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Research report

Aged APP23 mice show a delay in switching to the use of a strategy in the Barnes maze

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Abstract

Spatial learning and memory deficits in the APP23 transgenic mice have mainly been studied using the Morris water maze (MWM). However learning in the MWM relies on swimming abilities and may be confounded by the stressful nature of this test. We have therefore assessed spatial learning and memory in 12-month-old APP23 using a dry-land maze test developed by Barnes. Mice were given daily learning trials for a total of 41 successive days. After a 12-day interval the mice were re-tested for 4 additional days in order to examine the spatial memory retention. Immediately following this phase, reversal learning was examined for 13 additional days by moving the escape tunnel to the opposite position. During the initial learning phase, APP23 mice showed a significantly longer latency to find the escape tunnel as well as an increased number of errors compared to non-transgenic littermates. These deficits appeared to be due to a delay in switching from a "no strategy" to a spatial strategy. Indeed, this same delay in the use of spatial strategy was observed in the reversal phase of the study. Our results suggest that impairments in APP23 mice in learning and memory maze tests may be due to a specific deficit in the use of spatial strategy.

Keywords: Alzheimer's disease; APP23; Barnes maze; Morris water maze; Learning; Memory; Behavior; Dry-land maze

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that affects a large proportion of the elderly population. It is expected to increase in incidence in the next decades due to the increased life expectancy [8]. Several transgenic mouse lines over-expressing human APP have been developed to understand the mechanisms underlying this disease (PDAPP [9], Tg2576 [12], APP23 [19], TgCRND8 [6]). The APP23 mouse line mimics several histopathological and biochemical alterations typical for AD, such as amyloid plaques deposition in the neocortex and hippocampus, abundant neuritic dystrophy and a loss of pyramidal neurons in the hippocampal CA1 region [3,19]. In addition, cognitive and behavioural changes reminiscent of AD were found [21] and were most intensively studied with respect to spatial learning and memory in the Morris water maze [14,15].

Although frequently used for mice, the Morris water maze test (MWM) was initially developed to analyze the cognitive abilities of rats [7,18]. Applying this procedure to mice raises some problematic as these species use different behavioural strategies to solve cognitive tasks [22]. Overall mice perform less well in the MWM than rats, an effect not found in dry-land maze tests [22]. The reason for the impaired performance of mice specifically in a MWM is not entirely clear, but is likely related to the better natural adaptation of rats for swimming and

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to species-specific non-cognitive swimming strategies [23]. In addition, it has been shown that the MWM is particularly stressful in mice and this may interfere with the cognitive aspects of this test [10]. The impaired performance even of "normal" mouse strains might mask or decrease differences as compared to transgenic or knock-out lines and may make it more difficult to assess treatment effects.

In this study we, therefore, assessed spatial learning and memory in 12 months (Mo) old APP23 transgenic mice in a dry-land maze, the Barnes maze.

2. Materials and methods

All procedures described below strictly follow the Swiss legislation on research involving animal subjects. This research protocol is in accordance with the recommendations of the European Community Council for the Ethical Treatment of Animals (no. 86/609/EEC). All work performed at the Scripps Research Institute followed the guidelines of this institute's Animal Care and Use Committee. Adequate measures were taken in order to minimize pain and discomfort of the animals.

2.1. Animals

Male APP23 mice were generated and bred as previously described [21] and backcrossed to C57BL/6J for more than 10 generations. From the age of 4–5 weeks, the mice were reared in group-housed cages (3–4 mice per cage). After transportation to the laboratory where the test was performed, 9 Mo old mice were housed in a temperature-controlled room in which the lights were under a 12 h light/dark cycle with lights off at 8.00 a.m. All testing occurred during the dark (active) phase. Food and water were available *ad libitum*. After 3 months (Mo) of housing in the facilities 8 APP23-transgenic (tg) and 13 non-transgenic (non-tg) littermates (aged 12 Mo) were evaluated.

2.2. Barnes maze

The Barnes maze used was an opaque PVC disc 75 cm in diameter elevated 58 cm above the floor by a tripod. Twenty holes, 5 cm in diameter, were located 5 cm from the perimeter, and a black PVC escape box (19 cm × 8 cm × 7 cm) was placed under one of the holes. Distinct spatial cues were located all around the maze and were kept constant throughout the study. On the first day of testing, a training session was performed, which consisted of placing the mouse in the escape box and leaving it there for 1 min. One minute later, the first session was started. At the beginning of each session, the mouse was placed in the middle of the maze in a 10 cm high cylindrical black start chamber. After 10 s the start chamber was removed, a buzzer (80 dB) and a light (400 lux) were turned on, and the mouse was set free to explore the maze. The session ended when the mouse entered the escape tunnel or after 5 min elapsed. When the mouse entered the escape tunnel, the buzzer was turned off and the mouse was allowed to remain in the dark for 1 min. When the mouse did not enter the tunnel by itself it was gently put in it for one minute. The tunnel was always located underneath the same hole (stable within the spatial environment), which was randomly determined for each mouse. The platform was moved every day by 90° to avoid any odor cue but the spatial cues and the position of the tunnel remain at the same exact place. The platform was cleaned with 70% ethanol between each mouse.

Mice were tested once a day for 41 days and then allowed 12 days off. Mice were then retested for 4 days in order to examine retention of spatial memory. Immediately following this, the escape tunnel was moved 180° around the maze and reversal learning was examined for an additional 13 days. This final phase allowed the examination of learning of a new spatial contingency.

2.3. Data analysis

Every session was videotaped and scored by an experimenter blinded to the genotype of the mouse. Several measures were recorded including the time (second, *s*) between the start of the trial and entry into the escape tunnel, the number of errors made per session and the strategy employed by the mouse to locate the escape tunnel. Errors were defined as nose pokes and head deflections over any hole that did not have the tunnel beneath it. Search strategies were determined by examining each mouse's daily session and classifying it into one of three operationally defined categories: (1) random search strategy—localized hole searches separated by crossings through the center of the maze, (2) serial search strategy—systematic hole searches (every hole or every other hole) in a clockwise or counterclockwise direction, or (3) spatial search strategy—reaching the escape tunnel with both error and distance (number of holes between the first hole visited and the escape tunnel) scores of less than or equal to 3. Data were analyzed in blocks of four sessions. The significance of differences between mean values in time and errors was assessed with two-way ANOVA with genotype and blocks as sources of variation. χ^2-tests were used to compare non-tg and tg mice with respect to frequencies of spatial strategy. In addition, in order to qualitatively examine the time course of the switch from "no strategy" to "strategy", serial strategy was pooled with the spatial strategy and a Student's *t*-test was used to compare the switching time point between tg and non-tg mice.

3. Results

APP23 tg mice were slower in finding the escape tunnel across the initial learning phase in comparison to their non-tg littermates (sessions 1–41: $F_{(1,760)} = 5.94, p < 0.05$). This appeared to be especially true for the first trials (block 1–3, i.e. sessions 1–12; Fig. 1) where the APP23 tg mice needed significantly longer times to find the escape tunnel ($F_{(1,38)} = 9.55$, $p < 0.01$). There was no significant difference between APP23 tg and non-tg mice in the time to find the escape tunnel during the retention and the reversal tests (retention: $F_{(1,57)} = 1.70$, ns and reversal test: $F_{(1,216)} = 2.66$, ns).

Overall, there was no evidence of a difference between APP23 tg and non-tg in the number of errors made across the 41 days of the initial learning phase ($F_{(1,760)} = 1.70$, ns) nor during the retention ($F_{(1,57)} = 1.12$, ns) and the reversal phases ($F_{(1,216)} = 0.96$, ns). However, early during the initial learning phase, as represented by the first 3 blocks, the APP23 tg mice made significantly more errors than the non-tg mice (Fig. 2: $F_{(1,38)} = 4.89$, $p < 0.05$).

APP23 tg mice appeared to have a delay in the development of a strategy in this test, both during the learning phase ($X^2 = 3.9$, $p < 0.05$, Fig. 3), as well as during the reversal phase ($X^2 = 6.5$,

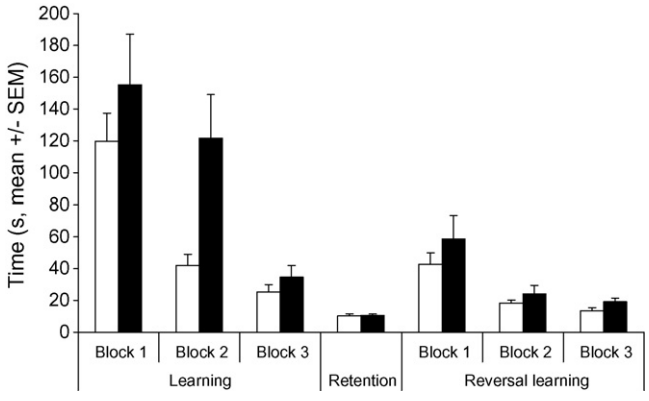

Fig. 1. Time to find the escape tunnel in APP23 tg and non-tg mice by block of four sessions. During the first three blocks of the initial learning phase APP23 mice (black squares) need longer than the non-tg mice (open squares; $p < 0.01$) to reach the tunnel. No significant difference was observed during the retention and reversal tests.