Age-Induced Cognitive Alterations in OF1 Mice

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CARRIE, I., M. DEBRAY, J. M. BOURRE AND H. FRANCES. Age-induced cognitive alterations in OF1 mice. PHYSIOL BEHAV 66(4) 651–656, 1999.—Female OF1 mice aged 17–18 months were compared with female OF1 mice aged 7–11 weeks for locomotor activity, pain sensitivity, and cognitive performance using the Morris water maze, passive and active avoidance, and the elevated plus-maze learning protocol. Performance of old mice was impaired compared to those of young mice for both locomotor activity, pain sensitivity, and the four cognitive tests including the elevated plus-maze not previously used in studies on aging. Using complementary experiments and a detailed analysis of the results, we have shown that the reduction of learning and memory do not result from a decline of sensory and motor capacities. We conclude that female OF1 mice aged 17–18 months show true cognitive deficits. © 1999 Elsevier Science Inc.

Aging Mice Morris water maze Passive avoidance Active avoidance Elevated plus-maze

THE aim of this article was to investigate whether the OF1 mouse strain used in our previous studies was suitable for use as a model for aging (3–5). To achieve this, learning and memory abilities of old mice were compared with those of young mice.

As aging is not a homogeneous process, and because several types of memory processes exist involving different neural circuits, a set of behavioral tests was performed allowing measurement of age-related learning deficits in the OF1 mice. Moreover, as it is difficult to separate the specific memory impairment from sensorimotor changes associated with aging, the use of various tests gave a better guarantee that results obtained represented true cognitive deficits.

OF1 female mice aged 17 months and over were compared with young female mice aged 7–11 weeks using various tests. The survival curve obtained from the breeder showed a 50% survival at 17 months. This agrees with our experience under our husbandry conditions. Female mice were used because of their lack of aggressiveness under usual maintenance conditions.

The Morris water maze is used to assess spatial learning, a special type of learning sensitive to hippocampal damage, an area affected by aging (2,6–8). Two protocols have been employed to investigate the spatial learning: cue learning (control test) to evaluate the motor and motivational abilities, and place learning which is a real memory test.

Associative learning has been tested by using two types of conditioning: passive and active avoidance. These tests allow investigation of the effects on learning/acquisition or memory processes such as consolidation, recall, and retrieval. Both passive and active avoidance have been used to study the role of age in learning of rodents. Indeed, a number of studies have shown a regular decrease in performances in old rodents (1,11,12,14,17,19,22).

The elevated plus-maze has been extensively studied in rats (18) and validated in mice (16). The test is based on the natural aversion of rodents for open spaces and for the height of the maze. This test is normally used to measure the effect of anxiolytic or anxiogenic agents. However, a new protocol has been developed to evaluate learning in rats and mice (13,20). It consists in measuring the time taken for the rodent to move from the open arm to the enclosed arm on Days 1 and 2, called transfer latency. Administration of drugs affecting learning and memory (scopolamine, MK801) allowed validation of the model: the treated rodents showed acquisition deficits, because the transfer latency on Day 2 was not significantly shortened. We considered this test, although not previ-

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ously used in old animals, well suited for studying the effect of aging.

Aging was associated with a sensory and motor decline. As motor, visual, and auditory capacities were all involved in learning tests and may influence the interpretation of cognitive results, locomotor activity, pain sensitivity, and additional experiments related to learning test were performed.

METHODS

Animals

Young 7-week-old (31.5 ± 0.6 g) and old 17–18-month-old (49.5 ± 1.5 g) female OF1 mice from IFFA-CREDO (L’Arbresle, France) were used. The mice grew old in the animal room and were used from 17 months of age when mortality is 50% according to the survival curve. Mice were housed in group cages, six animals of the same age per cage, in an air-conditioned animal room illuminated from 0800–2000 h, and maintained at 21 ± 1°C. They were allowed ad lib access to standard lab diet and water.

Experiments were performed between 1000 and 1700 h in rooms maintained at 21 ± 1°C. To minimize the influence of age-related physical incapacitation on performance, old mice were preselected. Animals with visible defects such as tumors or major motor incapacitation were not included in the test group. For each test, a new group of mice was used.

Locomotor Activity

Apparatus. A photocell actimeter (APELEX, 91300 Massy, France) was used. Mice were placed individually in Plexiglas cages (25 × 20 × 10 cm) equipped with two infrared photoelectric cells (in the middle of the longer and shorter sides) 2 cm above the floor. Cages were located in an aerated cupboard without illumination.

Protocol. Measurements were started immediately after introduction of the animals into the actimeter, and photo-beam breaks were recorded by 5-min periods for 1 h.

Pain Sensitivity

Tail-flick test. The apparatus used for this test was the Tail Flick Analgesi-Meter, model 33 (Innovators Instrumentation, IITC, Landing, NJ). It consisted of a radiant heat source, photocell, automatic timer, and power supply. The mouse’s tail was placed in a slit over the photocell, and its withdrawal stopped the timer. This represented and measured the animal’s reaction to the heat stimulus. The cutoff point was set at 10 s.

Hot plate. Pain threshold was determined using a 55 ± 1°C hot plate. The latency to paw lick or an attempt to escape by jumping was recorded.

Morris Water Maze

Apparatus. A white circular platform (6 cm in diameter) was placed on a pedestal 19 cm above the floor of a gray plastic tank, 80 cm in diameter and 30 cm high. The tank was filled with water (21 ± 1°C) to a level of 20 cm. The submerged platform was made invisible by adding a white opacifier: Lytron 631 (Norton International, distributed by Brenntag France, Sartrouville, France).

Place learning. Each mouse underwent four trials a day for 4 consecutive days. For each trial, the mouse was placed in the water facing the pool wall at one of eight possible starting locations, which were regularly distributed around the tank. In the protocol, there were visual cues in the room including posters on the walls, a light, and the experimenter, who always stood in the same position. Latency to finding the hidden platform was recorded. If a mouse did not find the platform after 120 s of swimming, it was gently put on it. Once the mouse located the platform (or was put on it) it was permitted to remain there for 30 s. At the end of the four trials the mouse was dried with paper towels and returned to a holding cage positioned 40 cm under a lamp.

Probe trial. On the fifth day of the learning test, the platform was withdrawn and the time the mouse swam in each of the four quadrants of the tank was recorded for 100 s. Learning was defined as a mouse spending a time significantly longer than 25 s only in the quadrant where the platform had been previously located (training quadrant).

Cue learning. In this protocol, the platform was rendered visible by attaching a cue to signal its position. The cue was a plastic square (5 × 5 cm) bearing slanting black and white 1 cm-wide stripes. As in the place learning protocol, the mouse was placed in the water facing the pool wall at one of the eight possible starting locations. The mice received three successive trials a day separated by 30 s of rest on the platform. The test was performed on 3 consecutive days. The latency to reaching the platform was recorded. The mice were dried at the end of each assay in the same way as described for place learning.

Avoidance Tests

Apparatus. This was an automated system coupled with a computer: the Gemini Avoidance System (San Diego Instruments).

Passive Avoidance

Seventeen hours before the test, mice were placed in the dark in the laboratory.

Acquisition test. A mouse was placed in the apparatus and allowed to explore for 3 min, the guillotine-type door being open. The door was then closed and the mouse placed in the right compartment. After 30 s of adaptation, the compartment lit up with an intensity of 543 lx, and the door opened. When the mouse entered the dark compartment (left), the door closed and an electric footshock of 0.3 mA was delivered for 10 s. Each mouse underwent one trial, and the maximum latency to entering the dark compartment was 300 s (cutoff time).

Retention test. The protocol was the same: 17 h in the dark before the test began; 3 min of exploration, door open; 30 s of adaptation, door closed; maximum latency: 300 s.

Latency to entering the dark compartment was measured. The retention test was performed either 3 h or 24 h after acquisition test. Learning occurred when the latency on trial 2 was significantly shorter than the latency on trial 1.

Active Avoidance

Protocol A. The test was comprised of 50 trials per day and lasted 5 days. A mouse was placed in the apparatus, the guillotine-type door was open, and the mouse allowed 2 min adaptation and exploration. After 2 min, the mouse was placed in the right compartment, and the test began.

Each trial lasted 30 s with 2 stimuli: first, a sound signal of 78 dB (conditioned stimulus) was emitted for 5 s, followed by an electric footshock of 0.3 mA (unconditioned stimulus) for 25 s. The intertrial interval ranged from 22 to 38 s, with a median of 30 s. During the trial, the guillotine-type door was open. It closed when the four paws of the mouse entered the
opposite compartment or at the end of the trial. The successive
trials started in the side where the mouse was located.
There were three possible types of response: 1) avoidance: the
mouse crossed during the sound signal; 2) escape: the mouse
crossed during the electric footshock; and 3) no response: the
mouse did not cross into the opposite compartment. Each
day, the number of avoidances were counted.

Learning was defined as an increase in the number of
avoidances and a reduction in the number of escapes or of "no
response."

Protocol B. Before performing the test, young and old
mice were selected using pinna reflex as an indicator of sound
perception. Only mice with a present pinna reflex were used.
The protocol was the same except for the duration of condi-
tioning stimulus (sound signal), which was 10 s instead of 5 s.

Elevated Plus-Maze Test (Learning Protocol)

Apparatus. A gray elevated plus-maze was used. Two open
arms (25 × 5 cm) and two (25 × 5 cm) closed arms were at-
tached at right angles to a central platform (5 × 5 cm). The
open arms and the central platform were covered with white
plastic-coated paper. The apparatus was 40 cm above the floor.

Protocol. The mouse was placed at the end of an open arm
with its back to the central platform. The platform for the mouse
to cross a line halfway along one of the closed arms was mea-
ured (transfer latency) on Days 1 and day 2. The mouse had
to have its body and four paws on the other side of the line. If
the mouse had not crossed the line after 90 s, it was placed be-
beyond it. After crossing the line, the mouse had 30 s for explor-
ing the apparatus.

Learning was defined as a reduced transfer latency on Day
2 compared to Day 1.

Statistical Analysis

Student’s two-tailed, unpaired t-test, was used to compare
between two groups: locomotor activity by 5-min periods,
pain sensitivity, and the probe trial of the Morris water maze.

Student’s two-tailed, paired t-test, was used to compare el-
vated-plus maze and passive-avoidance results between
Days 1 and day 2 for each group.

Locomotor activity (cumulative data for 60 min), Morris
water maze (place and cue learning), and active avoidance data
were examined by multivariate analysis (Systat Software): two-
way ANOVA for repeated measures, two factors (time, age).

All values are given as the mean ± standard error of the
mean (SEM).

RESULTS

Locomotor Activity

The number of photobeam breaks were recorded every
5 min for 60 min. Multivariate analysis of cumulative values
for 60 min (Fig. 1A) showed an effect of time, F(11, 198) = 57.5,

\[ p < 0.0001 \]

of age, F(1, 18) = 17.33, \[ p < 0.001 \], and no signifi-
cant time × age interaction, F(11, 198) = 0.77, \[ p = 0.07 \]. Old
mice were less active than young mice.

The number of photobeam breaks was calculated for each
5-min period by subtracting the previous cumulative value
(Fig. 1B). The comparison of scores between young and old
mice at 5, 10, and 15 min was significantly different (\( p < 0.05 \)).
For the subsequent times, there was no significant difference.

Considering that the first 15 min corresponded to the ex-
ploratory period, these results showed that a reduced locomo-
tor activity of old mice may be due to a decrease in exploration.

Pain Sensitivity

The latency to react to a heat stimulus (Table 1) was signifi-
cantly increased in old mice both in the tail-flick (\( p < 0.05 \))
and hot-plate (\( p < 0.001 \)) tests. Thus, aged mice appear to be
less sensitive to heat stimulus.

Morris Water Maze

Place learning. The latency to finding the hidden platform
(Fig. 2A) decreased significantly over the successive learning
trials in both groups, \( F(3, 63) = 18.06, p < 0.0001 \). However,
the mean latencies for old mice were consistently longer, indi-
cating a significant effect of age, \( F(2, 21) = 5.36, p < 0.01 \). There
was no time × age interaction, \( F(6, 63) = 1.11, p = 0.36 \). This
indicates that both old and young mice were able to learn
where the platform was located, but old mice were slower.

In the probe trial, after the 4 days of training, the time
spent swimming in the training quadrant Q1 (Fig. 2B) was sig-
ificantly longer than 25 s for young mice (\( p < 0.001 \)). The old
mice spent a time significantly longer than 25 s in both quad-
rants Q1 and Q2. So, the young mice remembered exactly the
location of the platform, whereas, the old mice confounded
the training quadrant with another quadrant.

Cue learning. The latency to find the platform (Fig. 2C) de-
creased significantly over the successive learning sessions in
both groups, \( F(2, 52) = 39.05, p < 0.001 \). There was no effect
of age; the old mice performed as well as the young mice. The
data indicate that aged mice have no motor deficits and were
motivated to escape from the water when the platform is ren-
dered visible by a cue.

FIG. 1. Locomotor activity was measured for 60 min by 5-min peri-
ods in an actimeter. Values are means ± SEM (n = 12 in each group).
Regarding cumulative data (A), the old group is significantly less
active than young group (\( p < 0.001 \)). The analysis by 5-min session
(B) showed a significant difference at 5, 10, and 15 min (\( *p < 0.05 \)).
TABLE 1

<table>
<thead>
<tr>
<th>Pain Sensitivity</th>
<th>Latency, Young (s)</th>
<th>Latency, Old (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot plate test</td>
<td>$5.33 \pm 0.4$</td>
<td>$8.6 \pm 0.7$</td>
</tr>
<tr>
<td>Tail flick test</td>
<td>$7.6 \pm 0.4$</td>
<td>$8.9 \pm 0.4$</td>
</tr>
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Values are means ± SEM ($n = 12$ in each group). Old mice were significantly less sensitive to a heat stimulus in the two tests.

**Passive Avoidance**

For young mice (Fig. 3A), latency was very significantly increased from Day 1 to Day 2 ($p < 0.01$). There was no significant difference for old mice 24 h after the acquisition test. The results of the experiment conducted with a 1-day interval between the acquisition and the retention test showed a good retention for young mice, whereas old mice did not remember the situation.

The measure of transfer latency 3 h after acquisition test (Fig. 3B) show a significant increasing in young ($p = 0.002$) and in old mice ($p = 0.005$). The results of the experiment conducted with a 3-h interval between the acquisition and the retention tests indicate a good retention for both young and old mice.

**Active Avoidance**

The percentage of avoidance was recorded for 5 days, with a conditioned stimulus duration of 5 s (Fig. 4A) or 10 s (Fig. 4B). The performance of young and old mice improved over 5 days [time effect; A: $F(4, 80) = 83.3$, $p < 0.0001$; B: $F(4, 80) = 41.2$, $p < 0.0001$] in both cases. There was a significant effect of age in the two experiences [A: $F(1, 20) = 47.815$, $p < 0.0001$; B: $F(1, 20) = 15.2$, $p < 0.001$]. The time × age interaction was significant for the two durations [A: $F(4, 80) = 16.47$, $p < 0.0001$; B: $F(4, 80) = 10.93$, $p < 0.0001$].

In the two experiences, the average magnitude of avoidance in young mice reached 80% on Day 5, whereas in old mice it reached only 34%. So, whatever the duration of sound signal, old mice exhibited learning impairments on this test. Moreover, the results of second experiment showed that learning deficits in old mice were not related to bad auditory ability since mice had a positive pinna reflex.

**Elevated Plus-Maze**

For young mice, transfer latency on Day 2 (Fig. 5) was significantly decreased compared to Day 1 ($p < 0.01$), indicating a learning effect. For old mice, transfer latency was similar on Days 1 and 2. On this test, old mice did not learn, unlike young mice.

**DISCUSSION**

The present results show that in old OF1 female mice a reduced locomotion and learning/memory ability for each of the four tests used. These results, however, require detailed analysis because of possible bias due to an alteration in the motor or sensory abilities of old mice.

The locomotor activity of old mice was significantly reduced compared to that of young mice. This may reflect impaired physical capacity. Another explanation is that because scores were significantly different only at 5, 10, and 15 min, this may indicate a decrease in exploration in old mice. A reduced curiosity and a diminished physical capacity may also act concomitantly to reduce the locomotor activity. These results are in agreement with those described in rats (10) and in mice (21).

The Morris water maze test indicated that old mice acquired place learning more slowly than young ones. As assessed by the evolution of escape latencies over the 4 training days. The old mice seemed to have a decreased ability to use spatial information; indeed, old mice need more time to see or to select and analyze the relevant cues (15). The probe trial showed good retention for young mice. Their swimming time in the training quadrant exceeded 25% of the total time. In contrast, although old mice spent more time in the training quadrant than in the other ones, they were unable to distinguish the training quadrant clearly. The nonspatial cue learn-
ing indicates that motor ability and motor coordination of old mice were not impaired; they had the same swimming abilities as young mice. Thus, the OF1 old mice showed impaired acquisition of the spatial learning task.

In the passive avoidance test, transfer latency on Day 2 increased compared to Day 1 in both young and old mice. However, this increase was significant in young but not in old mice. In this test, it is advantageous to stay in the lighted compartment so that reduced motor capacity cannot play a role in the reduced retention. Impaired perception of the light intensity or the electric footshock may, however, be involved in the poor retention of old mice. To discard these possibilities, we carried out a passive avoidance experiment with a time interval of 3 h between the acquisition and the retention tests. In this last experiment, old mice remembered the situation very significantly, and were as performant as young mice. We conclude that old mice are able to see the light, to understand the significance of the signal, and that they feel the electric shock. However, their memory of the situation decreased quicker than in young mice.

In the active avoidance test, the performance of old mice was considerably altered. In this test mice had to recognize the acoustic signal and to cross into the other compartment to avoid a footshock: apart from a decrease in learning/memory abilities, impairment of motor or auditory ability or pain sensitivity may explain the poor performance of old mice. The first experiment, using a standard protocol with 5 s as the conditioned stimulus duration, showed a significant learning default for old mice. But, 5 s has been described as perhaps being too short to allow aged mice to cross to the next compartment (11). Thus, a second experiment was performed in which the duration of sound signal was 10 s and only mice with a positive pinna reflex were used. The results showed

FIG. 3. Passive avoidance. Transfer latency to entering the dark compartment was measured on Days 1 and 2 (A). Values are means ± SEM. Transfer latency of young mice (n = 12) increased significantly on Day 2 (**p < 0.01). No significant difference was obtained between Days 1 and 2 for the old mice (n = 11). There was a significant learning in both groups 3 h (B) after acquisition test (n = 12 in each group; **p < 0.01).

FIG. 4. Active avoidance. The percentage of avoidance was recorded. Each point represents the mean group performance (±SEM) of 50 trials each day (n = 12 in each group). The conditioned stimulus duration (sound signal) was 5 s (A) or 10 s (B). The performance of old mice was significantly lower than that of young mice in two experiments (p < 0.001).

FIG. 5. Elevated plus-maze. Transfer latency to cross the midpoint of the closed arms was recorded. Values are means ± SEM. Transfer latency on Day 2 decreased significantly for young mice (n = 12; **p < 0.01). Old mice (n = 11) did not show any significant difference between Day 2 and 1.
that old mice had a significantly lower rate of avoidance responses compared to young mice. Despite the increasing of conditioned stimulus duration and a normal auditory ability, old mice exhibited a significant decline in active avoidance performance. Moreover, decreased pain sensitivity was not involved in the poorer performance of old mice because the same electric intensity was used in the passive and active avoidance tests, and we have shown that old mice are sensitive to the intensity (0.3 mA). Thus, neither motor nor auditory nor pain sensitivity impairments were the real cause for low rate of old mice in active avoidance. The poorer performance of old mice may be explained by a reduced motivation. Such an explanation has been advanced to explain the reduced avoidance of old C57BL6/J mice in another model of active avoidance (22).

Although old mice presented impaired sensitivity to the heat source, it may be different to electric shock (11). Indeed, an age-related decrease in shock sensitivity has not been found in Swiss mice (14). In addition, no difference was shown in nociception using the tail-shock test in rats (9). So, despite impaired sensitivity to heat sources, we have demonstrated that our mice are sensitive to the electric intensity used in two avoidance tests, and that this suspected bias is not involved in the poor cognitive performances of old mice.

In the elevated plus-maze test, latency on Day 1 did not differ between young and old mice. This indicates that, in this test, neither motor ability nor visual capacity differentiates old from young mice. Latency decreased between Days 1 and 2 in young mice indicating good learning. The lack of decrease in latency in old mice may be related to decreased motivation to escape from the uncomfortable situation or to impairment of learning.

Taken together, these results show that in old female OF1 mice learning and/or memory are impaired in each of the four tests used. This is the first time, to our knowledge, that the elevated plus-maze has been used to assess the decrease in cognitive performance of old mice. Although biases such as physiological deficits in pain perception, visual acuity, audition, and motor ability may interfere with cognitive processes, the tests we used did not require each of these physiological abilities to the same extent, and in addition, we have especially shown that each of these biases may be discarded using a detailed analysis and additional experiments. We, therefore, conclude 17-month-old OF1 mice can be used as a model to study age-related cognitive alterations.

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