Age-related cognitive decline in spatial learning and memory of C57BL/6J mice

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ABSTRACT

During the last decades, most of the preclinical neurodegenerative research was performed in mouse models of amyloidosis, tauopathies or α-synucleinopathies preferentially maintained on a C57BL/6J background. However, comprehensive neurobehavioural data from C57BL/6J mice outlining the critical point of spontaneous cognitive decline are incomplete. In this study, we aimed for the neurobehavioural phenotyping of hippocampus-dependent spatial learning and memory of aging C57BL/6J mice. Neurobehavioural phenotyping was performed by means of a Morris Water Maze (MWM) and a Novel Object Recognition (NOR) test. MWM measurements revealed signs of age-related memory loss in C57BL/6J animals from the age of 6 months onward. The NOR assessment strengthened latter finding by decreasing discrimination indexes (DI) and recognition indexes (RI) starting from the age of 6 months. Taken together, these findings contribute to the current knowledge of spontaneous cognitive behaviours of this perhaps most widely used mouse strain and serve as a benchmark for dementia mouse models to distinguish spontaneous from pathological neurodegenerative behaviour.

1. Introduction

C57BL/6J is perhaps the most frequently used inbred mouse strain in biomedical research. Numerous genetically engineered mouse (GEM) models of neurodegenerative conditions are referenced on this congenic/consonomic Morris water maze background [1]. For example, GEM models carrying genetic mutations related to the Alzheimer’s disease pathology, i.e., APP, PSEN1, TREM2, ABCA7, APOE4, BACE1, TAU among others, have been preferentially maintained on a C57BL/6J background [2–5]. A wealth of aging data on hippocampus-dependent cognitive behaviour and spatial memory assessments is already available for these mouse strains [2]. However, comprehensive studies detailing age-related, hippocampus-dependent cognitive behavioural and spatial memory assessment of C57BL/6J control mice are incomplete. To address this critical lack of knowledge, our study aimed to provide a comprehensive cognitive behavioural and spatial memory characterization of C57BL/6J mice. Our investigation differs from others in that C57BL/6J animals were studied over the course of their natural lifespan by including multiple age groups in order to identify the critical point of age-related cognitive decline. Our assessment reveals a decline in hippocampus-dependent learning and memory of aging C57BL/6J mice as early as 6 months of age. This finding not only provides an accurate understanding of age-related cognitive decline in such an important mouse strain, but also provides the opportunity to distinguish cognitive behaviour between pathological and age-related amnesia when, for example, comparing the performance of C57BL/6J mice with that of Alzheimer’s genetically engineered mice on a C57BL/6J background.

2. Material and methods

2.1. Animals

Male C57BL/6J mice were studied at the age of 2 (n = 14), 4 (n = 11), 6 (n = 25), 9 (n = 10) and 12 (n = 21) months from a colony that had been maintained in a genetically controlled condition based on The Jackson Laboratory’s Genetic Stability Program. At weaning, the...
animals were grouped according to their date of birth which was within a 5-day time range for all animals. On average, litters consisted of 5 pups. All animals were raised in the same conditions and were housed socially in standard mouse cages up to a maximum of eight animals per cage under conventional laboratory conditions with a constant room temperature (22 ± 2 °C), humidity level (55 ± 5%) and artificial 12 h/12 h day/night cycle (lights on at 8 a.m.). Food and water were supplied ad libitum. Experiments were approved by the Animal Ethics Committee of the University of Antwerp (ECD approval n° 2017/53) and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal and were performed in accordance to the ARRIVE Guidelines [6].

2.2. Neurobehavioural assessment

2.2.1. Morris water maze (MWM) test

Research on memory and spatial learning was conducted at the ages of 2, 4, 6, 9 and 12 months using the MWM test [7,8]. The MWM consisted of a circular pool (diameter: 150 cm, height: 30 cm) filled with opacified water using non-toxic white paint and kept at 25 °C. Invariable visual cues were placed around the pool. The MWM consisted of an acquisition phase and a probe trial which were performed with a regular day/night cycle with standardization of timing on each day. The acquisition phase was performed over the period of 4 days and consisted of 2 daily trial blocks (1 at 10:30 am and 1 at 03:00 pm) of 4 trials with a 15-minute inter-trial interval. During the acquisition phase, a round acrylic glass platform (diameter 15 cm) was placed 1 cm below the water surface on a fixed position in the center of one of the pool’s quadrants. Mice were placed in the water facing the wall and were recorded while trying to find the hidden platform for a maximum duration of 120 s. If the mice were not able to reach the platform within 120 s, they were guided to the platform where they had to stay for 15 s before being returned to their home cage. The starting positions varied in a semi-random order. The probe trial followed 4 days after the final acquisition trial. For this trial the platform was removed, mice were placed in the MWM at a fixed position and swimming trajectories were recorded during a period of 100 s. Both during acquisition and probe trials, the animals’ trajectories were recorded using a computerized video-tracking system (Ethovision, Noldus, The Netherlands), with path length, escape latency, and swimming speed recorded. Spatial accuracy was expressed as percentage of time spent in each quadrant of the MWM, i.e., the specific location of the platform during the acquisition phase.

2.2.2. Novel object recognition test

The NOR test was performed to assess recognition memory and was performed during four consecutive days with a regular day/night cycle with standardization of time on each day [9]. On the first two days of the protocol, mice were individually habituated to an empty arena (40 cm × 24 cm) during 10 min. On the third day (familiarization phase), two identical objects (brown-colored flasks) were placed 10 cm apart in the center of the arena and mice were allowed to freely explore the cage and objects for 5 min. On the fourth day (novel object phase) one object was replaced with a novel object (different color and shape, but similar in size). Mice were then placed in the arena and again allowed to explore for 5 min. Trajectories and nose-point locations were recorded. Exploration time was defined as the time during which the nose-point was directed towards one of the objects with a proximity of 3 cm. The recognition index (time spent exploring novel object divided by total time exploring both objects) was calculated as a measure of recognition [10]. Time spent investigating each object was scored using the behaviour tracking software (Ethovision, Noldus, The Netherlands).

2.3. Statistical analysis

Data are presented as mean ± SEM unless otherwise indicated. MWM trials were analyzed with a factorial ANOVA test for the factor of ‘age’. Statistics of age-compared results were analyzed with an Ordinary One-Way-ANOVA with a Tukey post-hoc test. Analyses were performed using GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com). MWM probe trial results were analyzed with Dirichlet distributions as described earlier [11] using R language programming in Jupyter Notebook [12] with the Dirichlet package from Eric Suh (Fitting the parameters of a Dirichlet distribution) available from: https://github.com/ericnush/dirichlet. For statistical accuracy, the number of animals was randomized to a total of 10 animals. Data were considered significant at p < 0.05. Applied statistical analyses are indicated in the figure legends.

3. Results

3.1. Age-related deterioration in MWM acquisition trial performances

An age-related trend was found for total path lengths (Fig. 1, A) and escape latencies (Fig. 1, C) during MWM training trials. When a sum of total path lengths and escape latencies over the total training phase was calculated for each animal, deteriorating performances were obtained with increasing age (Fig. 1, B, D), which were statistically significant from the age of 6 months onward. Average swim speeds during MWM trials (Fig. 1, E) knew a decreasing trend with aging. When averaged, swim speeds of each animal were calculated per age, significantly lower swim speeds were measured from the age of 6 months onward (Fig. 1, F).

3.2. Age-related performance deteriorations in MWM probe trials

Four days after the final acquisition trial, animals completed the MWM probe trial. Age-related deterioration of spatial memory was observed based on the average time spent by animals in the target quadrant and consequent randomized presence of animals in all four quadrants of the maze (Fig. 2).

3.3. Age-related performance deteriorations in the NOR test

The NOR applies another approach to assess the animal’s spatial learning and memory capacity relying on their natural proclivity for exploring novelty. The discrimination index (DI) [10,13] and recognition index (RI) [10] were calculated as follows:

$$DI = \frac{\text{Time (new)} - \text{Time (old)}}{\text{Time (total)}}$$

$$RI = \left(\frac{\text{Time (new)}}{\text{Time (new)} + \text{Time (old)}}\right)^{n} \times 100$$

From the age of 6 months, a significant downward trend in natural proclivity for discriminating and recognizing familiar and novel object was perceived (Fig. 3).

4. Discussion

In this study, hippocampus-dependent spatiotemporal learning and memory were assessed in four age cohorts of young adult (2 and 4 months), adult (6–9 months) and middle-aged (12 months) male C57BL/6J mice using a MWM and NOR test. More specifically, increasing total path lengths and escape latencies were measured alongside slower swim speeds with age. The latter observation, however, might be explained by a senescence-related deterioration in physical fitness. Similar findings were drawn from a recent study in aging C57BL/6N mice which displayed declining gait speed which was associated with a dramatic increase in the energetic cost of physical activity [14]. In addition, more randomized presences of animals in the MWM were observed during the probe trial. During the NOR test on the other hand, a decreased natural proclivity for exploring novelty was measured in aging C57BL/6J animals. This decreased trend in natural proclivity to explore novelty might...
on the one hand be explained by the deteriorating memory capacity of aging animals but on the other hand might also be explained by disininterest and attention deficits in aging mice. Further research is needed to pinpoint the reason for this decreased trend in more detail in order to determine the validity of this test in older mice. Overall, we found a consistent cognitive decline in both behavioural tests from the age of 6 months onward. These results align with previous findings reporting age-related behavioural changes from young adulthood (2–3 months of age) to middle aged (8–12 months) and old (18–31 months) male and/or female C57BL/6J mice [15–23]. In these studies, a battery of behavioural tests was performed including tests to assess sensory and motor functions, locomotor activity, social behaviour, anxiety-like behaviour, depression-related behaviour, and learning and memory functions. This wealth of data clearly indicates behavioural changes in aging C57BL/6J animals related to locomotor activity, anxiety-like behaviour, and memory functions. To our knowledge, this study is the first of its kind to report spontaneous (age-related) cognitive decline in spatial learning and memory as measured by an MWM test in aging male C57BL/6J mice, contributing to the current knowledge of this preferential background mouse strain. Moreover, this study is the first of its kind to establish the critical point of spontaneous cognitive decline because the animals were studied over their complete lifespan, including multiple age groups.

Over the past decades, extensive efforts have been made to study the neurodegenerative phenotypes of murine models displaying amyloidosis, tauopathies or α-synucleinopathies. Most of these GEM models of neurodegenerative conditions are referenced on the congenic/consomic C57BL/6J background making it perhaps the most frequently used inbred mouse strain in biomedical research. Appropriate neurodegenerative GEM models are not only selected on their genetic background strain, but also on their translatability of behavioural-cognitive performances compared to risk patients. While the human disease state comprises both mental and physical declines, GEM models often mimic only a fraction of these symptoms and therefore might need additional
manipulations on a behavioural level in order to recapitulate the full disease spectrum [24]. Most frequently used behavioural assays in neurodegenerative GEM models concern testing of spatial learning and memory such as the MWM, though simple tests such as the Y-maze and T-maze are also often employed [25]. This study provides necessary MWM reference data against which genetically engineered mouse studies can be compared to distinguish pathological cognitive aging from spontaneous (age-related) cognitive aging. From a future perspective, these findings will facilitate the study of dementia disorders and allow for the creation of appropriate genetically engineered mouse models that correctly mimic disease states.

In conclusion, the results of this study are critical because they reflect the natural cognitive aging of the C57BL/6J background strain and indicate the critical point of spontaneous (age-related) cognitive decline. Additional findings from neurodegenerative GEM mouse models can be substantiated by these results.

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Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

CRediT authorship contribution statement

JOH, SDM and EC: Investigation, Data curation. GDM, DVD and PPDD: Writing – reviewing and editing, Funding acquisition. JOH, DVD and GDM: Conceptualization, Writing – original draft preparation.

Among our findings, we all recognize that models reflecting only.

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