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Research report

Influence of cholinergic system on motor learning during aging in mice

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Abstract

Three, 12- and 20-month-old C57Bl6 mice, reared in standard conditions or in an enriched environment, were administered subcutaneously either scopolamine hydrobromide (SIGMA), 0.6 and 1.2 mg kg^{-1}, or physiological saline 15 min before testing their motor skills (muscular strength, dynamic equilibrium and motor coordination) and motor learning abilities (number of trials needed to reach a learning criterion on a rotorod rotating at 27 revolutions per min). The results demonstrated a lack of correlation between motor skill scores and between motor skill and motor learning scores, suggesting that the rotorod training procedure measures motor learning and not motor skills or is insensitive to changes in motor skills. They also demonstrated that motor skills decreased with age but were insensitive to environmental rearing and to scopolamine. In contrast, the learning scores, which also decreased with age, were very sensitive to scopolamine, particularly in the oldest mice. These results are discussed according to the role of cholinergic system in motor learning during aging.

Keywords: Motor skills; Motor learning; Enriched environment; Cholinergic system; Scopolamine; Aging; Mouse

1. Introduction

Motor skills and motor learning abilities are affected by aging and rearing environmental conditions and are also dependent on the integrity of the central cholinergic system. Indeed, it has been reported that motor performances in humans declined with age [13,32,65], and that, compared with young animals, spontaneous activity [37], runwheel activity [57] and complex motor performances, as measured by rodwalk, wire suspension, plank walk, inclined screen and accelerating rotorod performance [54] were decreased in old ones. Not only does motor performances declined with age, but also motor learning as demonstrated in humans [12,32,65] and in animals [5]. Aging is not the sole factor affecting motor skills and motor learning. Indeed, rearing environment also modifies motor behavior since spontaneous locomotor activity and exploration were reduced in animals reared in an enriched environment while learning abilities were enhanced [6,62]. At last, the central cholinergic system, particularly that of the neostriatum and of the septo-hippocampal formation, plays a role in locomotion and motor skills [39,50,51] as well as in cognitive functions [11,17,33,36,58], particularly in learning of motor abilities [53,61]. However, its role in cognition is still controversial [34].

If the effects of aging, rearing environment and cholinergic system have been studied separately on motor abilities and motor learning, the interactions between these factors have never been addressed systematically. The aim of the present study was, therefore, to search for such eventual interactions in motor skills and motor learning in mice. For this purpose, motor skills and motor learning performances of 3-, 12- and 20-month-old mice, reared in either standard or

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enriched environments, were studied after administration of scopolamine hydrobromide, a potent muscarinic receptor antagonist, and compared with those of control mice.

2. Material and methods

2.1. Animals

One hundred and seventy healthy C57Bl6 mice (sex ratio 1:1) were born and reared, in an authorized animal room, in standard conditions until weaning: 12-h light (08.00–20.00)–12-h dark (20.00–08.00), 20–22°C, food and water available ad libitum At weaning, they were separated according to their sex and housed five per cage in standard conditions or ten per cage in an enriched environment until 3-, 12- or 20-month 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), the 20-month-old animals were less numerous and scopolamine was administered at only one dose (1.2 mg kg^{-1}). Sixteen groups of ten mice each (five males and five females) were, therefore, studied.

- Six groups of 3-month-old mice reared in standard conditions (three groups) or in an enriched environment (three groups), received, before testing, either 0.6 or 1.2 mg kg^{-1} scopolamine hydrobromide, or physiological saline.
- Six groups of 12-month-old mice reared in standard conditions (three groups) or in an enriched environment (three groups), received before testing either 0.6 or 1.2 mg kg^{-1} scopolamjne hydrobromide, or physiological saline.
- Four groups of 20-month-old mice reared in standard conditions (two groups) or in an enriched environment (two groups), received before testing either 1.2 mg kg^{-1} scopolamine hydrobromide or physiological saline.

Ten 3-month-old additional mice 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

2.2.1. Standard conditions

After weaning, the animals were reared five per cage in small standard cages ($18 \times 12 \times 13$ cm) until they were tested. The mice were subjected to the same 12-h dark:12-h light cycle, the same temperature (20–22°C) and were provided with food and water ad libitum

2.2.2. Enriched environment

The enriched environment consisted of two large cages ($38 \times 26 \times 27$ cm) made of color plastic (Fig. 1). These two cages were interconnected and contained wheels, numerous pipes and small chambers. The cages were dismantled regularly and reassembled in another way in order to revive exploration. For the same reason, the location of food pellets and of the water bottle was also frequently changed. The animals were reared ten per cage from weaning until the age of testing.

2.3. Drug administration

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

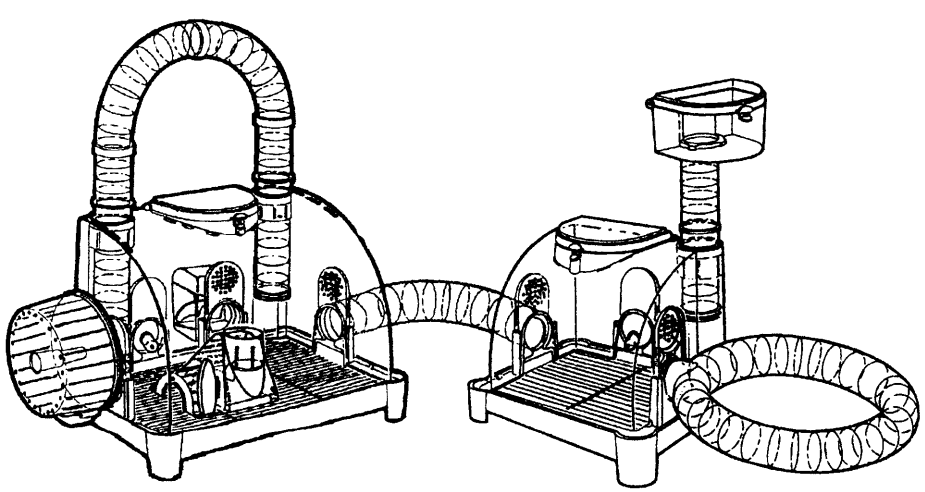

Fig. 1. Example of an enriched cage.

2.4. Behavioral procedure

All the animals were subjected to various behavioral experiments that always took place in the following order; [1] suspended string; [2] wooden beam; [3] hole board; [4] sensorimotor learning on a rotorod. The first three tests measured various motor skills — muscular strength (suspended string), dynamic equilibrium (wooden beam) and motor coordination (hole board). The last one (rotorod) measured the ability of the animals to improve walking on the rotorod during a training procedure; its aim was, therefore, to test motor learning abilities.

2.4.1. Suspended string

The aim of this test was to measure muscular strength of the animals. The mouse was hung by its two forepaws on an iron made wire (50 cm long, 3.5 mm in diameter), located 1 m above a thick carpet to cushion its eventual fall. The latency before falling was measured. Three tests were done, separated from each other by a 10-min interval and the best score was retained.

2.4.2. Wooden beam

The dynamic equilibrium was tested on a beam consisting of a scale ($2 \times 2 \times 100$ cm) located 40 cm above a soft carpet. At the beginning of the experiment, the mouse was placed on the middle of the beam, its body axis perpendicular to the beam long axis. During the test, the distance covered and the walking time were recorded in order to calculate the walking speed. The trial was stopped when the animal fell or after 3 min.

2.4.3. Hole board

The test was done to quantify the motor coordination skills of the animals. The apparatus consisted of an experimental box ($28 \times 28 \times 20$ cm). One centimeter above the bottom of the box was a platform (hole board) in which 36 holes, 2 cm in diameter and arranged in a 6×6 array had been drilled. The mouse was placed in the middle of the platform and allowed to explore it for 5 min. The walking time and the number of stumbles (one leg diving into a hole) were recorded. Then, the stumbling frequency (number of stumbles per min) was calculated.

2.4.4. Rotorod

The apparatus consisted of a wooden horizontal rod (3 cm in diameter and 40 cm long), covered with sticking plaster to increase roughness. It was located 30 cm above a landing platform covered with a thick sheet of soft plastic to cushion the eventual fall of the animal. This rod was rotated around its longitudinal axis by means of a DC electric motor, the rotation rate being 27 revolutions per min (rpm), as it has been shown that C57Bl6 mice were able to maintain balance efficiently even for higher rotation rates, i.e. 30 and even 40 rpm [10]. The mice were subjected every day to a session of ten trials (five trials in the morning and five in the evening). For each group, the ten trials were given in the following way — all the animals of the group were successively allowed to rotate for the first time; then, when the last mouse had completed its first trial, the first one was allowed to rotate for the second time, and so on. The time interval between two successive trials depended on the time during which the animals maintained balance without falling, but was never shorter than 3 min. For each trial, the mouse was placed on the rotating rod, its body axis being perpendicular to the rotation axis at the onset of training, and the head directed against the direction of rotation, so that the animal had to progress forward to maintain balance. The trial was stopped either when the mouse fell down or after 5 min (300 s), since mice staying on the rotating rod for 5 min were able to maintain their equilibrium for a much longer time [10]. The latency before falling and the behavior of the animals were quantified. Training was stopped, when the animals reached 300 s for two successive trials. For each trial, the scores reached by 10 mice of a group were averaged to calculate the mean score per trial (given $\pm$ S.E.M.: $\sigma/\sqrt{n}$). The relevant behavioral parameters recorded were hanging (consisting of the animals hanging on the rotorod while being passively rotated), and walking (consisting of the mice walking upon the rotating rod). The percentage of occurrence of each strategy was calculated, first for each mouse, then for each group (means of percentages $\pm$ S.E.M.).

2.5. Statistical analysis

Correlation analysis of the results of the various tests was made according to the '*r*' of Bravais-Pearson. For each experiment, ANOVAs were used to test the age $\times$ drug $\times$ environment interactions.

3. Results

3.1. Correlations between scores of the different tests

Whatever the age of the mice and the rearing environmental condition, and whether they were administered scopolamine hydrobromide or not, there was no correlation between motor skill scores (suspended string, wooden beam and hole board), as well as between motor skill scores and rotorod learning scores. For example, in 3-month-old control mice reared in standard conditions, the values of the correlation coefficient between suspended string and hole board scores, suspended string and wooden beam scores, and wooden beam and hole board scores, were -0.17, -0.26 and

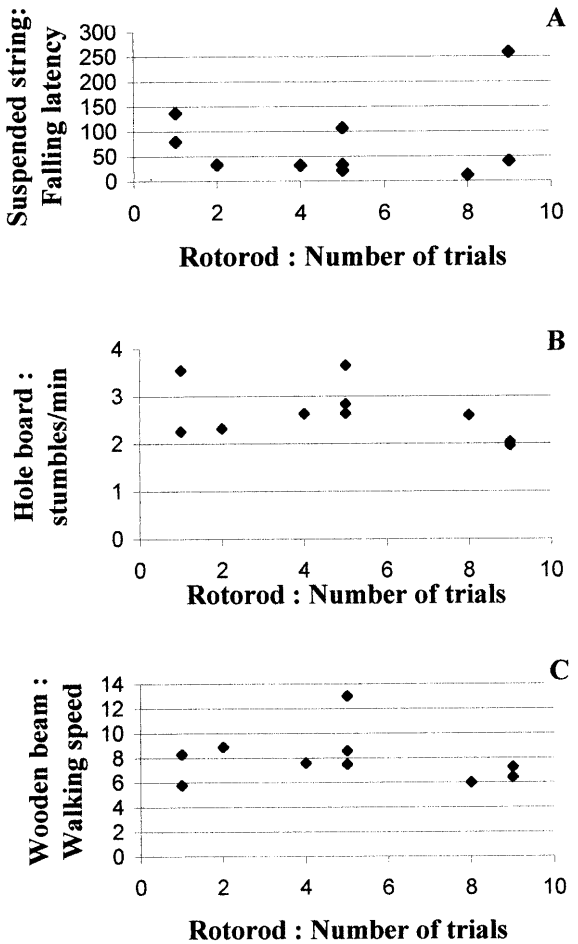

Fig. 2. Scatter plots of rotorod scores (number of trials to reach the learning criterion) on the one hand, and suspended string (falling latency in s; A), hole board (number of stumbles per min of walking; B), and wooden beam (walking speed in cm s^{-1}; C) scores, on the other hand.

0.27, respectively. Between the rotorod learning scores on the one hand, and suspended string, hole board and wooden beam scores, on the other hand, they were 0.15, −0.41 and −0.18, respectively (Fig. 2A–C). Therefore, the mice which needed a few trials to reach the learning criterion on the rotorod were not necessarily those which performed better in the motor skill tasks. Such a lack of correlation was also found when pooling control mice reared in standard and enriched conditions, in order to increase the sample size and, then, the statistical power.

3.2. Age, scopolamine and rearing environmental effects

Given that the oldest (20-month-old) mice were administered scopolamine hydrobromide at a single dose (1.2 mg kg^{-1}), a $3 \times 2 \times 2$ (age × drug × rearing environment) ANOVAs were used to test the eventual interactions in the data.

3.2.1. Motor skills

3.2.1.1. Suspended string. There was a significant age effect [$F(2,27) = 20.656$, $P < 0.0001$] but no drug [$F(1,18) = 0.078$, $P > 0.05$] or rearing environmental [$F(1,18) = 0.011$, $P > 0.05$] effects on the latency before falling from the suspended string (Fig. 3A and B). There was also no significant age × drug [$F(2,54) = 0.079$, $P > 0.05$] and drug × rearing environment [$F(1,36) = 0.000$, $P > 0.05$] interactions, demonstrating that the drug had the same effect (i.e. no effect) whatever the age of the animals and their rearing environment. The absence of an age × rearing environment interaction [$F(2,54) = 0.028$, $P > 0.05$] shows that the age effect was similar whatever the rearing environment. Indeed, as depicted in Fig. 3A and B, oldest mice fell sooner from the suspended string than young mice irrespective of the rearing environment and of the substance injected (saline or scopolamine hydrobromide).

3.2.1.2. Wooden beam. There was no significant age [$F(2,27) = 2.848$, $P > 0.05$), drug [$F(1,18) = 1.683$, $P > 0.05$) or rearing environmental [$F(1,18) = 0.014$, $P > 0.05$] effects on the walking speed on the wooden beam (Fig. 3C and D). The lack of drug effect whatever the age and the rearing environment was demonstrated by the absence of age × drug [$F(2,54) = 2.786$, $P > 0.05$] and drug × environment [$F(1,36) = 2.597$, $P > 0.05$] interactions.

3.2.1.3. Hole board. There was a significant age effect [$F(2,27) = 172.063$, $P < 0.0001$], but no drug [$F(1,18) = 0.801$, $P > 0.05$] or rearing environmental [$F(1,18) = 1.455$, $P > 0.05$] effects on the stumbling frequency (Fig. 3E and F). There was also no age × drug [$F(2,54) = 1.419$, $P > 0.05$] and no drug × rearing environment [$F(1,36) = 0.341$, $P > 0.05$] interactions, showing that scopolamine hydrobromide had the same influence (i.e. no influence) whatever the age and the rearing environment.

3.2.1.4. Rotorod: first trial. There were significant age [$F(2,27) = 37.864$, $P < 0.0001$] and rearing environmental [$F(1,18) = 4.616$, $P < 0.05$] effects, but no drug effect [$F(1,18) = 1.361$, $P > 0.05$] on the falling latency (Fig. 4 A–C). There was also no age × drug interaction [$F(2,54) = 0.027$, $P > 0.05$}], demonstrating that the drug had the same effect (i.e. no effect) whatever the age, and no age × rearing environment interaction [$F(2,54) = 0.142$, $P > 0.05$] showing that the effect of age did not depend on the rearing environment.

3.3. Rotorod: learning scores

When considering the number of trials needed to reach the learning criterion, there was an age effect

[$F(2,27) = 170.131$, $P < 0.0001$], a drug effect [$F(1,18) = 20.289$, $P < 0.0001$], but no rearing environmental effect [$F(1,18) = 3.224$, $P > 0.05$] (Fig. 5A and B). However, in young (3-month-old) control and scopolamine-treated mice, the number of trials needed to reach the learning criterion was smaller when they were reared in the enriched environment than in the standard one ($t = 3.416$, $P < 0.01$, and $t = 2.613$, $P < 0.05$, respectively). Such environmental rearing effect was not noted in 12 and 20-month-old animals (t between 0.516 and 1.340, $P > 0.05$). There was an age × drug interaction [$F(2,54) = 12.883$, $P < 0.0001$], showing that the effect of scopolamine hydrobromide depended on the age of the animals, being obvious in the oldest mice (Fig. 5A and B), but no drug × rearing environment interaction [$F(1,36) = 1.509$, $P > 0.05$], demonstrating that the effect of the drug did not depend on the rearing environment. The effect of age did not depend on the rearing environment as shown by the absence of age × rearing environment interaction [$F(2,54) = 1.382$, $P >$

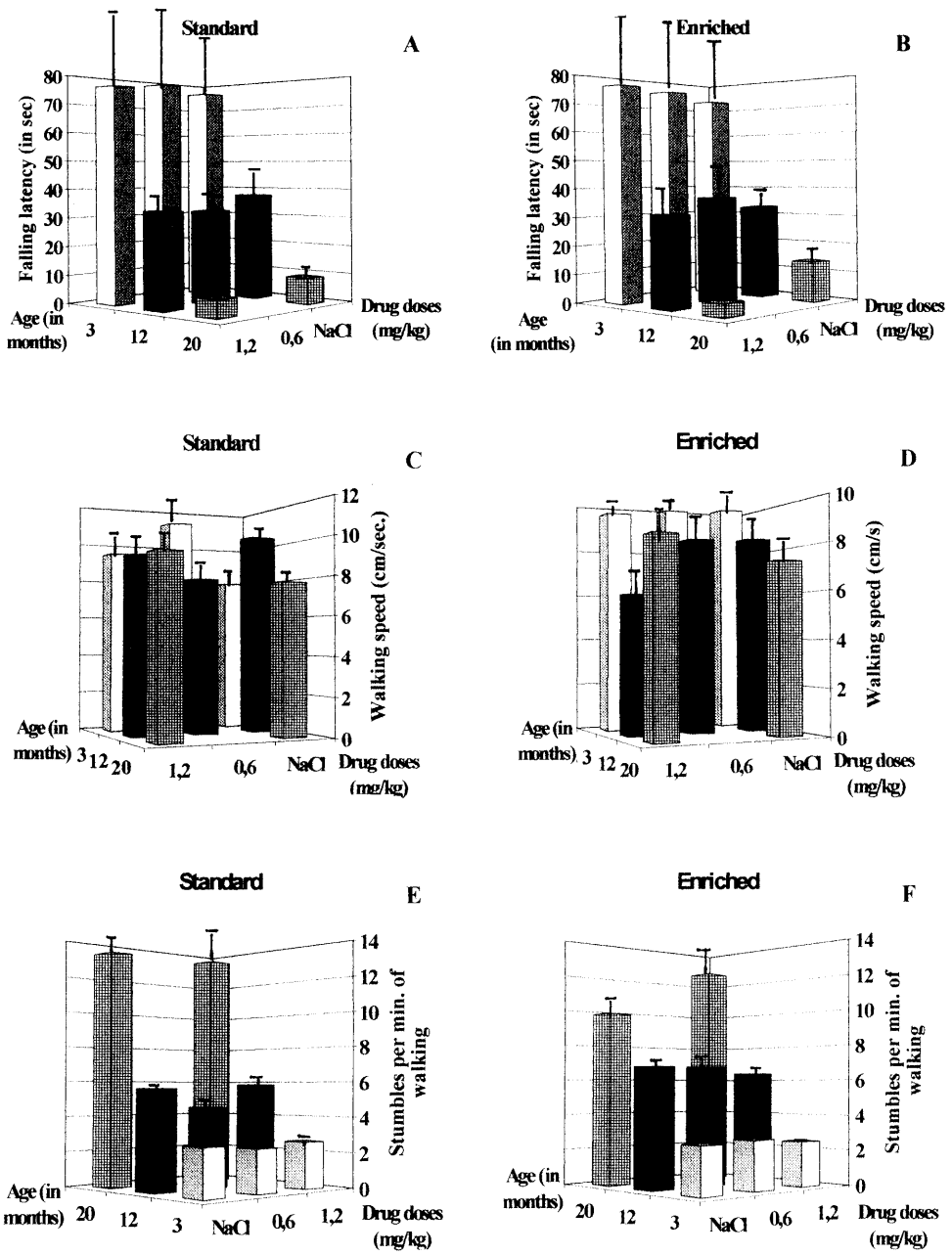

Fig. 3. Suspended string (mean falling latency in s ± S.E.M.; A and B), wooden beam (mean walking speed, in cm s^{-1} ± S.E.M.; C and D), and hole board (mean number of stumbles per min of walking, ± S.E.M.; E and F) scores as a function of age and drug dose in mice reared in standard (left figures) and enriched (right figures) conditions.

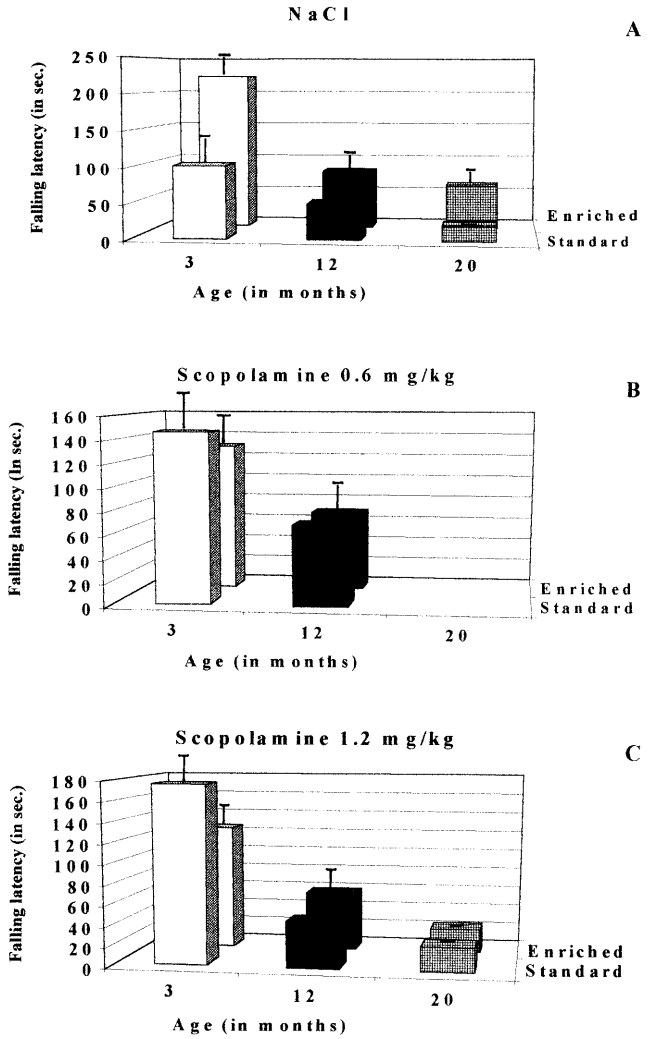

Fig. 4. Rotorod; first trial. Mean falling latency (in s ± S.E.M.) in 3, 12 and 20-month-old mice reared in standard or enriched environments, which have been injected with physiological saline; (A) 0.6 mg kg^{-1}; (B) or 1.2 mg kg^{-1}; (C) scopolamine hydrobromide.

0.05]. All 3- and 12-month-old mice, whatever their rearing environment and whether they received scopolamine hydrobromide or not, were able to reach the learning criterion. The percentage of walking increased from session to session, and, during the last trial, they all walked at least 75% of the time spent on the rotorod. Regarding the learning criterion and the percentage of time spent walking, the 20-month-old mice injected with saline were not greatly different from younger mice. Indeed, only one mouse reared in standard conditions and two mice reared in the enriched environment did not reach the learning criterion; the percentage of walking of these three mice did not increase throughout training and, during the last trial, they walked 20.7 ± 1.7% of the time. The behavior of the 20-month-old mice which were administered scopolamine hydrobromide was quite different — eight animals reared in the standard conditions and seven animals reared in the enriched environment did not reach the learning criterion, and their percentage of walking remained low during the whole training session (21.3 ± 3.7% of the time spent on the rotorod during the last trial).

3.4. Methylscopolamine effects

We compared the scores of 3-month-old mice reared in standard conditions, injected with methylscopolamine and injected with saline. Methylscopolamine had no effect on the latency before falling on the suspended string ($t = 0.103$, $P > 0.05$ Fig. 6A) and on the walking speed on the wooden beam ($t = 0.597$, $P > 0.05$ Fig. 6B). However, it increased the stumbling frequency on the hole board ($t = 5.218$, $P < 0.01$ Fig. 6C) and decreased the number of trials needed to reach the learning criterion on the rotorod ($t = 2.941$, $P < 0.05$ Fig. 6D).

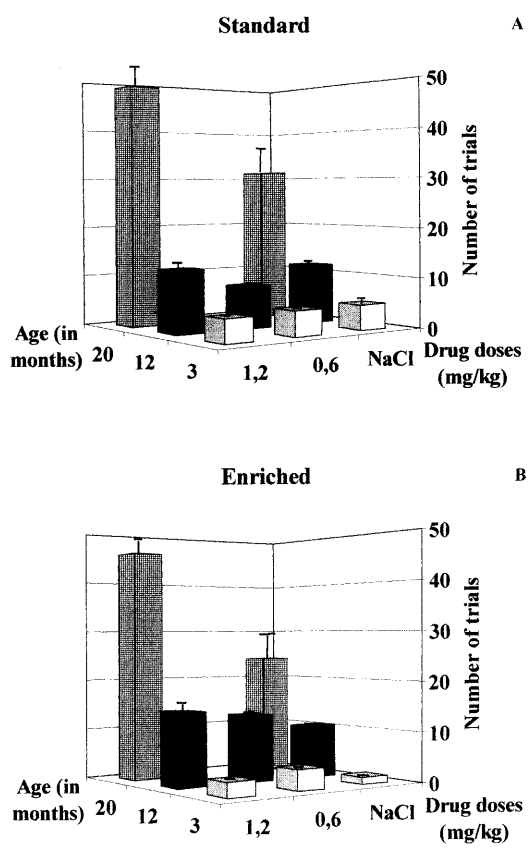

Fig. 5. Rotorod learning procedure. Mean number of trials ± S.E.M., needed to reach the learning criterion in 3-, 12- and 20-month-old mice which have been administered physiological saline, 0.6 or 1.2 mg kg^{-1} scopolamine hydrobromide. The animals were reared in standard (A) or in enriched (B) environments.

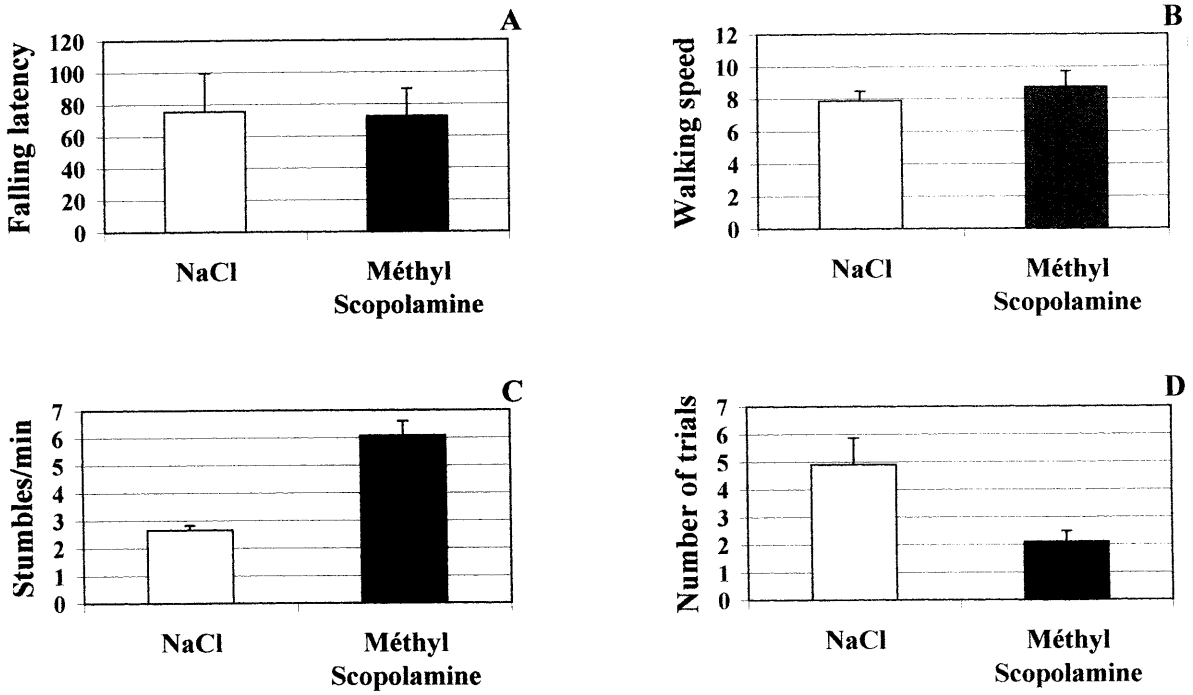

Fig. 6. Mean scores ± S.E.M. of NaCl and 1.2 mg kg^{-1} methylscopolamine-treated mice on the suspended string (falling latency, in s; A), the wooden beam (walking speed, in cm s^{-1}; B), the hole board (number of stumbles per min of walking; C), and on the rotorod (number of trials to reach the learning criterion; D). The mice were 3 months old and reared in standard conditions.

4. Discussion

In both control and scopolamine hydrobromide-treated mice, no correlation between the motor skill scores has been found. This is not surprising since the different tests used, measured different motor abilities — the suspended string measures the muscular strength of the animals, the wooden beam, their ability to keep balance, and the hole board, their capabilities to coordinate locomotion. This lack of correlation is in agreement with the results of a study showing that in Lurcher mutant mice, whose cerebellar cortex is almost completely degenerated and which are highly ataxic, all motor skills are not altered to the same degree, some of them being even preserved [30]. There was also no correlation between motor skill scores and the number of trials needed to reach the learning criterion on the rotorod. This means that the animals which learned the rotorod task quickly were not necessarily those which performed better in the motor skill tests. It can, therefore, be suggested that the rotorod training procedure measures motor learning and not only motor skills, or is insensitive to changes in motor skills as suggested earlier [47].

From the results of this study, it is clear that most of the motor skill scores of control mice decreased with age. The latency before falling from the suspended string was shorter in 12-month-old than in 3-month-old mice, and was even shorter at 20 months of age. This result is in agreement with the fact that the absolute performance of old people in a pinch force task, was inferior to that of young adults [32]. Such a latency decrease could be due to a reduction in muscle mass since it has been shown to be an age-related sarcopenia, at least in humans [45]. However, according to Jubrias et al. [29], atrophy of muscles is not sufficient to explain the strength loss associated with aging. Therefore, an alternative hypothesis would be a decreased efficacy of the mechanisms regulating muscular tone. These mechanisms, which are for a part cerebellar in origin, seem to be inefficient in Lurcher mutant mice whose cerebellar cortex is almost completely degenerated [22,31]. Given that the cerebellar cortex of aged rats is characterized by functional [15] and morphological [40] alterations, it is reasonable to think that decrease of muscular strength is not only muscular in origin but is also due to a dysfunctioning of the regulating mechanisms. Similarly, the stumbling frequency on the hole board increased with age; this lack of motor coordination could probably be explained by tremor which becomes pronounced with aging [24]. Tremor is a cerebellar and a basal ganglia syndrome. It develops after cerebellar lesions in humans and animals and is also a symptom of Parkinson's disease. It could be due, at least in part, to impairments in timing and time perception [42]. The cerebellum is involved in time perception [38] and timing of the movement [7,14,25,35,43,64] together with the basal ganglia [18,41]. Decrease in the

muscular strength and the tremor developed by the animals could well explain why the latency before falling from the rotorod during the first trial (that is before training) also decreased with age. Surprisingly, the walking speed on the wooden beam did not change with age. It can be stated that the animals compensate tremor by walking, as it is easier to maintain balance by walking that by being at rest. A similar phenomenon was observed in Lurcher mutant mice [22,23].

The environmental rearing had no effect on the hanging, wooden beam and hole board scores. However, when the motor task was more difficult, requiring more accurate motor skills, as it was the case on the rotorod, at least during the first trial, the scores were better in the animals reared in the enriched environment than in animals reared in standard conditions. Therefore, providing the task was difficult, enriched rearing increased motor skills more probably by enhancing timing capabilities.

The cholinergic system has been reported to be involved in tonic and phasic components of the movement [50,51]. Indeed, while methyscopolamine, which does not cross the blood-brain barrier and acts exclusively peripherally, had no influence on the suspended string and equilibrium scores, it increased the stumbling frequency on the hole board. Scopolamine hydrobromide, which crosses the blood-brain barrier, had no such effect at the same dosage (1.2 mg kg^{-1}). It can, therefore, be suggested that motor skills are more dependent on the integrity of the peripheral than of the central cholinergic system. However, such action, which is true for the motor tests applied, cannot be generalized. Moreover, to elucidate this issue, intrastructural microinjections of scopolamine should be required.

Motor learning decreased with age, at least between 12 and 20 months of age. Learning abilities of 3 month-old control and scopolamine-treated mice were increased when reared in the enriched environment while those of 12- and 20-month-old mice did not depend on the rearing condition. Therefore, the influence of rearing on motor learning abilities was obvious only in the youngest animals. As the spontaneous motor activity decreases with age [22], 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 be considered as such. Scopolamine hydrobromide decreased the motor learning abilities, such a decrease being even more drastic in aged mice (significant age × drug interaction), while methylscopolamine did not, showing that the central cholinergic system is involved in motor learning and that learning is more dependent on the integrity of the cholinergic system in old mice than in younger ones.

In young mice, administration of scopolamine had no effect on motor learning while aged scopolamine-treated mice were unable to learn the motor task. Moreover, in aged control mice, learning was delayed but not abolished. Therefore, the effect of scopolamine was obvious only in mice which learning abilities were impaired and it is reasonable to think that in aged mice, there was a cumulative effect of scopolamine and aging. If this is the case, one can wonder whether the mechanisms of the impairments due to aging and those due to scopolamine are different or similar. To say that learning deficits are different means that they are mediated by two different systems whose activity was reduced by aging and by scopolamine, respectively. The learning impairments resulting from aging could be a reduced activity of the catecholaminergic systems, as demonstrated by Arnsten et al. [2] and Gould and Bickford [20], those resulting from scopolamine administration being a reduced activity of the cholinergic system. To say that the learning deficits are similar means that they are both mediated by the same system, i.e. the cholinergic system, as it has been shown a cholinergic deficit in aged rats [19], such deficit being due to a neuronal atrophy rather than to a reduction of neuronal number [16].

Whether the hypothesis of the involvement of different mechanisms in the effects of aging and scopolamine in learning abilities cannot be excluded, there are many reasons to think that these two effects can be mediated by similar mechanisms involving the striatum. Indeed, the striatum is involved in motor learning in animals [1,3,4,26,44,59,60] and it is known that in Parkinson's and Huntington's diseases, which are characterized by striatal dysfunction, procedural learning or learning of motor skills is altered [21,48,49]. Besides, it has been shown that the cholinergic striatal system is involved in learning of motor tasks [52,53]. At last, there is a correlation between motor impairments and nigrostriatal dysfunction in aged rhesus monkeys [12] and reduced striatal muscarinic receptor densities in 12–20-month-old rats [8]. It cannot be entirely excluded that the decreased efficacy of training in performances of scopolamine-treated mice were due to a reduction of attention and increase of distractibility [9,27,28,46], to sedation and slowed information processing [63], or/and to an increase of anxiety [55,56]. However, this is unlikely since the scopolamine-treated mice performed as well as control mice in motor skill tests. It can, therefore, be suggested that, in our study, motor learning impairments in aged mice were due to a decrease of the cholinergic striatal activity. The lack of effect of scopolamine on motor learning in young mice could then be explained by the fact that, due to a high density, only a few striatal muscarinic receptors were blocked by the drug. Indeed, when scopolamine was administered at a high dose (1.8 mg kg^{-1}) to 3-month-

old mice, motor learning was impaired since the mice needed a number of trials three times higher than in control mice to reach the learning criterion (Thouvarecq, unpublished results). In the older animals, due to the reduced striatal muscarinic receptor densities [8], the action of scopolamine was more drastic. Therefore, one can think, as suggested above, that the cumulative effects of aging and scopolamine were mediated by a single system, i.e. the striatal cholinergic system. Intrastriatal microinjections of scopolamine could permit to support this proposal.

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