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Protective effect of 5-HT1B receptor gene deletion on the age-related decline in spatial learning abilities in mice

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Abstract

We previously observed that 5 months old serotonin 1B receptor knockout (5-HT1BKO) mice exhibited a facilitation of learning in a long-term spatial memory task in a water maze. In this study, we attempted to assess whether this effect might persist during aging. We compared the performances of young-adult (3 months old) and aged (22 months old) 5-HT1BKO and wild type (WT) mice in the same task. Young-adult and aged KO mice exhibited facilitated acquisition of the reference memory task as compared to their respective WT controls. Generally, the performance of aged KO was similar to that of young-adult WT on the parameters defining performance and motor (swim speed) aspects of the task. During probe trials, all mice presented a spatial selectivity, which was, however, less pronounced in aged than in young-adult WT. No such age-related effect was observed in KO mice. In a massed spatial learning task, aged KO and WT mice globally exhibited the same level of performance. Nevertheless, young-adult and aged KO mice were superior to their WT controls as concerns the working memory component of the task. The data suggest that 5-HT1BKO mice are more resistant than WT to age-related memory decline as concerns both reference/long-term and working/short-term spatial memory.

Keywords: Aging; Learning and memory; Serotonin; 5-HT1B; Knockout mice; Hippocampus; Morris water maze

1. Introduction

Memory capabilities that depend on the functional integrity of the hippocampus appear to be particularly vulnerable to the aging process [13]. Pharmacological strategies currently being pursued in Alzheimer's disease (AD) research aim to attenuate memory deficits which have been primarily attributed to the progressive degeneration of the basal forebrain cholinergic projections [27,31]. Age-induced cognitive dysfunctions are correlated to a combination of disturbances in both cholinergic and serotonergic functions [21,25]. The functional interaction between these neurotransmitter systems plays an important role in learning and memory performance [11,28]. Concurrent loss of cholinergic and serotonergic transmission has been reported to result in persistent memory impairments in rat, as compared to the loss of cholinergic transmission alone [19]. Intrahippocampal cografts of fetal cholinergic and serotonergic neurons are able to reduce impairments in long-term spatial memory induced by extensive lesions of the dorsal septo-hippocampal pathway [3].

The 5-HT1B receptor can be considered as a key target by which 5-HT is able to modulate hippocampal-dependent learning and memory [7,8]. Rats receiving a stimulation of hippocampal (CA1) 5-HT1B receptors were found to be impaired in a spatial learning task, exhibiting more reference than working memory errors [9]. These data suggest that the inactivation of 5-HT1B receptors might have positive effects on hippocampal functions.

The lack of availability of specific 5-HT1B antagonists has led us to adopt a molecular biological strategy for studying the implication of 5-HT1B receptor in cognitive functions, by using 5-HT1B receptor knockout (5-HT1BKO) mice [26] as subjects in different hippocampal-dependent and -independent learning tasks [18]. In the (classical) long-term spatial version of the Morris water maze, adult 5-HT1BKO mice showed facilitation in learning the task, as compared to their wild type (WT) controls, but this effect was not observed in a massed spatial learning assessing more short-term memory [18]. Furthermore, 5-HT1BKO

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did not differ from WT in contextual fear conditioning. 5-HT1BKO mice also exhibit longer periods of paradoxical sleep (without change in wakefulness or slow-wave sleep) as measured during a 12/12 h light/dark cycle [5], a result which, given the facilitatory influence of paradoxical sleep on memory consolidation [15], is in accordance with our previous behavioral results.

The aim of the present study was to evaluate whether the facilitation observed in adult 5-HT1BKO mice in long-term spatial memory performance would persist during aging. In other words, we wanted to determine whether age-related alterations in learning performance would benefit from inactivation of the 5-HT1B receptor. The subjects of the study were young-adult and aged 129/Sv mice. We focused our behavioral study on spatial learning in the Morris water maze, which was previously used to reveal the facilitatory effect of the mutation in adult mice and which is considered as being particularly sensitive to age-associated memory impairment [14]. Two basic spatial tasks were used to assess both long-term and short-term aspects of learning and memory capabilities of mice. A visually guided orientation task was also used to assess visual and sensori-motor abilities and especially to control for the existence of any visual defect in aged mice.

2. Materials and methods

2.1. Animals

The subjects were male 129/Sv WT and homozygous 5-HT1BKO (generation detailed in [26]) mice. On receipt from the breeding colony (Laboratoire de Transgénèse, Université de Bordeaux 2), mice were housed individually in standard transparent laboratory cages (26 cm × 12 cm × 14 cm) in a temperature-controlled (22±1 °C) colony room, adjacent to the experimental room. They were provided with food and water ad libitum, and maintained on a 12/12 h light/dark artificial cycle (lights on at 06:00 h). They were tested during the light phase between 10:00 and 17:00 h. At the beginning of the experiments the subjects were 3 months old (young-adult, Y, WT (Y-WT, $n = 12$) and KO (Y-KO, $n = 12$)) and 22 months old (aged, A, WT (A-WT, $n = 9$) and KO (A-KO, $n = 9$)). One week before the beginning of the experiments, the mice were handled and weighed each day by the experimenter. The genotype of each mouse was controlled at the end of the experiments. All experimental procedures were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Apparatus

The apparatus was a white circular tank (140 cm in diameter, 40 cm in height). It was located in a room with various distal cues and uniformly illuminated by a halogen lamp. The tank was filled with water (30 cm depth) maintained at 22 °C, and was rendered opaque by the addition of a non-toxic white paint (Pebeo, Gemenos, France). Located inside the pool was a removable circular (13 cm in diameter) Plexiglass platform positioned such that its top surface was 0.5 cm below the surface of the water. Data were collected using a video-camera fixed to the ceiling of the room and connected to a video-tracking system (Videotrack, Viewpoint, Lyon, France) located in an adjacent room.

2.3. General behavioral procedures

2.3.1. Pretraining

During each of the 3 days preceding the learning phase proper, each mouse received a pretraining session in order to acquire the procedural aspects of the task (see [18]).

2.3.2. Learning

Each mouse was submitted to daily sessions comprising either four or six trials (four trials for the reference memory learning, six trials for the massed learning). Each trial consisted of releasing the mouse into the water facing the outer edge of the pool at one of the virtual quadrants (except the quadrant where the platform was located), and allowing it to escape to the platform. A trial terminated when the animal reached the platform where it was allowed to remain for 15 s. During the earlier stage of learning, the few mice that failed to find the platform before 90 s elapsed, were invited to follow the finger of the experimenter which indicated the location of the platform, where they were allowed to remain for 15 s. At the completion of a trial, mice were removed from the pool and placed back to their home-cages which were placed beneath heat lamps to reduce core temperature loss, in the adjacent room.

2.4. Visually guided orientation task

On the day following the last pretraining day (day 1), each mouse performed a visually guided orientation session, i.e. a series of four trials in which a visible black cylinder (4 cm in diameter, 7 cm in height) was placed on the top of the submerged platform. Before the first trial only, the mouse was first placed for 15 s on the platform. For each trial, the platform location and the releasing points were different.

2.5. Spatial reference memory learning

The experiment was continued using a hidden platform to evaluate spatial reference memory performances. It was composed of two main steps: (1) learning (9 days: days 2–10), with the escape platform located in the center of the north quadrant; and (2) reversal (5 days: days 11–15), with the platform located in the center of the east quadrant. Before the first trial of the first session (day 2) only, the mouse was placed for 15 s on the platform. On the last day of learning (day 10) and of the reversal (day 15), the mouse was given

a fifth trial which was a probe trial. This trial consisted of letting the mouse swim in the pool for a fixed duration (90 s) while the platform was removed. The releasing point differed at each trial (for example, east, west, south, and east if the platform was located in the north quadrant), and different sequences of releasing points were used from day to day. WT and 5-HT1BKO mice ran alternately within each age group by squads of 10–11 mice; thus the duration of the inter-trial interval was approximately 15–20 min.

2.6. Massed spatial learning

Six weeks later, the same mice were submitted to a 5-day massed spatial learning (short-term repeated learning). The hidden platform was placed in a new location each day and the mouse had to reach it on six successive trials from different counterbalanced starting positions. The positions of the platform were different from those used in the former task. The first daily trial was preceded by an exposure trial (15 s on the platform). The mice were run as squads of three subjects (3–4 min inter-trial interval).

2.7. Data collection and analysis

The movements of the subjects were recorded using the Videotrack system as previously described [18]. The data were processed using Excel (Microsoft). This processing, applied on a given trial, allowed to calculate parameters of the performance of the mice: (1) the escape latency, i.e. the time required to escape to the platform from the releasing point (s); and (2) the path length, i.e. the distance traveled by the mouse until it reaches the platform (cm), a measure of accuracy. Finally, mean swim speed (path length/latency, cm/s) was calculated as a locomotor factor which might interfere with latency, and which can be considered as a basic behavioral component associated with aging. Genotype (WT versus KO) and age (young-adult, aged) were the between-subject factors, trial and day were the main within-subject factors of the ANOVA (using StatView 5.01, SAS Institute Inc.). Post-hoc analyses of individual group comparisons, using the Scheffe test ($P < 0.05$) was used when the effect of the main factors age and genotype were significant.

For the spatial reference memory learning, performance was evaluated across days (averaged over the four trials). We analyzed separately learning (days 2–10) and reversal (days 11–15) stages. Particular analyses were conducted, as for the probe trials, with the time spent (1) in the different quadrants (QUADRANT); or (2) in the exact platform zone (or equivalent virtual areas in the other quadrants: PLATFORM). ANOVAs were performed on these data with the genotype, the age and area (four zones of the same type: QUADRANT or PLATFORM) as the main factors.

For the massed spatial learning, performance was evaluated across trials (averaged over the 5 days). Two separate analyses were performed. In order to assess the working memory component of the task, we compared the difference in performance between trials 1 and 2 [23,29]. The accompanying (short-term) reference memory component of the task was analyzed across trials 2–6.

3. Results

3.1. Visually guided orientation task (day 1)

Both WT and KO mice, independent of their age, rapidly learned to locate the platform across the four successive trials in a relatively efficient manner (trial effect, path length, $F(3, 114) = 12.03$; $P < 0.0001$). All mice swam directly to the cued platform on the fourth trial in 7 s on average.

3.2. Spatial reference memory learning (days 2–15)

3.2.1. Learning stage (days 2–10)

All groups of mice learned the task and exhibited decreasing escape latencies across successive days (day effect, $F(8, 304) = 50.79$; $P < 0.0001$) as shown in Fig. 1A (days 2–10). ANOVA revealed significant effects of age ($F(1, 38) = 120.51$; $P < 0.0001$) and genotype

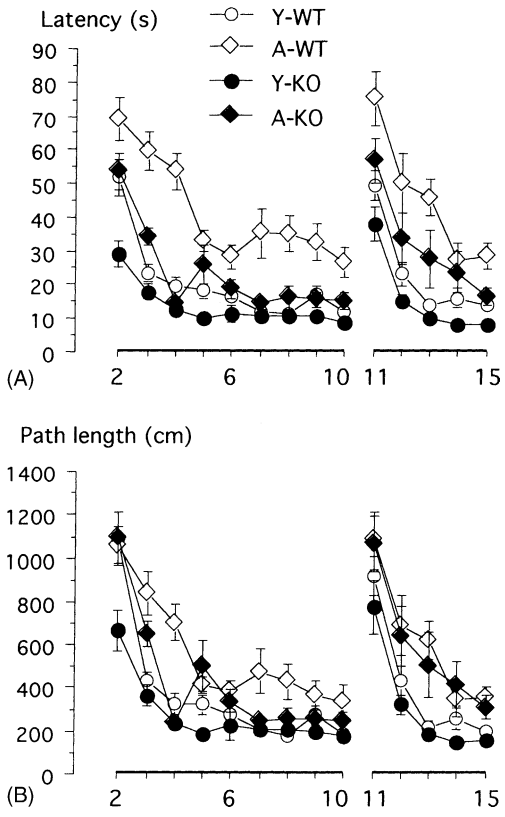

Fig. 1. Spatial reference memory learning. Performance across days 2–15 during the learning (days 2–10) and the reversal (days 11–15) stages of young-adult and aged WT (Y-WT and A-WT, respectively) and 5-HT1BKO (Y-KO and A-KO, respectively) mice. Mean (±S.E.) latency (s) to reach the platform (A), mean (±S.E.) path length (cm) (B).

($F(1, 38) = 77.82$; $P < 0.0001$). Young-adult mice exhibited shorter latencies than aged mice, as well as KO mice as compared to WT mice. The only non-significant between-group difference was due to the comparison between young-adult WT and aged KO mice. The distance covered by the mice to reach the platform decreased across days in all groups (Fig. 1B, effect of day, $F(8, 304) = 63.34$; $P < 0.0001$). ANOVA revealed significant effects of age ($F(1, 38) = 48.38$; $P < 0.0001$) and genotype ($F(1, 38) = 20.04$; $P < 0.0001$). Young-adult mice covered shorter distances than aged mice, as well as KO mice as compared to WT mice. The only non-significant between-group difference was due to the comparison between young-adult WT and aged KO mice.

3.2.2. Probe trial on day 10

All mice exhibited a bias in favour of the quadrant where the platform was previously located (QUADRANT effect, $F(3, 114) = 169.90$; $P < 0.0001$, Fig. 2A). When considering a more selective area, i.e. PLATFORM, the same overall preference was observed ($F(3, 114) = 121.84$; $P < 0.0001$, Fig. 2B), associated with significant age ($F(1, 38) = 13.39$; $P = 0.0008$), age × genotype ($F(1, 38) = 4.56$; $P = 0.04$), and age × area ($F(3, 114) = 8.62$; $P < 0.0001$) effects, but with a lack of genotype effect. This age effect was not due to KO mice ($F(1, 19) = 1.17$; $P = 0.29$) but to WT mice ($F(1, 19) = 16.63$; $P = 0.0006$; age × platform area, $F(3, 57) = 6.60$; $P = 0.0007$), with young-adult WT mice being more selective than aged WT mice.

3.2.3. Reversal (days 11–15)

The relocation of the platform induced a deficit concerning all parameters of performance in all groups of mice (Fig. 1A and B, day 11), which was compensated across subsequent days.

ANOVA conducted on escape latencies revealed a significant effect of day ($F(4, 152) = 75.39$; $P < 0.0001$), attesting to relearning (Fig. 1A, days 11–15). The analysis also revealed significant effects of age ($F(1, 38) = 32.38$; $P < 0.0001$) and genotype ($F(1, 38) = 10.09$; $P = 0.003$). Young-adult mice exhibited shorter latencies than aged mice, as well as KO mice as compared to WT mice in young-adult ($P = 0.0009$), but not significantly in aged ($P = 0.08$) mice. Path lengths decreased across days in all groups (Fig. 1B). ANOVA revealed significant effects of day ($F(4, 152) = 69.25$; $P < 0.0001$) and age ($F(1, 38) = 17.61$; $P = 0.0002$), but not genotype ($F(1, 38) = 1.14$; $P = 0.29$). Young-adult mice covered shorter distances than aged mice, as well as KO mice as compared to WT mice in young-adult ($P = 0.04$), but not in aged ($F < 1$) mice.

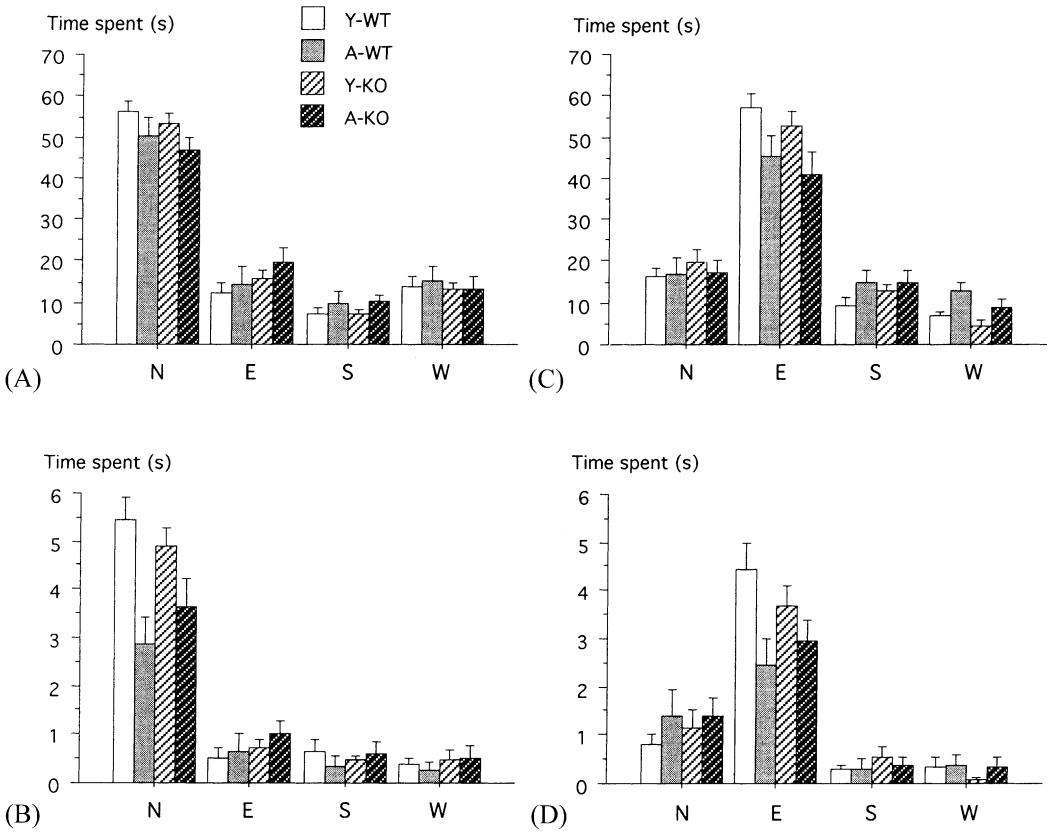

Fig. 2. Probe trials on days 10 (A, B) and 15 (C, D). Mean (±S.E.) time spent (s) in the different quadrants (A, C), and in PLATFORM (B, D). Y-WT, A-WT, Y-KO, A-KO (see Fig. 1) N, north; E, east; S, south; W, west.

3.2.4. Probe trial on day 15

All mice spent significantly more time in zones defining the more recent location of the platform as opposed to other equivalent zones (QUADRANT effect, $F(3, 114) = 130.99$; $P < 0.0001$, Fig. 2C; PLATFORM effect, $F(3, 114) = 70.71$; $P < 0.0001$, Fig. 2D). For these two measures, the analysis revealed significant effects of the interactions age × QUADRANT ($F(3, 114) = 5.59$; $P = 0.001$) and age × PLATFORM ($F(3, 114) = 5.09$; $P = 0.002$), which concerned WT mice only (age × QUADRANT ($F(3, 57) = 3.36$; $P = 0.02$); age × PLATFORM ($F(3, 57) = 4.51$; $P = 0.007$)), with young-adult WT mice being more selective than aged WT mice.

On both learning and reversal stages (days 2–15), mice differed in swim speed according to the age ($F(1, 38) = 28.32$; $P < 0.0001$) and the genotype ($F(1, 38) = 63.15$; $P < 0.0001$). Young-adult mice swam faster than aged mice, as well as KO mice as compared to WT mice. Interestingly, this genotype effect affected more aged mice than young-adult mice (overall percent difference between WT and KO: young-adult = −19%; aged = −38%). The two non-significant between-group differences were due to the comparisons between young-adult WT and aged KO mice, and young-adult KO and aged KO mice.

3.3. Massed spatial learning

The first to second trial improvement was evaluated to assess working memory. The second to sixth trial improvement was evaluated to assess short-term reference memory.

3.3.1. Working memory component (trials 1–2)

Mice reduced their latencies from trials 1 to 2 ($F(1, 38) = 32.07$; $P < 0.0001$, Fig. 3A). ANOVA revealed neither significant age ($F(1, 38) = 1.95$; $P = 0.17$), nor genotype ($F < 1$) effects, but a significant genotype × trial effect ($F(1, 38) = 5.30$; $P = 0.03$). The percent decrease in latency was higher in KO mice (young-adult = −33%, aged = −27%) than in WT mice (young-adult = −14.5%, aged = −13%). Mice decreased their path lengths between trials 1 and 2 ($F(1, 38) = 17.43$; $P = 0.0002$, Fig. 3B). ANOVA revealed significant effects of age ($F(1, 38) = 10.72$; $P = 0.002$), genotype ($F(1, 38) = 5.63$; $P = 0.02$), and genotype × trial, ($F(1, 38) = 8.26$; $P = 0.007$). KO mice improved their performance between trials 1 and 2 ($F(1, 19) = 29.45$; $P < 0.0001$), but not WT mice ($F < 1$). The percent decrease in path length was higher in KO mice (young-adult = −34%, aged = −26.5%) than in WT mice (young-adult = −12.5%, aged = −1%).

3.3.2. Short-term reference memory component (trials 2–6)

Mice improved their performances across trials 2–6 ($F(4, 152) = 35.07$; $P < 0.0001$, Fig. 3A). Young-adult mice exhibited shorter latencies than aged mice (age effect, $F(1, 38) = 19.50$; $P < 0.0001$; age × trial interaction ($F(4, 152) = 3.10$; $P = 0.02$). KO mice performed better

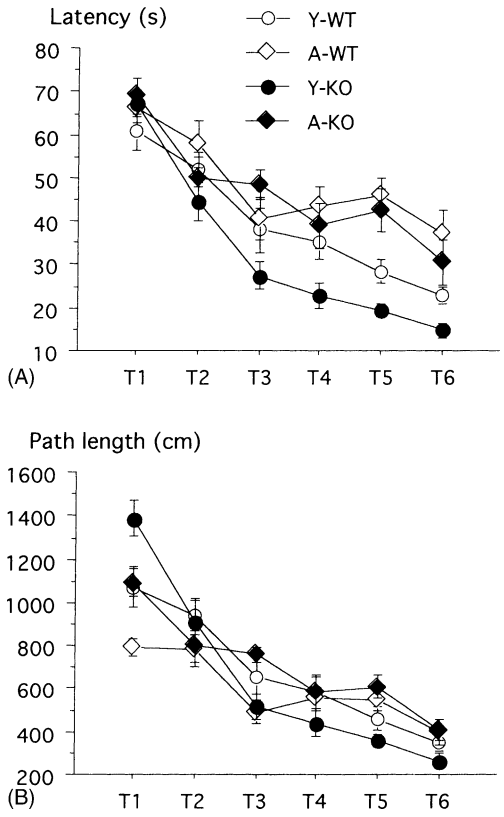

Fig. 3. Massed spatial learning. Performances across trials (T1–T6) averaged over the 5 days as measured by the mean (±S.E.) latency (s) to reach the platform (A), mean (±S.E.) path length (cm) (B). Y-WT, A-WT, Y-KO, A-KO (Fig. 1).

than WT mice (genotype effect, $F(1, 38) = 4.46$; $P = 0.04$). This genotype effect was revealed in young-adult ($F(1, 22) = 8.22$; $P < 0.009$) but not in aged ($F(1, 16) < 1$) mice. Mice improved their performances by shortening their path length across trials ($F(4, 152) = 41.13$; $P < 0.0001$, Fig. 3B). ANOVA revealed no significant effects of age ($F(1, 38) = 1$), or genotype ($F(1, 38) < 1$), but a significant effect of the interaction age × genotype ($F(1, 38) = 4.27$; $P = 0.05$). Young-adult mice exhibited shorter path lengths than aged mice in KO ($F(1, 19) = 7.34$; $P = 0.01$), but not in WT ($F(1, 19) < 1$), and KO mice did not perform better than WT in neither young-adult ($F(1, 22) = 2.42$; $P = 0.13$), nor aged ($F(1, 16) = 2.37$; $P = 0.14$) mice.

In this task, again, swim speed was associated with significant effects of age ($F(1, 38) = 37.58$; $P < 0.0001$) and genotype ($F(1, 38) = 24.64$; $P < 0.0001$). Young-adult mice swam faster than aged mice, and KO mice swam faster than WT mice. The only non-significant between-group difference was due to the comparison between young-adult WT and aged KO mice.

4. Discussion

The main results of the present study show that the facilitatory effect of the 5-HT1B receptor gene deletion previously

observed in young-adult mice [18] on long-term spatial learning performance generalizes to aged (22 months old) mice. The massed learning task also provided evidence for a similar facilitation, but in the working memory component of the task, only. In the short-term reference memory component of the task, an age effect was observed in KO mice only (with aged KO exhibiting lower performance than young-adult KO mice). The effects of the mutation might reflect an influence of the deletion on genes proximal to the 5-HT1B receptor locus, or a direct influence of the specific gene deletion per se, with possible effects of developmental plastic changes.

4.1. Non-mnemonic factors may contribute to the phenotype of 5-HT1BKO mice

One of the effects of aging is to decrease sensorimotor capabilities, which are particularly important as being the main "instrumental" supports of spatial navigation. In the present study, KO mice exhibited higher swim speeds than WT mice. This may constitute one aspect underlying the reduced age-related decline observed in KO mice, and this was demonstrated considering escape latency in the reference memory task. Although this factor may interfere with rapidity of spatial orientation, it does not interfere with performance accuracy as revealed by the measure of path length. Moreover, despite this overall difference in swim speed, the results of the massed learning protocol did not reveal differences in performance between aged WT and aged KO as measured by the escape latency. Our results also showed that, whatever their genotype or age, mice exhibited perfectly direct trajectories to the cued platform during the fourth trial of the visually guided test. Thus, visual and procedural aspects of spatial learning cannot alone account for genotype- or age-related differences in spatial learning performance and strategy.

4.2. 5-HT1BKO mice are more resistant to age-related memory decline than wild types

In general, aged mice differed significantly from young-adult mice by their reduced swim speed. Reduced locomotor speed is one of the symptoms of normal aging, but age-related alterations of cognitive capabilities are generally found to be linked only to a moderate extent to age-related differences in motor or locomotor activity (e.g. [30]). In the present study, aged mice were found to be able to exhibit correct retention of the region (QUADRANT) where the platform was recently located at the end of learning and reversal stages, but, in WT only, they were not as accurate as young-adult mice for searching in the exact platform location. These results are in agreement with those observed for rats in the same task [14,17,24].

Strain differences influence the rate of age-related decline in learning and memory in rats and mice [16,33]. The C57BL/6 strain presents preserved cognitive abilities during senescence [2]. In particular, it does not exhibit major age-related deficits in spatial learning as assessed in the reference memory version of the water maze [10], an observation which contrasts with our results. The present study indicates that the 129/Sv strain constitutes a good mouse model of cognitive aging, which may be useful in the framework of growing interest for genetic engineering [16,22,32].

As shown by the evolution of performance, our study clearly demonstrates that the absence of 5-HT1B receptors attenuates the age-related decline in learning a spatial navigation task as assessed in the reference memory version of the Morris water maze. However, this facilitation was not preserved when the mice had reached an asymptotic level of performance. After relocation of the platform, which can be considered as a relearning, aged KO and WT mice did not differ in their performance as they did during the acquisition stage. Performance related to the "working" memory component of the massed spatial learning again demonstrated the superiority of KO mice in solving a short-term spatial matching-to-sample task. However, in contrast to the long-term reference memory task, results assessing the short-term "reference" memory component during massed learning showed that the performance of aged WT and KO mice did not differ. Furthermore, this task is not sensitive to age-related performance decline in WT mice, so one may ask the question as to why aged KO mice showed reduced performance as compared to young-adult KO mice.

The reference memory version of the water maze task reveals larger age-related deficits in rats than a working memory version of the task [17], a result which is consistent with our present results in mice. Furthermore, if the reference memory task reveals a quasi linear function for age-related performance decline (from 11 to 24 months of age), the massed learning (or repeated acquisition) task reveals deficits only in the oldest group of rats [14]. In our study, we did not observe any age-related change in performance of WT mice in this test. As for rats, this test has a lower capacity for discriminating age-related impairments. One aspect of this task is linked to the reduction of the inter-trial interval which facilitates learning and which, as a possible consequence, reduces cognitive demand.

4.3. Effects of the 5-HT1B receptor gene mutation: integrating behavior and neurobiology

The facilitatory effect of deletion of the 5-HT1B receptor on spatial learning was clearly demonstrated in aged mice which exhibit impairments in a task with high cognitive demand, such as the reference memory task. This effect was not found to be so great in the short-term version of the task. This dissociation supports the idea that serotonin, via 5-HT1B receptors, may play a crucial role in memory tasks involving a high cognitive demand [8]. It was previously demonstrated that the mutation did not interfere with spontaneous alternation (a recognized model of working memory), with cue and contextual fear conditioning [18], nor

with the acquisition of an operant food-reward conditioning [6].

The facilitatory effect of the mutation may result from different neurobiological mechanisms. The blockade of 5-HT1B receptors located on medial septal cholinergic terminals might facilitate cholinergic transmission in the hippocampus [12,20], and thus the acquisition of spatial learning. The blockade of 5-HT1B receptors located on glutamatergic CA1-subiculum terminals [1,4] might facilitate the transfer of processed information from the hippocampus to cortical areas and thus might participate in long-term memory storage and consolidation mechanisms.

5. Conclusions

The present study demonstrated that the 5-HT1B gene deletion is associated with an improvement in long-term spatial learning which is evidenced in young-adult as well as aged mice. This improvement is especially interesting in the context of age-dependent memory dysfunctions. The effect of the deletion depends on the type of task, which entails different levels of cognitive demand. The effect of the mutation cannot be directly interpreted within the context of the possible pharmacological efficacy of 5-HT1B antagonists, but these drugs, which may interact with serotonin–acetylcholine cross-talk pathways, may be investigated as interesting compounds for future research on the neurobiological mechanisms of age-associated memory decline.

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