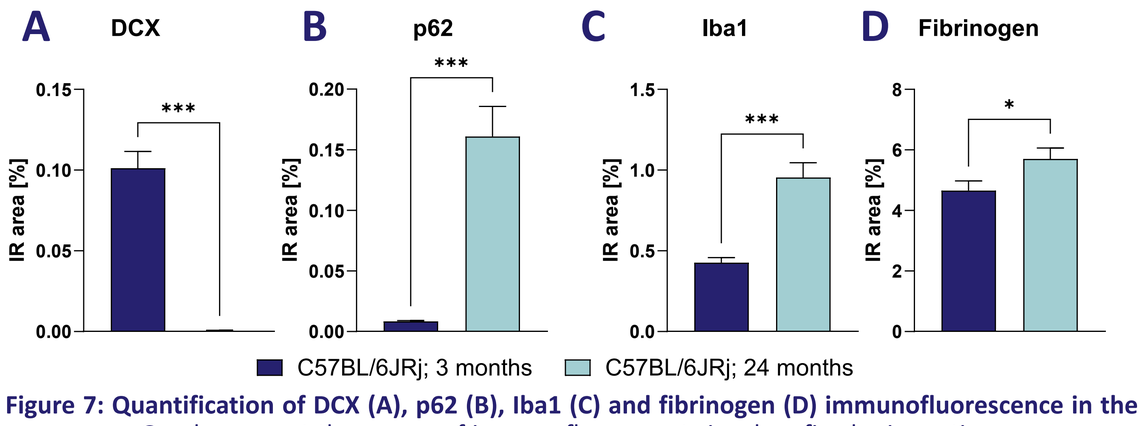
Histological phase



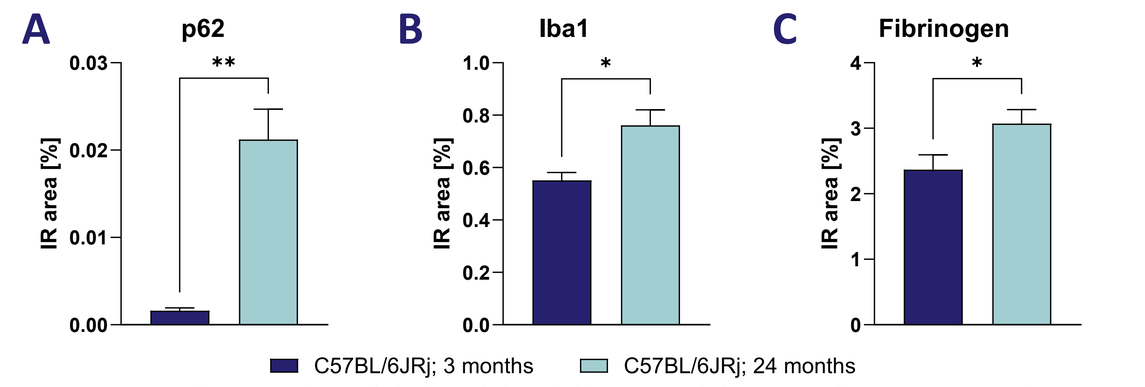
▲ **Figure 5: Immunohistochemical analysis of terminal brain tissue.** Representative immunofluorescent labeling of doublecortin (DCX), p62/SQSTM1, and Iba1/AIF1 (ionized calcium-binding adaptor molecule 1) in hippocampus (HC) and cortex (Cx) of young (3-month-old) and aged (24-month-old) mice. Single-channel magnifications show labeling in the dentate gyrus (DG), CA1 subfield, and cortical regions. Nuclei are counterstained with DAPI. AF: autofluorescence.



▲ **Figure 6: Immunohistochemical analysis of terminal brain tissue.** Representative immunofluorescent labeling of fibrinogen and claudin in hippocampal tissue from young (3-month-old) and aged (24-month-old) mice. Nuclei were counterstained with DAPI. AF, autofluorescence.



▲ **Figure 7: Quantification of DCX (A), p62 (B), Iba1 (C) and fibrinogen (D) immunofluorescence in the hippocampus.** Graphs present the means of immunofluorescent signal on five brain sections per mouse. Unpaired t-test with Welch's correction (DCX, p62, fibrinogen) or Mann Whitney's test (Iba1). Mean + SEM. n young = 10 and n aged = 7; *p <0.05; ***p <0.001.



▲ **Figure 8: Quantification of p62 (A), Iba1 (B) and fibrinogen (C) immunofluorescence in the cortex.** Graphs present the means of immunofluorescent signal on five brain sections per mouse. Unpaired t-test with Welch's correction. Mean + SEM. n young = 10 and n aged = 7; *p <0.05; **p <0.01.

CONCLUSIONS

Aged wild-type mice displayed several key histological markers of aging, including increased p62, near-zero DCX expression, and increased neurovascular permeability. These cellular hallmarks were consistent with earlier cognitive declines. Taken together, combining these behavioral and histological read-outs offers a robust framework for evaluating candidate therapies targeting mechanisms of neurodegeneration and cognitive impairment.