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Physiology & Behavior 90 (2007) 155–164

PHYSIOLOGY & BEHAVIOR

Cholinergic system, rearing environment and trajectory learning during aging in mice

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Received 11 April 2006; received in revised form 17 September 2006; accepted 20 September 2006

Abstract

Three, 12- and 20-month-old C57BL6/J mice, reared in standard conditions or in enriched environments, were administered subcutaneously either scopolamine hydrobromide, 0.6 or 1.2 mg kg^{-1}, or physiological saline (control mice) 15 min before testing their abilities to find an invisible platform in a modified version of the Morris water maze, the starting point being kept unchanged throughout the experiment to allow the aged animals to solve the task. The results demonstrated that: 1) All control mice, whatever their age, were able to learn the platform location, but the number of trials needed to reach the learning criterion (3 consecutive trials in less than 8 s) increased with age; 2) All the scopolamine-treated mice, whatever their age, were also able to learn the platform location. However, compared to age-matched controls, the number of trials needed to reach the learning criterion was greater; 3) Rearing the animals in an enriched environment antagonized the effect of scopolamine, but only in the youngest (3 month-old) mice. All control and scopolamine-treated mice, whatever their age and their rearing environment, remembered, 7 days later, the platform location.

Keywords: Trajectory learning; Spatial memory; Enriched environment; Cholinergic system; Scopolamine; Aging; Mouse

1. Introduction

Spatial learning and memory alterations occur in aged humans [1–3] as well as in aged laboratory rodents [4–9]. Such dysfunctions have been correlated with functional or/and morphological changes in cholinergic structures in the central nervous system, such as the hippocampus [10], the dentate gyrus [11–13], the septal area [14], the septo-hippocampal system [15,16] and the basal forebain [10,17]. Moreover, there is evidence that deficits of spatial memory during aging are reduced, at least in part, by restoration of the cholinergic system functioning [18]. Besides, in adult animals, lesion of cholinergic structures alters spatial memory [19–22], and such impairments are restored by pharmacological enhancement of the cholinergic activity [23] as well as by grafts of embryonic cholinergic neurons [24–26]. At last, manipulations of the cholinergic system interfere with spatial learning and spatial memory: transgenic mice that express human acetylcholinesterase in brain neurons are impaired in spatial learning [27]; scopolamine, a potent muscarinic antagonist, impairs spatial cognition as measured on the radial maze or in the Morris water maze [28–31]. On the contrary, enhancement of cholinergic activity by anticholinesterasic drugs [32], or by administration of NGF [33], increases spatial abilities. Administration of NGF also restores spatial memory in aged rats [34]. Similarly, knockout mice lacking the p75 low affinity neurotrophin receptor that induces apoptosis in various neuronal cell types and which is expressed in the cholinergic neurons of the basal forebrain are expected to show an increased number of neurons in this region; they also improve their spatial learning abilities [17].

Aging and cholinergic system are not the sole factors affecting spatial cognitive functions. Indeed, rearing environment also modifies the development of behavior and learning abilities. Rhesus monkeys reared in isolation do not take care of their infants and are likely to be more aggressive [35,36]. Conversely, rearing in

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0031-9384/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.physbeh.2006.09.024

enriched environments increases the behavioral capabilities of the animals [37], as demonstrated by learning tests [38–40], as well as sensory system development, in particular the visual system [41]. Restoration of the abilities following lesion of a nervous structure also depends on sensorimotor experience [42–44], even when the lesion was of genetic origin [45–47]. The effects of enriched environments and sensorimotor experience on behavior are correlated with the development of nervous structures which is reduced in restricted environments [48,49] and increased in enriched environments [38,50–52].

If the effects of aging, rearing environment and cholinergic system have been studied separately on spatial abilities, the interaction between these factors has never been addressed systematically. The aim of the present study was, therefore, to search for such eventual interactions in spatial abilities in mice. For this purpose, spatial behavior of 3-, 12-and 20-month-old mice, reared in either standard or enriched environments, was studied after administration of scopolamine hydrobromide, a potent muscarinic receptor antagonist, and compared with that of control mice. However, preliminary studies have shown that aged mice have difficulties in learning spatial tasks through the acquisition of a cognitive map. This observation agrees with many results demonstrating that the number of mice and rats which have spatial learning and spatial memory impairments increases with age [4,5,7,9,13,53,54]. Since the aim of this study was to demonstrate the eventual differential effect of scopolamine in young and old mice, it was necessary to use a protocol that the oldest animals were able to learn. It is the reason why we have chosen to modify the standard protocol of the Morris water maze and to keep the starting point unchanged throughout the experiment. In such a protocol, the learning process would be associative in nature, the mice associating a proximal cue and the platform location [55]. It is therefore reasonable to think that the animals learned the platform location through a trajectory learning rather than through the acquisition of a cognitive map [56].

All the animals of this study were subjected, 2 weeks before, to several motor tests and to a motor learning task on a rotorod [57]. Moreover, since the decline of acetylcholine release with age is controversial [58–60], we have tested the presynaptic status of the cholinergic system by evaluating choline uptake by synaptosomes prepared from the striatum and the hippocampus in 3- and 20-month-old mice.

2. Material and methods

2.1. Animals

One hundred and seventy healthy C57BL6/J mice (sex ratio 1/1) were born and reared in an authorized animal room in standard conditions until weaning: 12 h light (8.00–20.00)–12 h dark (20.00–8.00), 20–22 °C, food and water available ad libitum.

At weaning (22 days of age), they were separated according to their sex and housed 5/cage either in standard conditions, or 10/cage in an enriched environment until 3, 12 or 20 months of age. Before testing, the animals were administered either scopolamine hydrobromide or physiological saline. Scopolamine was administered at two doses (0.6 and 1.2 mg kg^{-1}) in 3- and 12-month-old mice. Due to mortality (46% from the beginning of the 20th month, that is 34 mice among 74, with no significant difference between males and females), the 20-month-old animals were less numerous and scopolamine was administered at only one dose (1.2 mg kg^{-1}). Sixteen groups of 10 mice each (5 males and 5 females) were therefore studied:

- 6 groups of 3-month-old mice reared in standard conditions (3 groups) or in an enriched environment (3 groups), received, before testing, either 0.6 mg kg^{-1} or 1.2 mg kg^{-1} scopolamine hydrobromide, or physiological saline.
- 6 groups of 12-month-old mice reared in standard conditions (3 groups) or in an enriched environment (3 groups), received, before testing, either 0.6 mg kg^{-1} or 1.2 mg kg^{-1} scopolamine hydrobromide, or physiological saline.
- 4 groups of 20-month-old mice reared in standard conditions (2 groups) or in an enriched environment (2 groups), received, before testing, either 1.2 mg kg^{-1} scopolamine hydrobromide or physiological saline.
- Ten 3-month-old additional mice (5 males and 5 females) reared in standard conditions were administered, before testing, methylscopolamine (1.2 mg kg^{-1}) that does not cross the blood–brain barrier.

2.2. Rearing environments

After weaning, the animals were subjected to the same dark–light cycle (12h/12h), the same temperature (20–22 °C) and were provided with food and water ad libitum.

2.2.1. Standard conditions

The animals were reared 5/cage in small standard cages (18 × 12 × 13 cm) until they were tested.

2.2.2. Enriched environment

The animals were reared in the enriched environment from weaning until they were tested.

The enriched environment consisted of two large cages (38 × 26 × 27 cm) made of color plastic. These two cages were interconnected and contained wheels, numerous pipes and small chambers. The cages were dismantled every 2 weeks and reassembled in another way in order to revive exploration [57]. For the same reason, the location of food pellets and of the water bottle was also changed once a week. The animals were reared 10/cage from weaning until the age of testing.

2.3. Drug administration

Scopolamine hydrobromide (0.6 and 1.2 mg kg^{-1}) (SIGMA), methylscopolamine (1.2 mg kg^{-1}) (SIGMA) or physiological saline were administered subcutaneously 15 min before testing.

2.4. Behavioral procedure

The water maze learning task was an adaptation of that used by Morris et al. [61], consisting of a circular pool 90 cm in diameter and 40 cm in height. The temperature of the water was 22 ± 1 °C.

An escape platform (8 cm in diameter), hidden 1 cm beneath the water surface (made opaque with small recyclable plastic lentils), was placed in a fixed position (north-west) relative to three visual cues (a black spot, 5 cm in diameter, a black square, 5 × 5 cm, and a black triangle, 5 cm in height) located around the perimeter of the pool. The mice could also use extramaze visual cues. The position of the cues and of the starting point (south) were kept unchanged throughout the experiment. Such a protocol was more adapted to aged animals than that consisting of varying the starting point from trial to trial as preliminary experiments have suggested that aged mice have difficulties to find the platform through the acquisition of a cognitive map. Such a learning process would rather be associative in nature, the animals associating a proximal item and the platform location [55]. When the mouse was placed in the water for the first time, it was allowed a maximum of 60 s to find the platform. If the animal reached the platform (successful trial), it was allowed to stay on it for 10 s, then was taken away and placed on a dry linen. The intertrial interval was 5 min and between each block of five trials a 15 min rest was observed. The maximal number of trials was 10/day. If the mouse did not find the platform within 60 s, it was placed on it for 10 s. According to a previous study [62], the learning criterion was reached once the mouse found the platform in three consecutive trials within a maximum latency of 8 s. Therefore, the number of trials was different according to each mouse. Escape latencies were measured. Seven days later, the animals were subjected to a retrieval test in the same conditions as previously described: the platform was located in the north-west quadrant and the starting point was south. The retrieval test was achieved once the animals reached the learning criterion. All the control and scopolamine-treated mice, either reared in standard or enriched environments, were subjected 1 h later to a second retrieval test consisting of four trials with different starting points (west, north, east and south), and the mean escape latency was measured for each trial. In the results section are given only the scores reached by the oldest mice (20 months-old).

2.5. Choline uptake

All experimental procedures for the preparation of tissues were carried out at 0–4 °C. For preparation of the synaptosomes, ten 3-month-old mice and ten 20-month-old mice were killed by decapitation 7 days after learning completion, and the striatum and hippocampus were dissected and homogenized in 5 ml of 0.32 M sucrose using 10 up-and-down strikes of a Teflon glass homogenizer (800 rpm). Nuclear material was removed by centrifugation at 1000 *g* for 5 min. The resultant pellet was suspended in 800 µl of ice-cold Krebs–Ringer medium previously oxygenated (95% O_2–5% CO_2). The medium contained (mM): NaCl = 109, KCl = 3.6, KH_2PO_4 = 1.1, $CaCl_2$ = 2.4, $MgSO_4$ = 0.6, $NaHCO_3$ = 25, glucose = 5.5, pH = 7.6. Protein concentration was determined by the method of Bradford [63] on 10 µl aliquots of each striatal and hippocampal synaptosomal preparation. [^{3}H]-choline uptake was evaluated on 100 µl aliquots of the synaptosomal preparations, in conditions close to those of Ferguson et al. [64]. After a 5 min preincubation at 37 °C in Krebs–Ringer buffer, [^{3}H]-choline (83.1 Ci/moml,; Amersham, Les Ulis, France) was added to 0.1 µM or 0.4 µM final concentrations. Five-minute incubations were stopped by dilution into ice-cold Krebs–Ringer medium followed by filtration in vacuo on Whatman GF/B filters. Filters were washed twice with 3 ml cold Krebs–Ringer medium and dried. Tissue radioactivity retained by synaptosomes was determined by liquid scintillation spectrometry. Blank values, obtained by incubation parallel samples at 0 °C, were substracted.

2.6. Statistical analysis

Analyses of variance (ANOVAs) were used to test the age × drug × environment interactions. They were followed by Tukey post hoc analyses.

3. Results

Due to the lack of significant difference in the scores between males and females, both genders were pooled.

3.1. Learning curves

In all groups, the escape latency decreased from the first to the last trial. This is illustrated in Fig. 1 which depicts the learning abiLities of 3- and 20-month-old mice reared in standard (Fig. 1A) or enriched (Fig. 1B) conditions, and administered with either saline or 1.2 mg kg^{-1} scopolamine hydrobromide, as well as in 3-month-old mice administered with 1.2 mg kg^{-1} methylscopolamine.

A three-way ANOVA (age × drug × session) with repeated measures on the last factor, demonstrated, in standard conditions, an age effect [$F(1,36) = 4.288$, $p = 0.046$], the escape latencies being always higher in 20-month-old than in 3-month-old mice, a drug

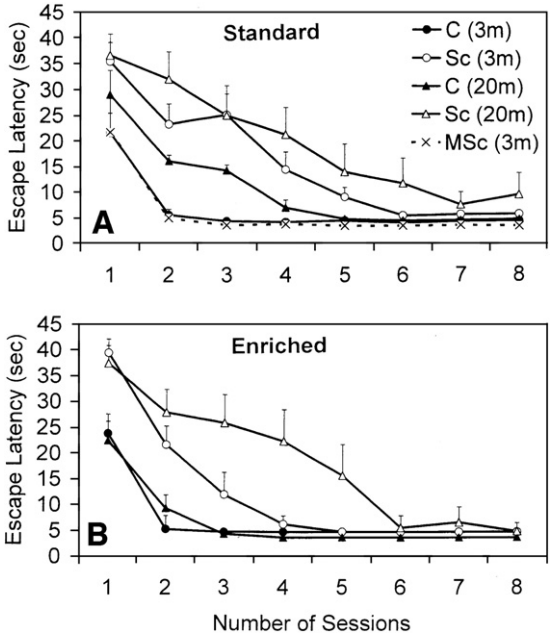

Fig. 1. Evolution of the escape latency (in s ± SEM) with the number of sessions in 3- and 20-month-old control (C) and scopolamine hydrobromide-treated (Sc) mice, and in 3-month-old mice which were administered with methylscopolamine (MSc).

effect [$F(1,36)=25.3184$, $p=0.00001$], the escape latencies of scopolamine-treated mice being always greater than those of control mice, whatever their age, and a session effect [$F(7,252)=39.399$, $p=0.00000$], showing that in all the animals the escape latencies decreased with the sessions (Fig. 1A). There was no age × drug interaction [$F(1,36)=0.003$, $p=0.954$] which shows that the drug effect did not depend on age (whatever the age, the escape latencies of scopolamine-treated mice were always higher than those measured in control mice). There was also no age × session interaction [$F(7,252)=1.084$, $p=0.374$] demonstrating that evolution of the scores with the sessions did not depend on age (the escape latencies always decreased with the sessions whatever the age of the animals). However, there was a drug × session interaction [$F(7,252)=3.754$, $p=0.0007$] indicating that the evolution of the scores with the sessions depended on the drug (see Fig. 1), the escape latencies decreasing slower in scopolamine-treated mice than in controls, especially in 3-month-old animals.

It is worthy to note that at 3 months of age, there was a rearing environment × drug interaction [$F(1,36)=6.819$, $p=0.013$] demonstrating that scopolamine had not the same effect in mice reared in standard and in enriched environments. Indeed, comparing Fig. 1A and B shows that the learning curves which are similar in controls whatever the rearing environment, are not in scopolamine-treated mice, learning scores being better in mice reared in enriched conditions than in mice reared in standard conditions. However, the scores during sessions 6, 7 and 8 were similar in both groups, showing that the enriched environment increased learning velocity but had no effect on the final score. In 20-month-old mice, there was no rearing environment × drug interaction [$F(1,36)=0.231$, $p=0.633$] showing that the drug effect was similar whatever the rearing environment (enriched or standard).

3.2. Learning and retrieval abilities in the different groups

Given that only one dose of scopolamine was administered in old mice, global analyses, including the three ages and the two rearing environments, were done only for animals injected with saline or 1.2 mg kg^{-1} scopolamine. Concerning the number of trials needed to reach the learning criterion, a 3-way ANOVA (age × drug × rearing environment) demonstrated an age effect [$F(2,108)=19.696$, $p<0.0001$], a drug effect [$F(1,108)=128.520$, $p<0.0001$] but no rearing environment effect [$F(1,108)=1.534$, $p=0.218$].

3.2.1. Age effect

In mice reared in standard conditions and administered physiological saline, the number of trials needed to reach the learning criterion increased with age (Fig. 2A–C). From the above-mentioned ANOVA, post hoc comparisons demonstrated a significant difference between 3- and 12-month-old mice ($p=0.004$), between 3- and 20-month-old mice ($p=0.003$), but not between 12- and 20-month-old mice ($p=0.181$).

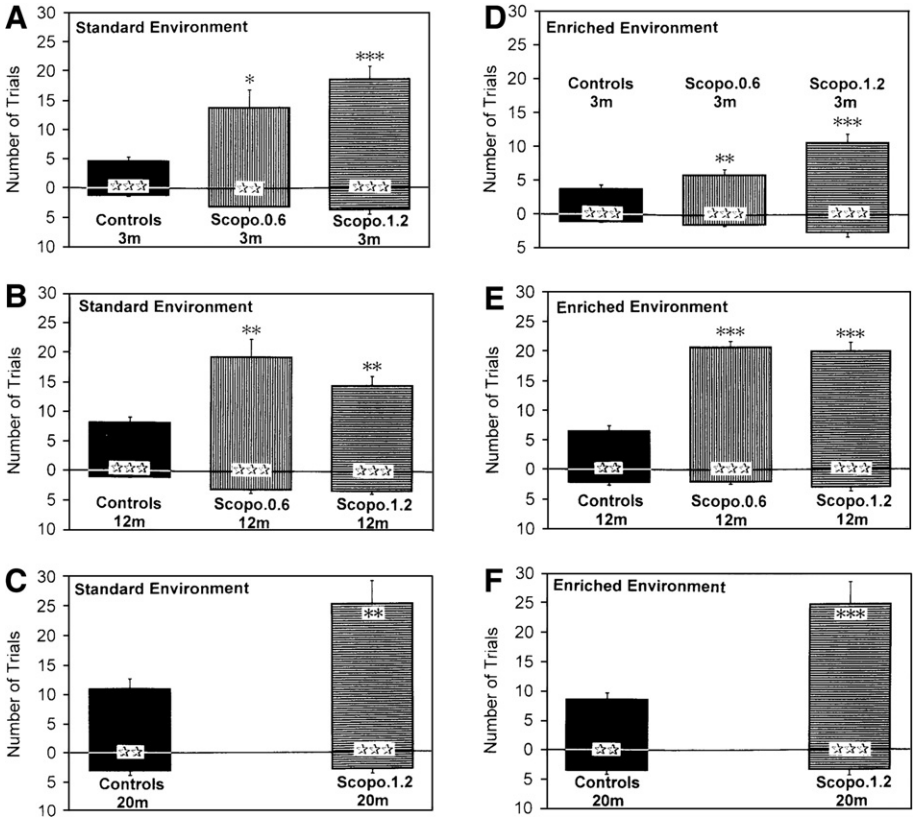

Fig. 2. Number of trials (±SEM) needed to reach the learning criterion during the learning session (upper bars) and the retrieval test (lower bars) in 3- (A,D), 12- (B,E), and 20-month-old (C,F) mice reared in standard (A,B,C) or enriched (D,E,F) environments. Mice administered scopolamine hydrobromide at the dose of 0.6 mg kg^{-1} or 1.2 mg kg^{-1} are noted Scopo 0.6 and Scopo 1.2, respectively. Asterisks: differences relative to 3-month-old mice significant at $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***); stars: differences between learning and retrieval sessions significant at $p<0.01$ (★★), $p<0.001$ (★★★).

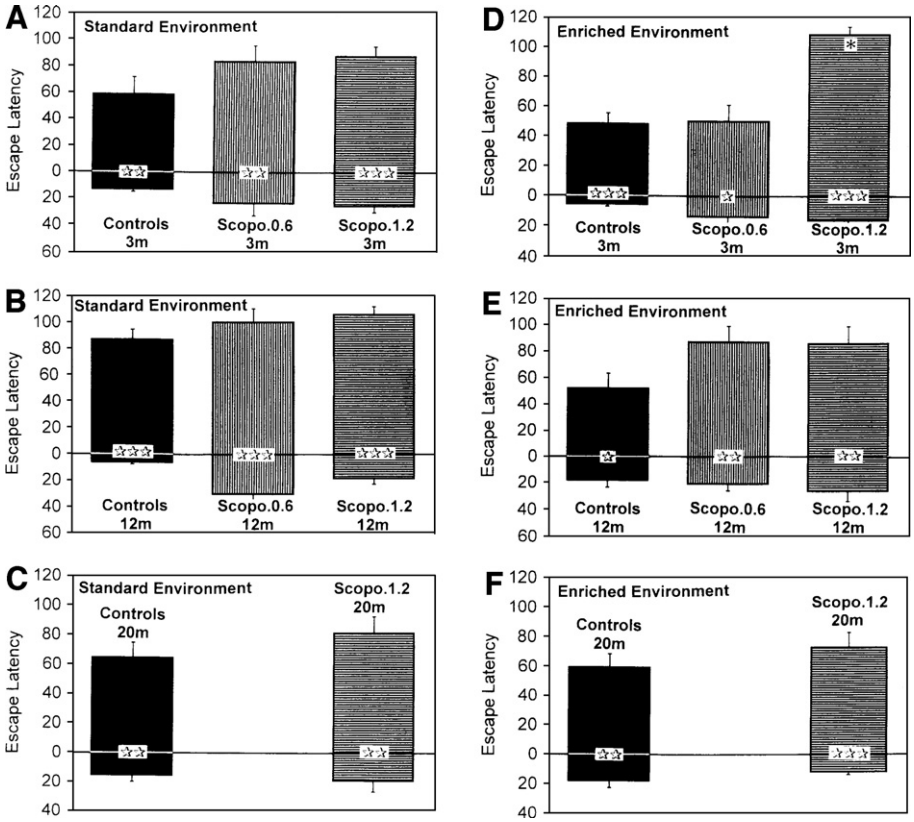

Fig. 3. Cumulative escape latency (in s±SEM) during the first two trials of the learning session (upper bars) and of the retrieval test (lower bars) in 3- (A,D), 12- (B,E), and 20-month-old (C,F) mice reared in standard (A,B,C) or enriched (D,E,F) environments. Mice administered with scopolamine hydrobromide at the dose of 0.6 mg kg^{-1} or 1.2 mg kg^{-1} are noted Scopo 0.6 and Scopo 1.2, respectively. Asterisks: differences relative to 3-month-old mice significant at $p<0.05$ (∗); stars: differences between learning and retrieval sessions significant at $p<0.05$ (⋆), $p<0.01$ (⋆⋆), $p<0.001$ (⋆⋆⋆).

All the animals remembered the platform location. Indeed, in 3-, 12- and 20-month-old mice, the number of trials needed to reach the criterion was significantly lower during the retrieval phase than during the learning phase ($p=0.001$, $p<0.0001$, and $p=0.002$, respectively) (Fig. 2A). Moreover, the cumulated time needed to find the platform during the first two retrieval trials was shorter than that measured during the first two learning trials ($p=0.007$, $p<0.0001$, and $p=0.005$ in 3-, 12- and 20-month-old mice, respectively) (Fig. 3A–C).

3.2.2. Drug effect

Since 3- and 12-month-old mice were administered with 0.6 or 1.2 mg kg^{-1} scopolamine and 20-month-old mice only 1.2 mg kg^{-1} scopolamine, drug effect was estimated from three ANOVAs done in 3-, 12-, and 20-month-old mice, respectively.

A two-way ANOVA (drug × rearing environment) demonstrated a drug effect at 3 months [$F(2,54)=20.03$, $p<0.0001$] as well as at 12 months [$F(2,54)=32.96$, $p<0.0001$] and 20 months [$F(1,36)=35.96$, $p<0.0001$]. From the above-mentioned ANOVA, post hoc comparisons demonstrated that in mice reared in the standard condition as well as in mice reared in the enriched environment, scopolamine administered at either 0.6 or 1.2 mg kg^{-1} increased the number of trials needed to reach the learning criterion (p between 0.014 and <0.0001) (Fig. 2).

All the scopolamine-treated mice remembered quite well the platform location. Indeed, the number of trials needed to reach the criterion was always smaller during the retrieval test than during the learning session (p between 0.010 and <0.0001) (Fig. 2), and the cumulated time needed to find the platform during the first two retrieval trials was shorter than that measured during the first two trials of the learning session (p between 0.010 and <0.0001) (Fig. 3).

3.2.3. Rearing environment effect

From global analysis including the three ages, only one dose of scopolamine (1.2 mg kg^{-1}) and the two rearing environments, there was no rearing environment effect [$F(1,108)=1.53$, $p=0.216$] but a just missing statistical significance to an age × rearing environment interaction [$F(2,108)=2.96$, $p=0.056$] suggesting that the effect of rearing environment depended on the age, but no drug × rearing environment interaction [$F(1,108)=0.10$, $p=0.751$], indicating that the influence of the drug did not depend on the rearing environment. Given the significant tendency of the age × rearing environment interaction, we performed separate ANOVAs for the three ages. At 3 and 12 months of age (Fig. 4A,B) there was a rearing environment effect [$F(1,36)=11.01$, $p=0.002$, and $F(1,36)=4.30$, $p=0.045$, respectively], but not at 20 months [$F(1,36)=0.230$, $p=0.633$] (Fig. 4C).

The existence of an age × drug × rearing environment interaction [$F(2,108)=3.85$, $p=0.024$] indicated that if globally there was no significant rearing environment effect, such an effect could emerge according to drug and age. Indeed, from the above-

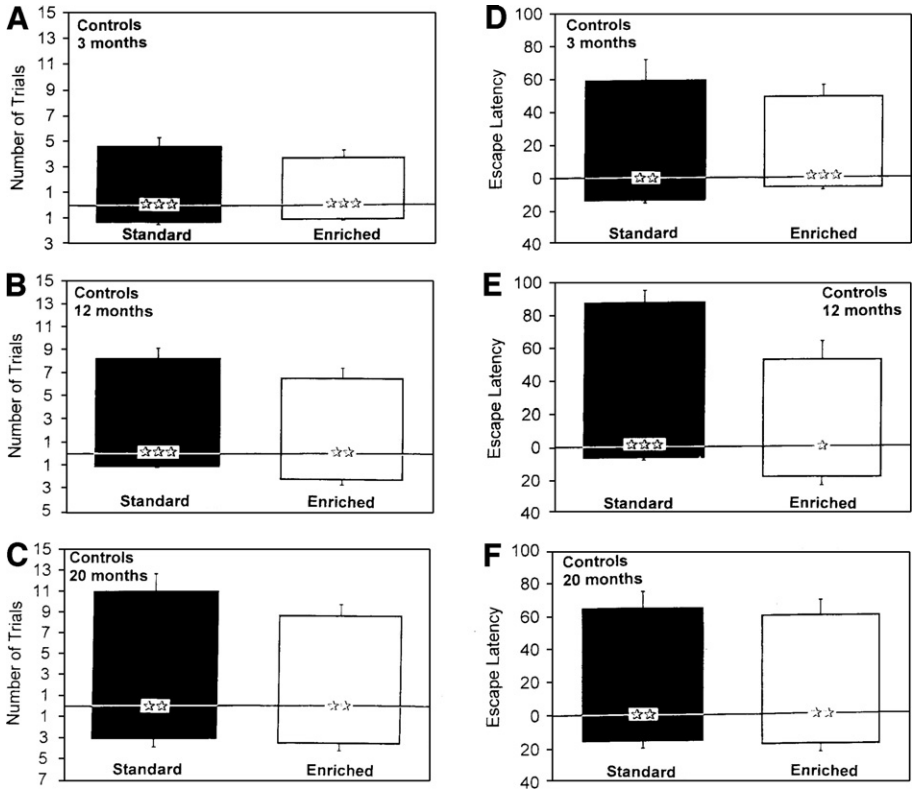

Fig. 4. A–C: Number of trials (±SEM) needed to reach the learning criterion during the learning session (upper bars) and the retrieval test (lower bars) in 3- (A), 12- (B), and 20-month-old (C) mice reared in standard (full bars) or enriched (open bars) environments. Stars: differences between learning and retrieval sessions significant at $p<0.01$ (⋆⋆), $p<0.001$ (⋆⋆⋆). D–F: Cumulative escape latency (in s±SEM) during the first two trials of the learning session (upper bars) and of the retrieval test (lower bars) in 3- (D), 12- (E), and 20-month-old (F) control mice reared in standard (full bars) or enriched (open bars) environments. Stars: differences between learning and retrieval sessions significant at $p<0.05$ (⋆), $p<0.01$ (⋆⋆), $p<0.001$ (⋆⋆⋆).

mentioned ANOVA, post hoc comparisons showed that in 3-month-old scopolamine-treated mice, the number of trials needed to reach the learning criterion was significantly smaller when the animals were reared in the enriched environment than when they were reared in standard conditions (in 0.6 and 1.2 mg kg^{-1} scopolamine-treated mice, $p=0.013$ and $p=0.026$, respectively) (compare Fig. 2A and D).

All the animals, whatever their age and their rearing environment, remembered the platform location. Indeed, the number of trials needed to reach the criterion during the retrieval test was smaller than that needed during the learning session (p between 0.010 and <0.0001) (Fig. 4A–C), and the cumulated time needed to find the platform during the first two retrieval trials was shorter than that measured during the first two trials of the learning session (p between 0.019 and <0.0001) (Fig. 4D–F).

3.3. Learning abilities of methylscopolamine-treated mice

As depicted in Fig. 1, the learning curves of 3-month-old mice administered physiological with saline or 1.2 mg kg^{-1} methylscopolamine were similar. Moreover, the number of trials needed to reach the learning criterion was the same in both groups (4.7±0.6 and 4.9±07 in control and methylscopolamine-treated mice, respectively: $p=0.794$) and smaller that that measured in 1.2 mg kg^{-1} scopolamine hydrobromide-treated mice (18.5±2.2) ($p<0.0001$).

3.4. What did the animals remember?

In 20-month-old mice which were administered physiological saline (controls) or 1.2 mg kg^{-1} scopolamine hydrobromide, and which were reared in standard conditions or in the enriched environment, we performed two retrieval tests spaced by a one-hour interval. During the first retrieval test, the starting point was similar to the position during learning (south). During the second retrieval test, it was successively west, north, east and south. The mean escape latencies measured during the last four trials of the first retrieval test and those measured during the four trials of the second retrieval test are depicted in Table 1. For each group, there was no significant difference between the mean escape

Table 1
Escape latencies (in s±SEM) of 20-month-old control and scopolamine-treated mice reared in the standard or in the enriched environment, during the last four trials of the first retrieval test, when the starting point was kept unchanged and was that of the learning session, and during the four trials of the second retrieval test, when the starting point was changed from trial to trial

	First retrieval test	Second retrieval test	p value
Control standard	4.8±0.8	4.8±0.7	$p>0.05$
Scopo 1.2 standard	4.1±0.5	4.5±0.6	$p>0.05$
Control enriched	5.8±0.9	5.1±1.4	$p>0.05$
Scopo 1.2 enriched	5.7±1.5	4.1±0.7	$p>0.05$

For each group, there was no significant difference between the scores of the first and the second retrieval tests ($p>0.05$).

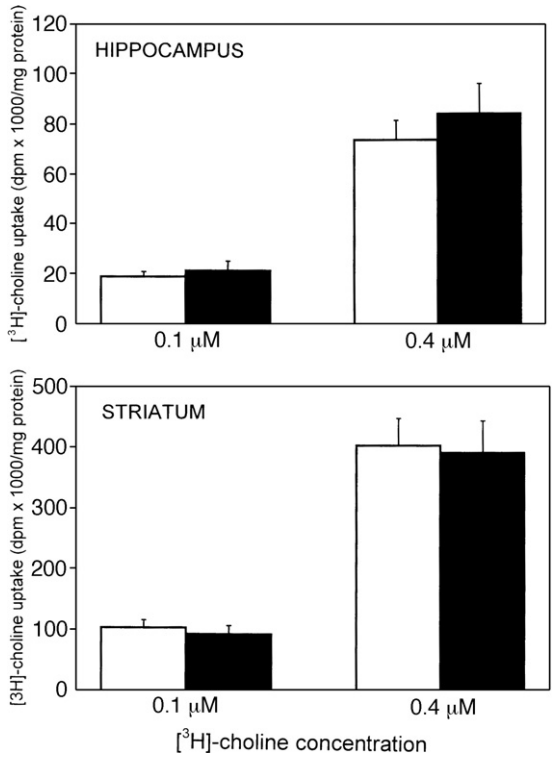

Fig. 5. [^{3}H]-choline uptake by synaptosomes prepared from the striatum and the hippocampus of 3- (open bars) and 20-month-old (full bars) mice. Means are given ± SEM.

latencies measured during the first and the second retrieval tests ($p > 0.05$), suggesting that the animals remembered, 7 days later, the spatial location of the platform and, then, that during the learning session they learned not only a trajectory but were able to compute a cognitive map of their environment.

3.5. Choline uptake

Preliminary experiments at increasing concentrations of [^{3}H]-choline indicated that, in our experimental conditions, [^{3}H]-choline uptake was maximal for [^{3}H]-choline concentrations between 0.1 and 0.4 μM in both striatum and hippocampus (data not shown). [^{3}H]-choline uptake was not different between 3- and 20-month-old mice at the two [^{3}H]-choline concentrations either in striatum or hippocampus (Fig. 5).

4. Discussion

The results of this study demonstrated that all the mice, whatever their age, were able to find the platform location through a trajectory learning, the escape latency decreasing with the number of trials. However, the number of trials needed to reach the learning criterion was higher in 12- than in 3-month-old mice, and was even higher in 20-month-old animals. While it has previously been demonstrated that aged mice have motor deficits, these deficits do not affect all motor skills and, particularly, the walking speed on a wooden beam was the same whatever their age [57]. Besides, old mice were able to each the learning criterion. This took more time than for younger ones, but once the learning criterion was reached, the escape latency was similar in old and young mice (3–4 s); this would not be the case if motor troubles of aged mice prevented trajectory learning. Moreover, Lurcher mutant mice whose cerebellar cortex is almost completely degenerated [65] and which are highly ataxic [66,67] are able to learn to escape about as well as non-mutant mice [55]. Whatever their age, the animals remembered quite well the platform location during the retrieval test performed 7 days after the learning test, showing that the long-term memory was not altered in aged mice and that they reached the platform as quickly as young ones. This could seem surprising, but one has to remember that the old mice constitute a biased group since it represents a selected group of healthy animals. For all these reasons, it seems unlikely that the delayed learning of the platform location in aged mice was due to motor disabilities. Therefore, such a delayed acquisition cannot be explained by memory deficits, but rather by acquisition impairments. It might be that the delayed associative learning process with age could be due to the development of encoding disabilities, as suggested by Lebrun [68] and Boller and Deweer [2]. Such alterations would be explained, at least in part, by attentional deficits as demonstrated in aged rats [8].

All the animals, whatever their age, reached the learning criterion when they were administered scopolamine hydrobromide. This is not surprising since it is known that the nicotinic system (and not only the muscarinic system) is involved in spatial cognition. However, compared to controls, learning was much delayed in scopolamine hydrobromide-treated mice. The effect of scopolamine hydrobromide on trajectory learning is a central effect since methylscopolamine, which does not cross the blood–brain barrier and acts exclusively peripherally, had no influence on the scores of the animals.

It is known that the cholinergic activity decreases with age. Particularly, the number of choline acetyltransferase positive cells is reduced in the forebrain [53], the septal area [54], the dentate gyrus and the striatum [12] with aging. However, the fact that, in our experiments, choline uptake by synaptosomes was maximal in both striatum and hippocampus and not different in 3- and 20-month-old mice, suggests that reduction of cholinergic activity in old mice was not presynaptic in origin. However, as demonstrated by Decker et al. [69], whether hippocampal choline uptake was similar in young and aged rats, it was reduced in place-trained rats compared to naive and cued-trained animals. Moreover, old rats displayed impaired place learning and a corresponding dampening of the training-induced change in choline uptake, suggesting that there is a task-specific engagement of cholinergic function in young animals that does not occur in aged animals. Since the scores of 20-month-old control mice were similar to those of 3-month-old mice which were administered 0.6 mg kg^{-1} scopolamine, it can be suggested (but this has to be demonstrated) that the muscarinic receptors in aged mice are either decreased in number or less sensitive to acetylcholine, and, therefore, that reduction of the cholinergic activity in old mice is postsynaptic in origin. Long-term memory was preserved in scopolamine-treated mice since the animals remembered the platform location 7 days after the learning session. This agrees with the fact that cholinergic hypofunction alters spatial

learning but does not impair recall (see [70] for review), and suggests that the great number of trials needed by the scopolamine-treated mice to find the platform during the learning session was not due to memory impairments. It can better be explained by attentional deficits. Indeed, removal of cholinergic neurons in the basal forebrain alters cued target detection and visuospatial attention in the rat [19]; visuospatial learning was also impaired following lesion of the cholinergic projection to the hippocampus in marmosets [71]; scopolamine slows orienting of attention to cued visual targets in the monkey [72] and alters attentional capabilities in humans [73,74]. Cholinergic lesions produced by 192 IgG–saporin in the basal forebrain and the medial prefrontal cortex also produced attentional deficits [75,76]. One could think that it would have been better to destroy the basal forebrain by injecting 192 IgG–saporin, as done in a number of studies, instead of blocking the cholinergic system as a whole by scopolamine. However, the aim of the present study was not to inactivate definitely cholinergic structures but rather to inactivate them temporarily (during the learning procedure) to test the ability of the animals, when intact, to remember information acquired during the period of cholinergic blockade.

The effects of scopolamine increased with age, suggesting a cumulative effect of scopolamine and aging.

The environmental rearing had no effect on the scores of control mice. This is, a priori, surprising since it has many times been demonstrated that enriched environments enhance the behavioral capabilities of the animals and, especially, learning abilities [39]. However, in 3-month-old control mice reared in standard conditions, the number of trials need to reach the learning criterion was so small (4.6±0.7) that it could hardly be smaller. In older mice, such a number of trials was greater (8.2± 0.9 and 10.9±1.7 in 12- and 20-month-old mice, respectively); it was slightly reduced in the animals reared in the enriched environment, but not significantly. It could be that the mice did not explore more the enriched cages than the standard ones and the former did no longer constitute an enriched environment. This suggests that active exploration is a necessary condition for the enriched environment to be considered as such. In 3-month-old scopolamine-treated mice, whatever the dose of scopolamine administered, the number of trials needed to reach the learning criterion was high in the animals reared in standard conditions (13.8±3.0 and 18.6±2.2 for 0.6 and 1.2 mg kg^{-1}, respectively). In this case, rearing in the enriched environment significantly increased the score, and in 0.6 mg kg^{-1} scopolamine-treated mice, the number of trials needed to reach the learning criterion (5.9±0.8) was of the same order of magnitude than that needed by 3-month-old mice reared in standard conditions (4.6±0.7). The sensorimotor experience was therefore well-suited to restore abilities following an alteration of nervous structures, as already demonstrated [42–47,57]. Particularly, it increases the cholinergic system activity [77], enhances the number of hippocampal neurons [78], as well as hippocampal NGF levels, which is one of the factors involved in regulation of the survival and differentiation of developing basal forebrain neurons [79], alters the PKA-dependent LTP in the hippocampus [80] and restores the hippocampal-dependent behavior after lesion of the hippocampus [81].

Acknowledgments

This work was supported by Fondation Simone and Cino del Duca.

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