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Research report

The double-H maze test, a novel, simple, water-escape memory task: Acquisition, recall of recent and remote memory, and effects of systemic muscarinic or NMDA receptor blockade during training

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ABSTRACT

To explore spatial cognition in rodents, research uses maze tasks, which differ in complexity, number of goals and pathways, behavioural flexibility, memory duration, but also in the experimenter's control over the strategy developed to reach a goal (e.g., allocentric vs. egocentric). This study aimed at validating a novel spatial memory test: the double-H maze test. The transparent device made of an alley with two opposite arms at each extremity and two in its centre is flooded. An escape platform is submerged in one arm. For experiments 1–3, rats were released in unpredictable sequences from one of both central arms to favour an allocentric approach of the task. Experiment 1 (3 trials/day over 6 days) demonstrated classical learning curves and evidence for recent and nondegraded remote memory performance. Experiment 2 (2 days, 3 trials/day) showed a dose-dependent alteration of task acquisition/consolidation by muscarinic or NMDA receptor blockade; these drug effects vanished with sustained training (experiment 3; 4 days, 3 trials/day). Experiment 4 oriented rats towards a procedural (egocentric) approach of the task. Memory was tested in a misleading probe trial. Most rats immediately switched from response learning-based to place learning-based behaviour, but only when their initial view on environmental cues markedly differed between training and probe trials. Because this simple task enables the formation of a relatively stable memory trace, it could be particularly adapted to study consolidation processes at a system level or/and the interplay between procedural and declarative-like memory systems.

1. Introduction

Spatial cognition, which relies upon declarative memory in humans, can be weakened in elderly (e.g. [1,2]), and is markedly altered in Alzheimer's disease (AD) patients (e.g. [3–6]). Similar alterations have been induced in laboratory rodents by lesions of selected brain regions or transmitter systems, or by the administration of drug treatments activating or blocking receptors of interest, among which cholinergic muscarinic receptors and glutamatergic NMDA receptors have awaked much interest (e.g. [7]). Spatial memory deficits have also been characterized in aged mice and rats, as well as in a variety of transgenic mice developed to reproduce one or more of the neurodegenerative features or histopathological signatures of various diseases (e.g. [8,9]), or in which essential steps of learning-triggered intracellular signaling pathways have been knocked-out (e.g. [10–12]).

To characterize spatial memory in rodents, research usually assesses the effects of experimental treatments in a variety of maze tasks (e.g. [13,14]). In these tasks, animals may achieve good performance by using strategies based on their acquired knowledge of the salient landmarks of their testing environment (a so-called "allocentric" strategy) or on bodily cues becoming central for the organization of displacements (a so-called "egocentric" strategy). These tasks can also be distinguished according to the degree of flexibility with which an animal may try to solve them. In some of them (e.g., the Stone maze), flexibility is weak: the task consists in acquiring the only correct route connecting a start point with a goal, leaving no space to alternative strategies or short cuts, reducing the spatial load on memory function in a training level-dependent manner, and facilitating the emergence of cognitive routines or motor response-based automatisms. In other tasks leaving more room to flexibility, such as Olton's radial maze [40] or the ziggurat maze [15] – formerly called the cone field task [16] – there are several goals, food (or other rewards) being provided at various locations. Good performance may be achieved by an allocentric or an egocentric strategy. In the largely used Morris water maze (e.g.

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[17]), a dry version of the latter (e.g. [18]) or the Barnes maze (e.g. [19]), animals have to learn a given location to which they have to navigate. Although the item to be learned and remembered is single, the search patterns and routes to this location are virtually infinite. Even with such tasks, animals do not necessarily use an allocentric strategy. Indeed, in e.g. the water maze, rats and mice can reach the escape point by swimming in circle along the pool border at about the distance from the border at which the platform is placed: they can know how to reach the platform without knowing precisely where it is immersed [20].

The problem with the tasks in which a specific route must be learned is that animals can solve them without having to use a spatial memory. In many if not all of the others, the problem is less that animals can solve them with alternative strategies, and thus without having to form a spatial memory, than the fact that the experimenter has no or relatively poor control over which spontaneous strategy an animal is going to develop during training. In addition, the allocentric solution to these tests, which are often used to screen the effects of cognition-enhancing drug candidates in preclinical approaches, requires relatively complex mental processing; if one goes back to the notion of model, especially of human memory systems, it is noteworthy that not all of our daily behaviours rely upon such complex operations. Under some instances, it might be interesting to know the effects of cognition-enhancing drug candidates on relatively simple behaviours. Regarding the aforementioned drawbacks on the use of alternative strategies, on the lack of control by the experimenter of an animal's strategy and on task complexity, the recently introduced starmaze (e.g. [21,22]) appears an interesting compromise as, being a relatively simple navigation task preventing possible deviations from an ideal start-to-goal trajectory, it enables an extremely fine a posteriori analysis of an animal's spontaneous strategy during a retention test. However, as in the other tasks, the experimenter still has limited control over the strategy used by the animal to achieve good performance; for instance, mice can be forced into procedural routines, but the protocol is based on using a mobile goal, which is not very "ecological". We therefore conceived a novel test device, which we call the *double-H maze*, and in which rodents have to learn to reach the location of an escape platform submerged in water, but the pathway possibilities from the start to the goal are limited to a reasonably low number and training may be adapted such as to shape an allocentric or an egocentric strategy.

2. Materials and methods

2.1. Subjects

For the currently reported four experiments, we used a total of 219 adult, male Long-Evans rats weighing between 240 and 268 g at the start of each experiment. They were provided by the Centre d'Elevage R. Janvier, Le Genest St-Isle, France. All rats were kept in individual transparent Makrolon cages (42 cm × 26 cm × 15 cm) in temperature-controlled (23 ± 1 °C) rooms that were maintained on a 12: 12 h dark–light cycle (light on at 7:00 AM). All rats were housed with *ad libitum* access to food and water throughout the experiment. All procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with the national council directive no. 87848, October 19, 1987, *Ministère de l'Agriculture et de la Forêt*: (C-67-482-13), the French law on researcher agreement (licence 67-215 to JCC; other authors under the former's responsability), and international (NIH publication no 85-23, revised 1985) laws and policies. All efforts were made to minimize the number of animals used with respect to statistical constraints.

2.2. The double-H maze test

Regarding the device, the principle of the test and the possible protocols, we aimed at setting up a test that, compared to all existing ones, would (i) assess spatial memory, (ii) be simple enough to be learned in a rather short period of training or, using sustained training, under conditions of moderate neuronal dysfunctions, (iii) enable a control over the strategy that an animal may develop to solve the task (i.e., maintain the animal in a strategy based on declarative-like memory vs. a procedural memory-based strategy), (iv) establish a memory that would result in a minimal performance decline over time, as usually seen in fear conditioning tasks, but more seldom in tasks assessing spatial memory in the absence of an overtraining program.

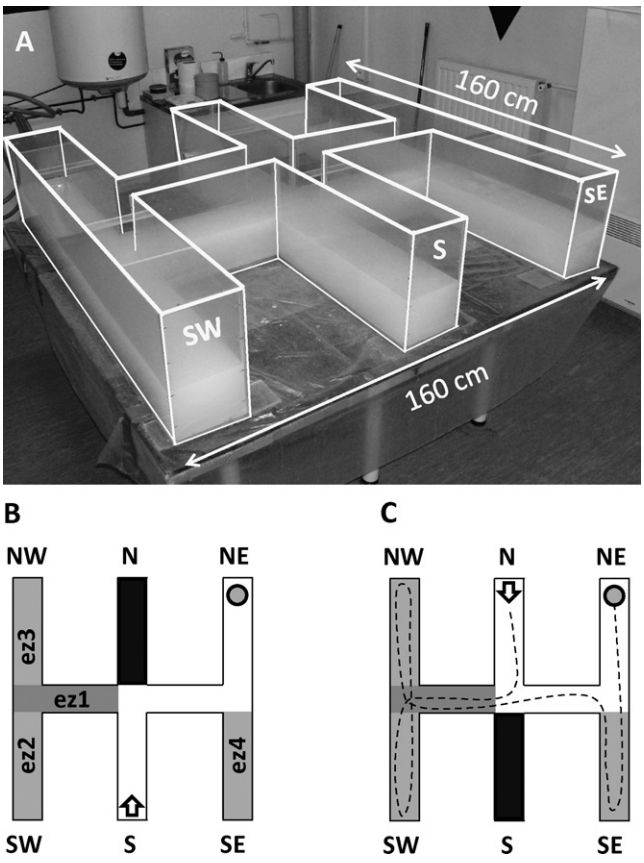

Fig. 1. The double-H maze. (A) Photograph showing the general aspect of the maze. The edges have been redrawn in white to generate a more contrasted illustration. The maze, holding in a square of 160 cm × 160 cm, has an internal surface of 10,949 cm^2. For the photograph, it has been filled with opaque water to about 14 cm in height. (B) Bird view drawing of the double-H maze, on which the error zones (ez1–ez4) are indicated in grayish. In this example, the error zones are defined according to a task in which the rat is released from the S arm, from where it has to swim to a platform submerged at the extremity of the NE arm (O). The arm facing the start arm has been filled in black to indicate that it is closed. To close it, we use a transparent guillotine door. (C) Same drawing as in B, but with a start from the N arm (and this time the S arm is closed) and modification of the error zones according to the new start point. The hatched line indicates a theoretical example of a rat's swim path in which 4 errors are counted (ez1, ez3, ez2 and ez4) before the platform is reached; all these errors are counted as initial errors as none of them was repeated.

2.2.1. Apparatus

In a bird view, the general layout of the apparatus roughly corresponds to the shape of two contiguous Hs. It is made of three parallel run arms, 160 cm in length and 20 cm wide, connected to each other at the level of their centre by a perpendicular one (see Fig. 1A). Each of these arms is equipped with side walls, 35 cm high. By convention, the intermediate arm's extremities are designed as north (N) and south (S), respectively. One or alternatively both of these extremities are used as start arms in our basic training protocols (see below). The extremities of both other side arms are corresponding to our four potential target locations. They are termed north-west (NW), north-east (NE), south-west (SW) and south-east (SE) hereafter (see Fig. 1A–C). All elements of the apparatus are made of transparent Plexiglas. They have been glued and subsequently screwed to each other, and all joints are waterproofed by application of silicone joints in all internal angles. The double-H is placed on a table, 80 cm from the floor, in a large room with well-contrasted cues on the walls.

2.2.2. General behavioural procedures

The double-H is flooded with water (21 °C) to a height of 15 cm, thus with an approximate volume of 170 L. The water is rendered opaque with powdered milk (about 250 g). A platform, 11 cm in diameter, 14 cm high, ballasted by gravel, is immersed at one extremity of one of the four goal arms, 1 cm underneath the water surface. For the rat, the task consists in learning to swim from the start point – which

can be constant from trial to trial to force a procedural memory-based strategy, or can be randomly alternated between N and S to prevent such a strategy and rather force the animal into an approach based on spatial navigation – to the escape platform. After a first day of pre-training aiming at familiarizing the rats with the testing device and the water, the platform was immerged and training could be started. For the pre-training session, the water was left without milk, the platform protruded 1 cm above the water surface, and the rats were given four consecutive trials, for which the platform was located at the end of the NE arm. The rats were always released from the S start point. On the next day, rats were given three (or four, depending on the experiment; see below) trials. These trials were separated from each other by a 10-s gap. Depending on the kind of strategy the experimenter wanted to be preferentially developed by the rats, release occurred either from the N or the S arm in a randomized order (e.g., S, N, N, then N, S, N, then S, N, S, . . .) to favour an allocentric strategy, or systematically from the same arm with an alternation from N to S from one rat to the next one, but with the same start point over all trials for the same rat, in order to favour an egocentric strategy. Whatever the release protocol, when the rats were released from the N, the S arm was closed by a transparent guillotine door to prevent any entry, and vice versa. Each trial, whatever the protocol, lasted for a maximum of 60 s. When the rat did not reach the platform within this delay, it was gently guided to the platform by the experimenter. Once a rat had climbed on the platform, it was left there for 10 s.

During the acquisition phase, the experimenter noticed several variables: the latency (in s) and distance (in cm) to reach the platform, the swim pattern of each trial, which was drawn on a data sheet, as well as the number of errors, for which initial and repetitive errors were distinguished. An error was counted each time a rat either returned into the start arm (which was rarely observed) or entered into an arm defined as an error zone relatively to the start point and the location of the platform (see ez1–ez4 in Fig. 1B), i.e., an arm or arm portion not directly on the shortest way to the platform from where the rat was located. For instance, if the platform was submerged in the NE arm and the rat was released from the N, the error zones corresponded to the NW, SW and SE arms, as well as to the portion of the middle arm opposite to that which the rat had to swim through to reach the NW arm directly (see Fig. 1B and C). In this configuration, if a rat turned on its right after leaving the start arm instead of going left (Fig. 1C), then went to the SW arm, from there swam to the NW and then to the SE ones, before reaching the platform, the experimenter would count 4 errors (1 for having turned left when leaving the start arm, 1 for having entered into the SW arm, 1 for having been in the NW one, and finally one for having gone to SE). Repetitive errors were errors committed more than once in a given zone. A rat was considered to enter an arm when the four paws were in there.

2.3. Drugs

Scopolamine methylbromide (ScoMBr), scopolamine hydrochloride (ScoHCl) and MK801 hydrogen maleate (MK801) were provided by Sigma–Aldrich (France). The drugs were prepared daily in 0.9% saline. The solutions used for the administration of the low doses were prepared by dilution of the solutions used for that of the high doses. All drugs/doses were administered intraperitoneally (i.p.) in a volume of 1 mL/kg, 10 min before the start of each training session. To control for the effects of ScoHCl, a high dose of ScoMBr was used (0.51 mg/kg). The control for MK801 was 0.9% saline (same volume as with the drug).

2.4. Experiments

2.4.1. Experiment 1: learning the place, retrieving the trace (post-acquisition delays of 1, 5 and 18 days)

This experiment used 27 rats. It aimed at assessing the overall layout of the learning curve as well as the stability of the memory over time. We recorded the latency to the platform and the number of errors over a series of 6 training sessions, 1 per day. The platform was located in the NW arm. The rats were given 3 trials per day as described above, with start points being balanced between N and S in different sequences from day 1 to day 6. The maximal duration of a trial was of 60 s. Using a different set of rats at each delay, 1, 5 or 18 days after the last training session, we performed a probe trial for which the platform was removed from the maze. The rats were released from the S and left in the maze for 60 s. The experimenter recorded the latency to enter and that to reach the extremity of the NW arm, which is the arm where the platform was located during training, as well as the time spent in this arm (see Fig. 2 for track examples and corresponding time in target arm).

2.4.2. Experiment 2: influence of muscarinic or NMDA receptor antagonist on place learning and subsequent drug-free recall performance (light training; 2 days)

This experiment used 48 rats which were allocated to one of six groups, each corresponding to a drug/dose or a control treatment. As experiment 1 had shown clear-cut performance improvement from day 1 to day 2, we decided to first test the drug effects with only two sessions of training. Drugs injected were the muscarinic receptor antagonist scopolamine hydrobromide (ScoHCl), which was administered i.p. at the dose of 0.17 or 0.51 mg/kg, and the NMDA receptor antagonist dizolcipine (MK801), which was admnistered i.p. at the dose of 0.03 or 0.09 mg/kg. These doses were relatively comparable to those used by others to validate protocols or tasks testing learning capacities (e.g. [23]). Controls consisted in an administration

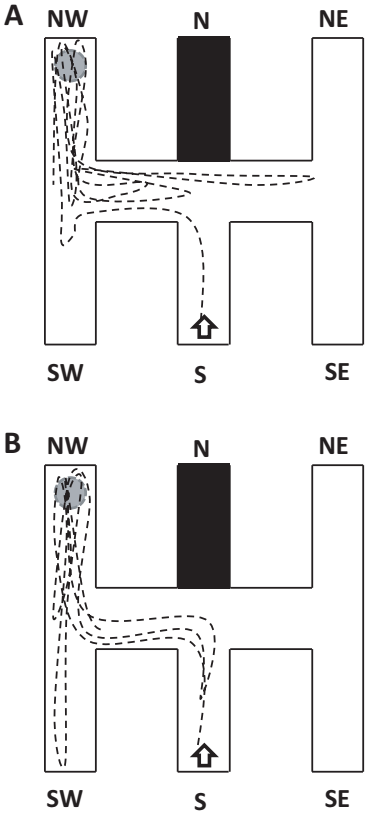

Fig. 2. Two examples of swim tracks recorded during a probe trial given with a one-day delay after a 6-day training period (3 trials/day). For the probe trial, the start was from the S arm (white arrow). The platform was located in the NW arm during the training and has been removed from the maze for the probe trial. The track shown in A (hatched lines) corresponds to a latency to access the former platform location of 6.62 s, and a time spent in the platform arm of 25.16 s (chance at 8.2 s). For the track shown in B, the latency to the former platform location was of 14.02 s and the time spent in the platform arm of 26.45 s. These scores were obtained in rats that were intact and did not receive any drug treatment before the training or probe trial sessions.

of the high dose of scopolamine methylbromide (ScoMBr-0.51), a form of scopolamine poorly crossing the blood–brain barrier, or 0.9% saline solution in order to control for ScoHCl and MK801 effects, respectively. All injection volumes were of 1 mL/kg. The injections were made 10 min before the first training trial. The rats were trained over only two consecutive days and were given 3 trials per day. They were released from the S or N arms in a balanced way and the platform was located in the NE one. After a 1-day rest, they were subjected to a drug-free probe trial which lasted for 60 s. The platform was removed from the maze. The latency to enter the target arm as well as the time spent in the former target arm were recorded and analyzed.

2.4.3. Experiment 3: influence of muscarinic or NMDA receptor antagonist on place learning and subsequent drug-free recall performance (sustained training; 4 days)

This experiment used 60 rats which were allocated to one of six groups, each corresponding to a drug/dose or a control treatment. Drugs and doses, control injections and all other aspects of the protocol were as in experiment 2, except that the rats were trained over 4 days (instead of 2). This 4-day training protocol was chosen on the basis of experiment 1, which showed that after the fourth training day, performance had reached a stable floor level.

2.4.4. Experiment 4: influence of muscarinic or NMDA receptor antagonist on procedural memory and subsequent drug-free recall performance (sustained training; 4 days)

This experiment used 84 rats which, as in experiments 2 and 3, were allocated to one of six groups, each corresponding to a drug/dose or a control treatment. Drugs and doses, control injections and all other aspects of the protocol were as in experiment 3, except that the rats were always released from the same start arm during the training trials (i.e., N), whatever the trial or the day, that there were 4 trials/day and that the platform was always located in the NW arm. For the drug-free probe trial, half the rats were released from the NE arm, the other half being released from the S arm. The variables recorded and analyzed were the first arm chosen by

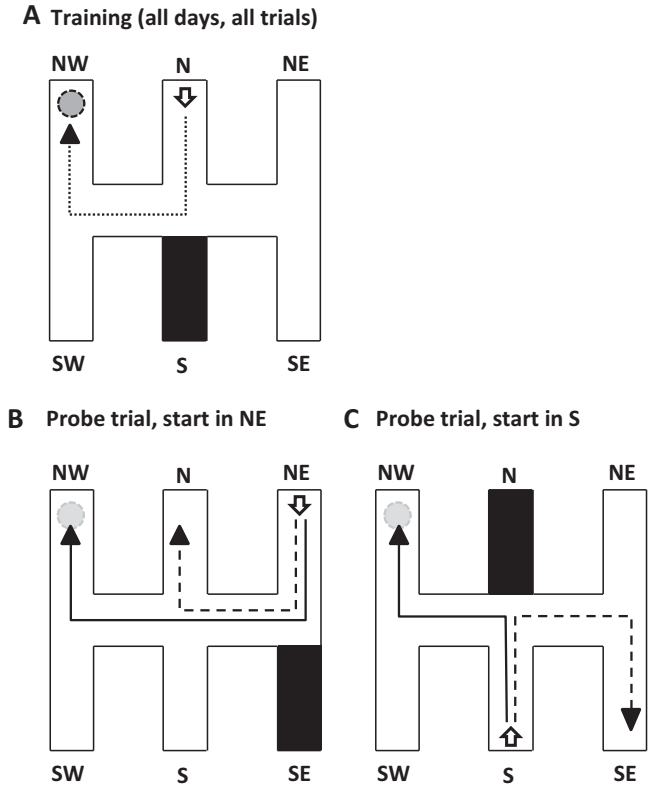

Fig. 3. Protocol used in experiment 4. For the training (A), the rats were always released from the N (white arrow; the S arm was closed by a guillotine door). They had to swim to the NW arm to find the platform. All rats were trained over 4 consecutive days for 4 consecutive daily trials. Before each training session, they were administered saline, 0.51 mg/kg ScoMBr, 0.17 or 0.51 mg/kg ScoHCl, 0.03 or 0.09 mg/kg MK801. For the probe trial, half the rats were tested with the protocol based on a release from the NE arm (B, white arrow), and we considered their first choice as indicating the engagement of a response relying upon either declarative-like memory (swim to NW) or procedural memory (swim to N). The other half was tested with the protocol based on a release from S (C, white arrow), and their first choice indicated if they first engaged a declarative-like memory (swim to NW) or a procedural one (swim to SE). The probe trial lasted 60 s and we also recorded the time spent in the NW arm.

the rat as well as the time spent in the former target arm. If the rats exhibited a procedural response, the ones released from the NE went directly into the N one (right–right turn), and the ones released from the S arm went directly into the SE one (right–right turn). It is noteworthy that for the rats released from the NE arm, the environmental perspective was relatively comparable to that of the training trials, whereas for those released from the S arm, it was completely different as they faced the opposite wall of their training wall, and the walls on their right and left during training were now on their left and right, respectively, at the start of the probe trial (see Fig. 3 for an illustration).

2.5. Statistical analyzes

All data were analyzed using parametric statistics. Depending on the experiment or the variables that had to be considered, we used one- or two-way analyses of variance (ANOVA). These were followed, when necessary, by post hoc comparisons using the Newman–Keuls multiple comparisons test [24]. When a given performance had to be compared with a reference value, such as a chance level in a probe trial, we used a *t*-test. For analyses of performance during the training period, we considered the latency and distance to reach the platform, as well as the number of initial and repetitive errors (see above for their definition). Because the analyses of latencies and distances yielded strictly similar conclusions, only the latencies will be considered hereafter. For analyses of the probe trial performance, we considered the latency to enter the target arm and the time spent in this arm. When an automatic motor response was trained during task acquisition (experiment 4), in the subsequent probe trial we also considered the very first arm entered by a rat as accounting for an initial response based on a procedural memory system (egocentric response learning), on a declarative-like memory system (allocentric place learning), or on another, and then inappropriate type of task approach (fail). When relevant, the rats' first choice classified as "egocentric" or "allocentric" under two different probe trial conditions (see above) but after exactly the same training protocol was analyzed with a χ^2 statistic. All parametric and nonparametric analyses were performed using Statistica (Version 8.0; Statsoft). In all groups under all testing conditions of our four experiments, the two variables recorded during the probe trial, namely the latency to enter the target arm and the time spent in this arm, were also compared to chance level. For the latency to enter the target arm, we computed the average latency of all first trials of all first sessions, to which probe trial performance was compared; it amounted 42.8 s. It will be called "average first trial latency" hereafter. For the time in the target arm corresponding to chance, we computed the relative inner surface of this arm (1327.28 cm^2/9644.4 cm^2 = 13.76%) and considered 13.76% of the 60 s probe trial duration; it amounted 8.25 s.

3. Results

3.1. Experiment 1: learning the place, retrieving the trace (post-acquisition delays of 1, 5 and 18 days)

The data for the acquisition period are shown in Fig. 4A–C. Concerning the number of initial and repetitive errors, a Delay (1d, 5d, 18d) × Day (1, 2, ...,6) ANOVA showed no overall Delay effect (*initial errors:* $F(2,24) = 0.28$; *repetitive errors*: $F(2,24) = 1.21$) and no significant Delay × Day interaction (*initial errors:* $F(10,120) = 0.37$; *repetitive errors:* $F(10,120) = 1.29$), accounting for similar performances across groups. The overall Day effect (*initial errors:* $F(5,120) = 87.72$; *repetitive errors:* $F(5,120) = 12.59$, $p < 0.05$) reflected a significant reduction of the number of each type of errors across acquisition sessions. A similar ANOVA of the latencies showed no overall Delay effect ($F(2,24) = 0.06$) and no significant Delay × Day interaction ($F(10,120) = 0.67$), meaning that also this variable accounted for comparable performance across groups. There was a significant Day effect ($F(5,120) = 198.37$, $p < 0.05$), due to the progressive decrease of the latencies, confirming learning of the platform location.

The data for the probe trials are shown in Fig. 4D and E. ANOVA of the latencies to enter the target arm or of the time spent in this arm showed no Delay effect ($F(2,24) = 2.19$ and 2.46, respectively), indicating similar performance at each delay considered. Moreover, whatever the group, the average time to enter the target arm was significantly below the average first trial latency (i.e., 42.8 s), and the time spent in this arm was significantly above chance (i.e., 8.25 s), reflecting retention of the platform location (Student's test, $p < 0.05$ in each group). Taken together, these results showed that performance did not undergo a significant time-dependent degradation until almost 3 post-acquisition weeks.

3.2. Experiment 2: influence of muscarinic or NMDA receptor antagonists on place learning and subsequent drug-free recall performance (light training; 2 days)

3.2.1. Effects of the muscarinic receptor antagonist scopolamine

The data for the acquisition period are shown in Fig. 5A–C. Concerning the number of initial and repetitive errors, a Drug (ScoMBr-0.51, ScoHCl-0.17, ScoHCl-0.51) × Day (1, 2) ANOVA showed a significant Drug effect for both types of errors (*initial errors:* $F(2,21) = 4.25$; *repetitive errors:* $F(2,21) = 4.25$, $p < 0.05$) and a significant Day effect for initial errors ($F(1,21) = 9.79$, $p < 0.05$) but not for repetitive errors ($F(1,21) = 2.02$). There was no significant Drug × Day interaction, whatever the error type (*initial errors:* $F(2,21) = 0.39$; *repetitive errors:* $F(2,21) = 0.32$). Overall, the number of both error types was significantly higher in the ScoHCl-0.51 group than in both other groups ($p < 0.05$, in each case). Similar conclusions were drawn from the analysis of the latencies. The Drug (ScoMBr-0.51, ScoHCl-0.17, ScoHCl-0.51) × Day (1, 2) ANOVA showed significant Drug ($F(2,21) = 6.14$, $p < 0.05$) and Day effects ($F(1,21) = 70.06$, $p < 0.05$), but no significant Drug × Day interaction ($F(2,21) = 0.62$). Overall performance was still worse in the ScoHCl-0.51 group as compared to the two other groups.

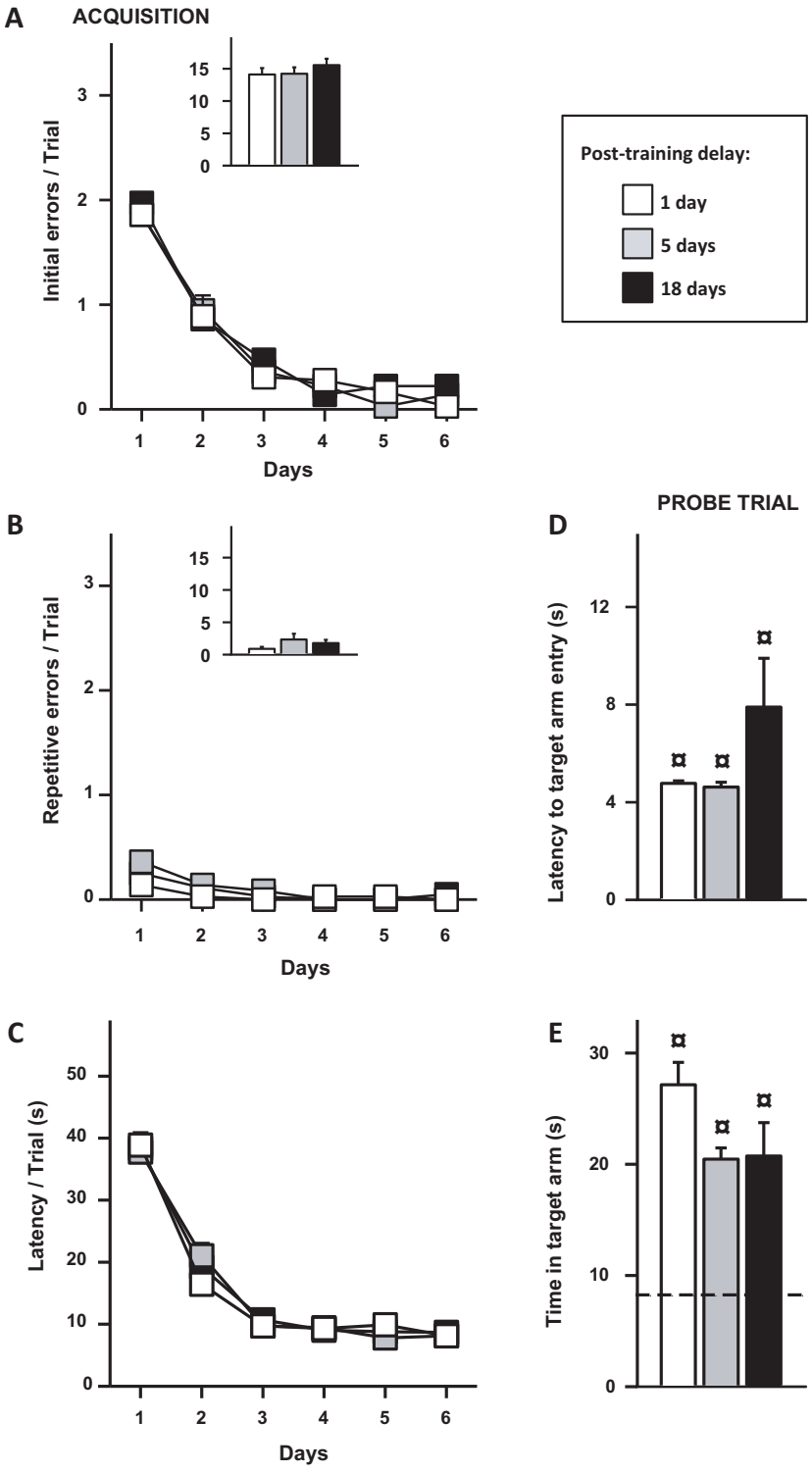

Fig. 4. Acquisition (left panel) and retention (right panel) of the platform location in experiment 1. Rats were given 3 trials/day over 6 days, and a probe trial 1 day, 5 days or 18 days after the last training trial. The figure shows the average (+s.e.m.) number of initial errors (A), repetitive errors (B) and latencies to the platform (C). The standard error of the mean was small enough to be masked by the size of the symbols. The inserts in A and B show the average number of errors in each group cumulated over all days. The probe trial performance is shown as the latency to reach the target arm (D) and the time spent in this arm (E). In D, the average first trial latency is of 42.8 s. In E, the chance level (8.25 s) is indicated by the interrupted line. Statistics: ¤ indicates a significant difference from average first trial latencies or chance, $p < 0.001$.

The data of the subsequent probe trial are shown in Fig. 5D and E. ANOVA of the latency to enter the target arm or of the time spent in this arm revealed no significant Drug effect ($F(2,21) = 0.60$ and $F(2,21) = 1.51$, respectively). In all groups, the average time to reach the target arm was significantly lower than 42.8 s. The average time spent in this arm did not differ from chance (=8.25 s), except in the ScoMBr-0.51 control group (Student's test, $p < 0.05$). Taken together, these results demonstrated impaired performance in the groups treated with the centrally active scopolamine, suggesting information acquisition/consolidation failure despite improvement of performance during the acquisition trials.

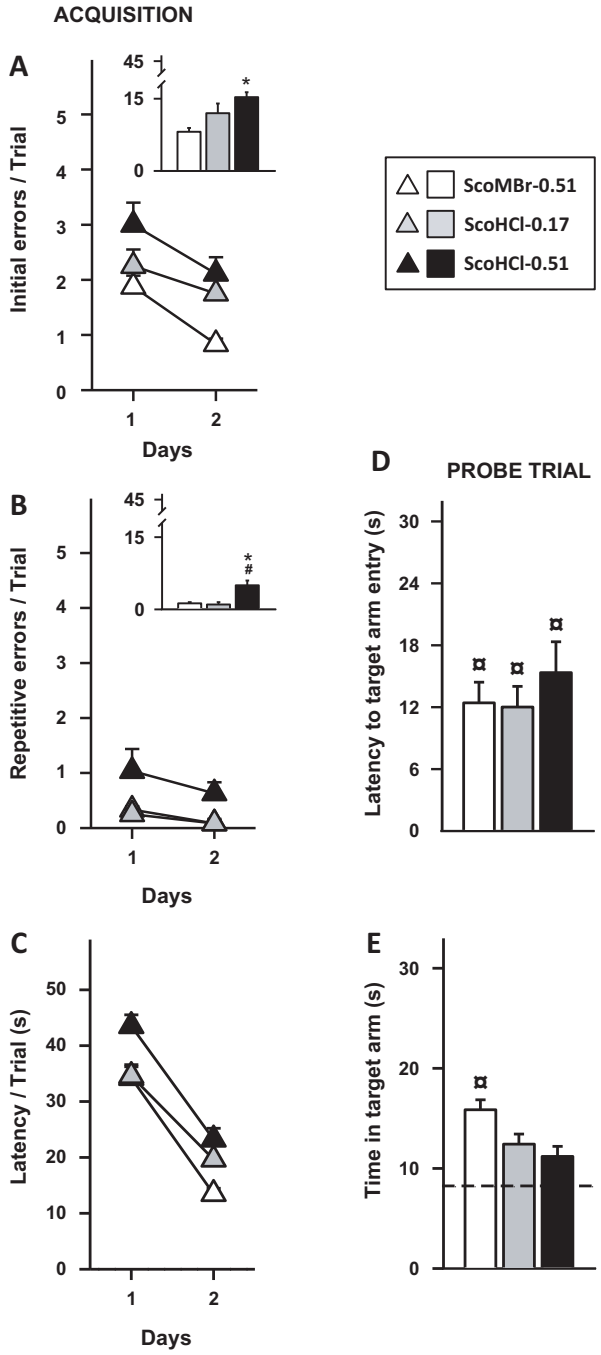

Fig. 5. Acquisition (left panel) and retention (right panel) of the platform location in experiment 2. Rats were given 3 trials/day over 2 days, and a probe trial 1 day after the last training trial. Ten minutes before each training session, the rats were injected i.p. with 0.51 mg/kg scopolamine methylbromide (SCoMBr-0.51), or with 0.17 mg/kg or 0.51 mg/kg scopolamine hydrochloride (ScoHCl-0.17 and ScoHCl-0.51, respectively). The figure shows the average (+s.e.m.) number of initial errors (A), repetitive errors (B) and latencies to the platform (C). The inserts in A and B show the average number of errors in each group cumulated over all days. The probe trial performance is shown as the latency to reach the target arm (D) and the time spent in this arm (E). In D, the average first trial latency is of 42.8 s. In E, the chance level (8.25 s) is indicated by the interrupted line. Statistics: * indicates a significant difference with the control condition, $p < 0.05$; # indicates a significant difference with the low dose of scopolamine, $p < 0.05$; ¤ indicates a significant difference from average first trial latencies or chance, $p < 0.001$.

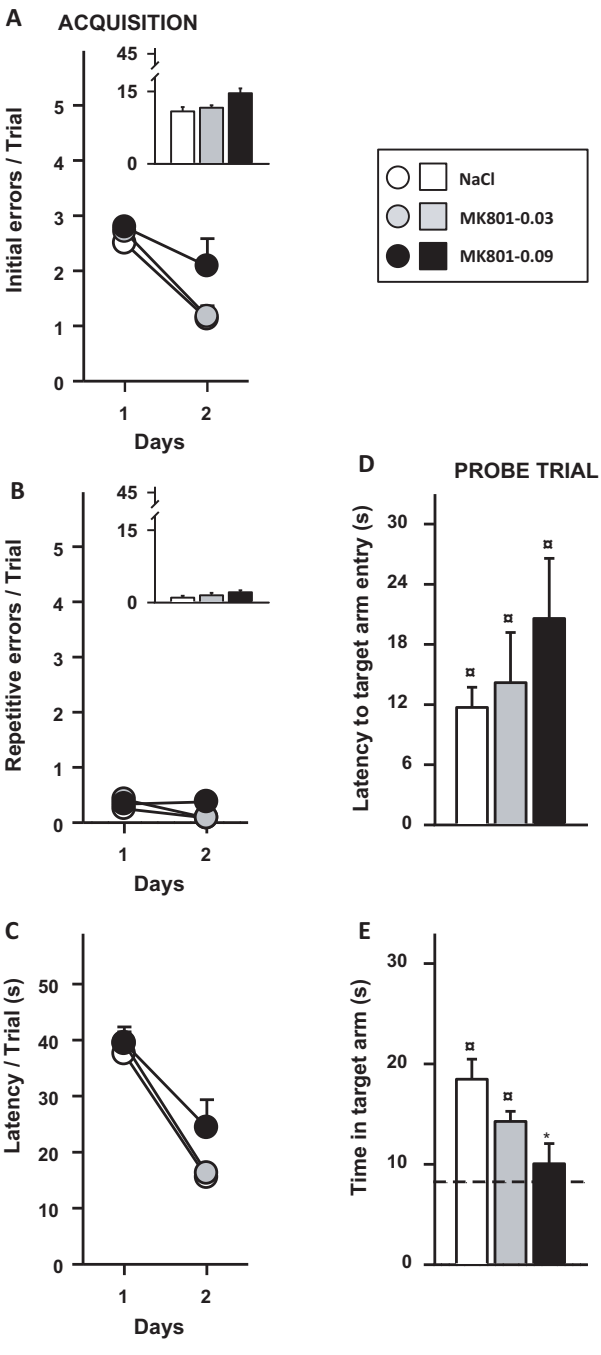

Fig. 6. Acquisition (left panel) and retention (right panel) of the platform location in experiment 2. Rats were given 3 trials/day over 2 days, and a probe trial 1 day after the last training trial. Ten minutes before each training session, the rats were injected i.p. with a saline solution (NaCl), or with 0.03 mg/kg or 0.09 mg/kg dizocilpine (MK801-0.03 and MK801-0.09, respectively). The figure shows the average (+s.e.m.) number of initial errors (A), repetitive errors (B) and latencies to the platform (C). The inserts in A and B show the average number of errors in each group cumulated over all days. The probe trial performance is shown as the latency to reach the target arm (D) and the time spent in this arm (E). In D, the average first trial latency is of 42.8 s. In E, the chance level (8.25 s) is indicated by the interrupted line. Statistics: * indicates a significant difference with the control condition, $p < 0.05$; ¤ indicates a significant difference from average first trial latencies or chance, $p < 0.001$.

3.2.2. *Effects of the NMDA receptor antagonist MK801*

The data for the acquisition period are shown in Fig. 6A–C. Concerning the number of initial and repetitive errors, a Drug (NaCl, MK801-0.03, MK801-0.09) × Day (1, 2) ANOVA showed a significant Day effect for initial errors ($F(1,21) = 23.35$, $p < 0.05$) but not for repetitive errors ($F(1,21) = 3.43$). There was neither a significant Drug effect for both types of errors (*initial errors:* $F(2,21) = 3.36$; *repetitive errors:* $F(2,21) = 1.37$), nor a significant Drug × Day inter-

action (*initial errors:* $F(2,21)=1.04$; *repetitive errors:* $F(2,21)=1.73$). The number of repetitive errors was so low that there was no difference among groups and no improvement across acquisition sessions. A Drug (NaCl, MK801-0.03, MK801-0.09) × Day (1, 2) ANOVA of the latencies showed no significant overall Drug effect ($F(2,21)=1.39$) and no significant Day × Group interaction ($F(2,21)=1.29$). The overall Day effect, however, was significant ($F(1,21)=82.33$, $p<0.05$), accounting for a progressive shortening of the latencies to reach the platform across acquisition sessions.

The data of the probe trials are shown in Fig. 6D and E. The ANOVA of the latency to enter the target arm showed no significant Drug effect ($F(2,21)=0.78$) and a Student's test ($p<0.05$) revealed that in all groups, the average time to reach the target arm was significantly below 42.8 s. Concerning the time spent in the target arm, the ANOVA showed a significant Drug effect ($F(2,21)=3.90$, $p<0.05$); this effect reflected that only NaCl and MK801-0.03 rats spent an average time in the target arm that was significantly above chance level (=8.25 s; Student's test, $p<0.05$). These results point to an altered memory function, but only in the rats that were given the high dose of MK801 during training.

3.3. Experiment 3: influence of muscarinic or NMDA receptor antagonists on place learning and subsequent drug-free recall performance (sustained training; 4 days)

This experiment was performed to determine if a more sustained training could enable acquisition and remembering the platform location despite the drug treatments. Therefore, training was extended to 4 daily 3-trial sessions (drugs given before).

3.3.1. Effects of the muscarinic receptor antagonist scopolamine

The data for the acquisition period are shown in Fig. 7A–C. Concerning the number of initial and repetitive errors, a Drug (ScoMBr-0.51, ScoHCl-0.17, ScoHCl-0.51) × Day (1, 2, 3, 4) ANOVA showed significant Drug (*initial errors:* $F(2,27)=6.76$; *repetitive errors:* $F(2,27)=3.93$, $p<0.05$) and Day effects (*initial errors:* $F(3,81)=22.46$; *repetitive errors:* $F(3,81)=10.08$, $p<0.05$), but no significant Drug × Day interaction (*initial errors:* $F(6,81)=0.48$; *repetitive errors:* $F(6,81)=1.85$). It is noteworthy that the overall number of both types of errors was significantly higher in the ScoHCl-0.51 group as compared to the ScoMBr-0.51 group (initial and repetitive errors; $p<0.05$) or to the ScoHCl-0.17 one (only initial errors; $p<0.05$). A similar pattern of performance was observed for the latencies. The Drug (ScoBr-0.51, ScoHCl-0.17, ScoHCl-0.51) × Day (1, 2, 3, 4) ANOVA showed significant overall Drug ($F(2,27)=4.35$, $p<0.05$) and Day effects ($F(3,81)=54.92$, $p<0.05$), but no significant interaction between the two factors ($F(6,81)=0.34$). While there was an improvement of performance over days in all groups, rats given the high dose of ScoHCl took a significantly longer overall time to reach the platform as compared to both other groups ($p<0.05$). It is noteworthy that, on the last training day, all rats had reached comparable performance levels.

The data of the probe trials are shown in Fig. 7D and E. The ANOVA of the latency to enter the target arm or the time spent in this arm showed no significant Group effect ($F(2,27)=0.26$ and $F(2,27)=2.35$, respectively). Moreover, in all groups the average time to reach the target arm was largely and significantly below 42.8 s, and the average time spent in this arm was significantly above chance (=8.25 s), reflecting retention of the platform location, whatever drug/dose was given (Student's test, $p<0.05$ in all groups). Altogether, these results indicate that with two additional days of training, scopolamine did not prevent consolidation of the memory for the platform location.

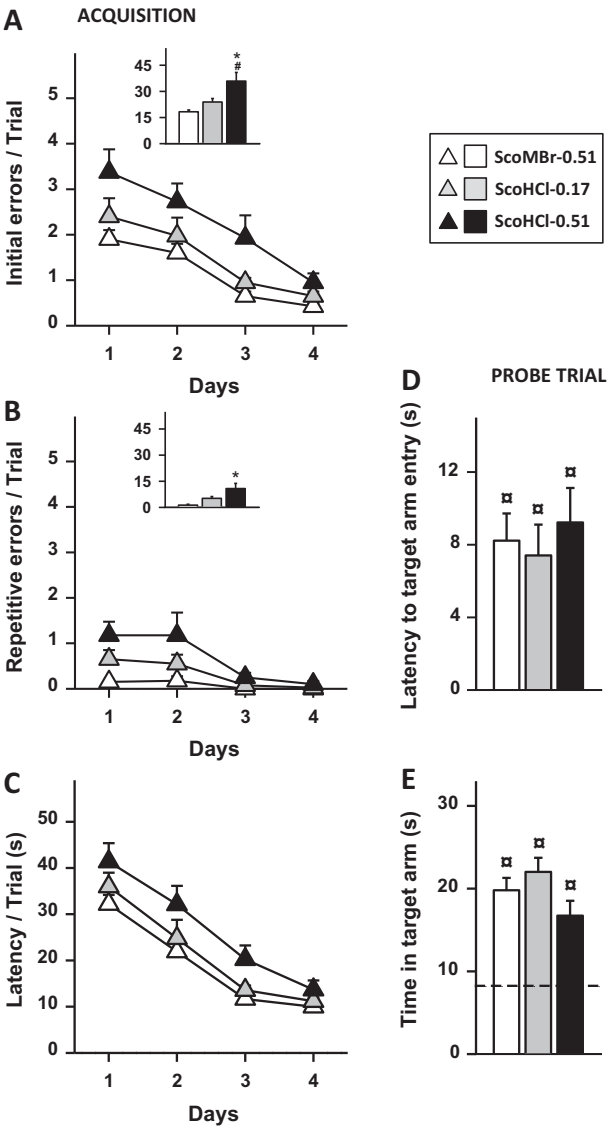

Fig. 7. Acquisition (left panel) and retention (right panel) of the platform location in experiment 3. Rats were given 3 trials/day over 4 days, and a probe trial 1 day after the last training trial. Ten minutes before each training session, the rats were injected i.p. with 0.51 mg/kg scopolamine methylbromide (SCoMBr-0.51), or with 0.17 mg/kg or 0.51 mg/kg scopolamine hydrochloride (ScoHCl-0.17 and ScoHCl-0.51, respectively). The figure shows the average (+s.e.m.) number of initial errors (A), repetitive errors (B) and latencies to the platform (C). The inserts in A and B show the average number of errors in each group cumulated over all days. The probe trial performance is shown as the latency to reach the target arm (D) and the time spent in this arm (E). In D, the average first trial latency is of 42.8 s. In E, the chance level (8.25 s) is indicated by the interrupted line. Statistics: * indicates a significant difference with the control condition, $p<0.05$; # indicates a significant difference with the low dose of scopolamine, $p<0.05$; ¤ indicates a significant difference from average first trial latencies or chance, $p<0.001$.

3.3.2. Effects of the NMDA receptor antagonist MK801

The data of the acquisition period are shown in Fig. 8A–C. Concerning the number of initial and repetitive errors, a Drug (NaCl, MK801-0.03, MK801-0.09) × Day (1, 2, 3, 4) ANOVA showed a main Drug effect on the number of initial errors ($F(2,27)=10.12$, $p<0.05$), but not on the number of repetitive errors ($F(2,27)=1.26$): the number of initial errors was significantly higher in the MK801-0.09 group compared to the MK801-0.03 and the NaCl groups ($p<0.05$, in each case). There was also a significant Day effect for both types of errors (*initial errors:* $F(3,81)=61.11$; *repetitive errors:* $F(3,81)=7.84$, $p<0.05$), indicating that overall performance underwent improvement across acquisition sessions.

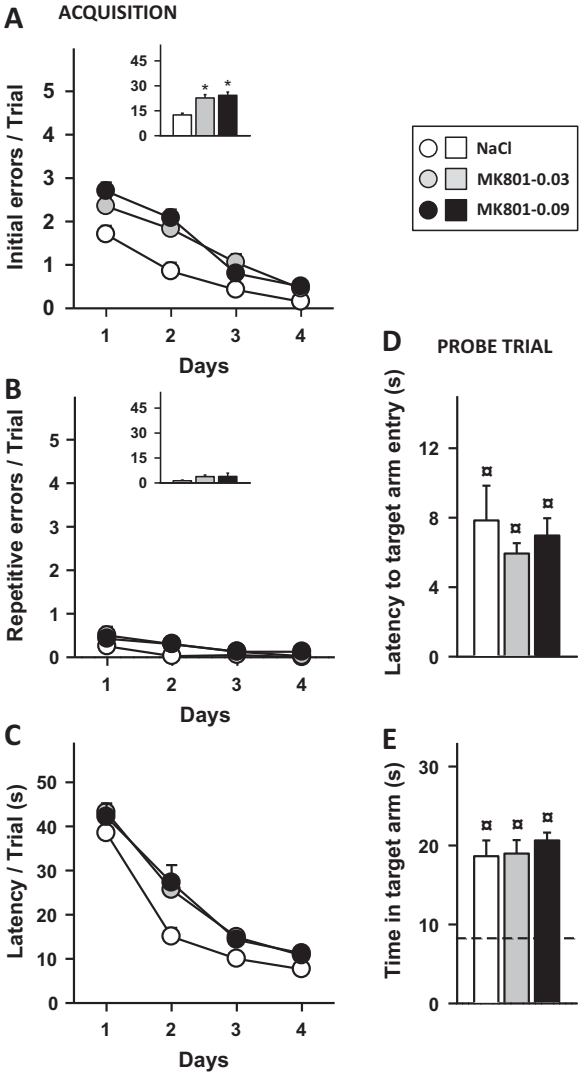

Fig. 8. Acquisition (left panel) and retention (right panel) of the platform location in experiment 3. Rats were given 3 trials/day over 4 days, and a probe trial 1 day after the last training trial. Ten minutes before each training session, the rats were injected i.p. with a saline solution (NaCl), or with 0.03 mg/kg or 0.09 mg/kg dizocilpine (MK801-0.03 and MK801-0.09, respectively). The figure shows the average (+s.e.m.) number of initial errors (A), repetitive errors (B) and latencies to the platform (C). The inserts in A and B show the average number of errors in each group cumulated over all days. The probe trial performance is shown as the latency to reach the target arm (D) and the time spent in this arm (E). In D, the average first trial latency is of 42.8 s. In E, the chance level (8.25 s) is indicated by the interrupted line. Statistics: * indicates a significant difference with the control condition, $p < 0.05$; ¤ indicates a significant difference from average first trial latencies or chance, $p < 0.001$.

The ANOVA did not reveal any Drug × Day interaction (*initial errors:* $F(6,81) = 1.63$; *repetitive errors:* $F(6,81) = 0.53$). Concerning the latencies, a Drug (NaCl, MK801-0.03, MK801-0.09) × Day (1, 2, 3, 4) ANOVA showed significant Drug ($F(2,27) = 3.58$, $p < 0.05$) and Day effects ($F(3,81) = 164.78$, $p < 0.05$), but no interaction between the two factors ($F(6,81) = 1.36$). The overall latencies were significantly longer in the MK801-treated groups as compared to the NaCl group. It is noteworthy that, on the last training day, all rats had reached comparable performance levels.

The probe trial data are shown in Fig. 8D and E. The ANOVA of the latency to enter the target arm or of the time spent in this arm revealed no significant Drug effect ($F(2,27) = 0.32$ and $F(2,27) = 0.35$, respectively). Furthermore, in all groups the average time to reach the target arm was significantly lower than 42.8 s and the average time spent in this arm was significantly above chance (=8.25 s), reflecting retention of the platform location in all three treatment groups (Student's test, $p < 0.05$, in each case).

3.4. Experiment 4: influence of muscarinic or NMDA receptor antagonists on procedural memory and subsequent drug-free recall performance (sustained training; 4 days)

In this experiment we assessed the effects of the same drugs/doses as in experiments 2 and 3. This time, the rats were always released from the same start arm (N). For the drug-free probe trial, half of them were released from the S, from where they had a view of the environment opposite to (and clearly different from) that of the training trials. The other half was released from the NE, from where the view was very close to that of the training trials, as the release point corresponded to only a 60 cm translation towards the E. This analysis considered an additional variable, namely the number of rats which had their very first arm choice based on an egocentric response (two successive right turns, accounting for procedural memory and leading them e.g., into the N when starting from NE) and the number of rats in which this first choice was based on an allocentric response (a direct swim to the appropriate target arm, namely NW, accounting for declarative-like memory).

3.4.1. Effects of the muscarinic receptor antagonist scopolamine

The data of the acquisition period are shown in Fig. 9A–C, with no distinction according to the arm from which the rats would be released for the future probe trial. The performance of the rats that were released from S for the probe trial, and which are called PTS (probe trial S) rats hereafter, is shown in D and E, left. The performance of the rats that were released from NE, and which are called PTNE rats hereafter, are shown in the same panels, but on the right. Concerning the number of initial and repetitive errors, the ANOVA only showed a significant Day effect (*initial errors:* $F(3,111) = 61.4$; *repetitive errors:* $F(3,111) = 16.2$, $p < 0.001$), which reflected performance improvement. There was also a significant overall Drug effect, but only on *initial errors* ($F(2,37) = 4.5$, $p < 0.05$); this effect was due to overall performance that was impaired in ScoHCl-0.51 vs. ScoHCl-0.17 and ScoMBr-0.51 rats ($p < 0.05$ in each case). The Drug × Day interactions were not significant ($F(6,111 < 1.0)$. A relatively similar pattern of performance was observed for the latencies: the Day effect was significant ($F(3,111) = 103.0$, $p < 0.001$).

The data collected during the probe trial are shown in Fig. 9D and E. The ANOVA of the latency to enter the target arm showed no significant Drug effect, whether in PTS ($F(2,17) = 1.9$) or PTNE rats ($F(2,17) = 1.5$). ANOVA of the average time spent in the target arm, however, showed a Drug effect that was significant in PTS rats ($F(2,17) = 4.89$, $p < 0.05$), but not in PTNE ones ($F(2,17) = 1.5$). In PTS rats, this effect was due to significantly impaired performance in the ScoHCl-0.51 rats as compared to either ScoHCl-0.17 or ScoMBr-0.51 rats. We also compared the first choice made by PTS and PTNE rats according to whether it accounted for a response relying upon procedural or declarative-like memory. To this end, we considered the number of rats that made an egocentric (response learning) vs. those making an allocentric choice (place learning) for each drug condition (Fig. 11). When given ScoMBr, 6 out of 7 PTS rats but none of the 7 PTNE rats made an allocentric choice (swim to NW, $\chi^2 = 10.5$, $p < 0.01$), the others making an egocentric one (swim to N). When given ScoHCl-0.17, all 6 PTS rats but only one of the 6 PTNE rats made an allocentric choice ($\chi^2 = 8.6$, $p < 0.01$), all others making an egocentric one. Finally, in rats given ScoHCl-0.51, 5 out of 7 PTS rats but only one of the 7 PTNE rats made an allocentric choice ($\chi^2 = 4.7$, $p < 0.05$), all others making an egocentric one.

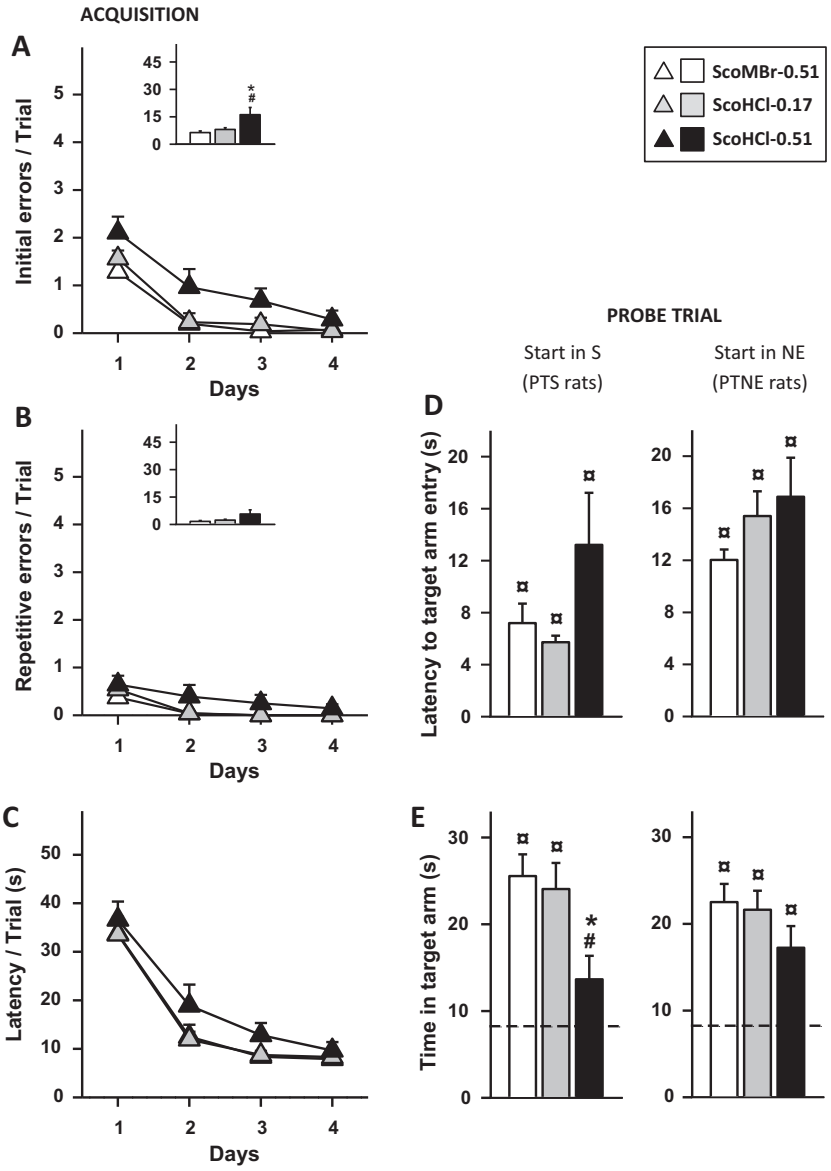

Fig. 9. Acquisition (left panel) and retention (right panel) of the platform location (in the NW arm) in experiment 4. Rats were given 4 trials/day over 4 days, for which they were released from the N arm, and then a probe trial 1 day after the last training trial. For this probe trial, half of them were released from the S arm, the other half being released from the NE arm (see Fig. 11). Ten minutes before each training session, the rats were injected i.p. with 0.51 mg/kg scopolamine methylbromide (SCoMBr-0.51), or with 0.17 mg/kg or 0.51 mg/kg scopolamine hydrochloride (ScoHCl-0.17 and ScoHCl-0.51, respectively). The figure shows the average (+s.e.m.) number of initial errors (A), repetitive errors (B) and latencies to the platform (C). The inserts in A and B show the average number of errors in each group cumulated over all days. The probe trial performance is shown as the latency to reach the target arm (D) and the time spent in this arm (E) in PTS (start in S) and PTNE (start in NE) rats. In D, the average first trial latency is of 42.8 s. In E, the chance level (8.25 s) is indicated by the interrupted line. Statistics: ¤ indicates a significant difference from average first trial latencies or chance, $p < 0.001$.

3.4.2. Effects of the NMDA receptor antagonist MK801

The data of the acquisition and probe trials are shown in Fig. 10A–C, which is constructed as Fig. 9. Concerning the number of *initial* and *repetitive errors*, the ANOVA showed a significant Drug effect on the number of initial errors ($F(2,37) = 6.6$, $p < 0.01$). There was also a significant Day effect ($F(3,111) = 78.9$, $p < 0.001$) as well as a significant Drug × Day interaction ($F(6,111) = 3.0$, $p < 0.01$). The interaction was due to performance, which, during the first session, was impaired in the rats given MK801, whatever the dose, as compared to NaCl ($p < 0.05$, in each case). On subsequent sessions, there was no significant drug-induced impairment. There was no significant Drug effect on the number of *repetitive errors* ($F(2,37) = 2.7$), but the Day effect was significant ($F(3,111) = 25.8$, $p < 0.001$); the Drug × Day interaction was not significant ($F(6,111) = 1.2$). When the latencies were analyzed, we found significant Drug ($F(2,37) = 4.7$, $p < 0.05$) and Day ($F(3,111) = 87.6$, $p < 0.001$) effects. The Drug effect was due to an impairment of overall performance in MK801-0.09 rats as compared to both other groups. The Day effect reflected performance improvement in sessions 2, 3 and 4 as compared to session 1 ($p < 0.05$, in each case).

The data collected during the probe trial are shown in Fig. 10D and E (PTS rats on the left; PTNE rats on the right). The ANOVA of the latency to enter the target arm showed no significant Drug effect in PTS ($F(2,16 < 1.0$) and PTNE rats ($F(2,18 < 1.0$). ANOVA of the average time spent in the target arm, however, showed a Drug effect which achieved significance in PTNE rats ($F(2,18) = 7.6$, $p < 0.01$), not in the PTS ones ($F(2,16 < 1.0$). In PTNE rats, this effect was due to a significant MK801-induced impairment of performance at both

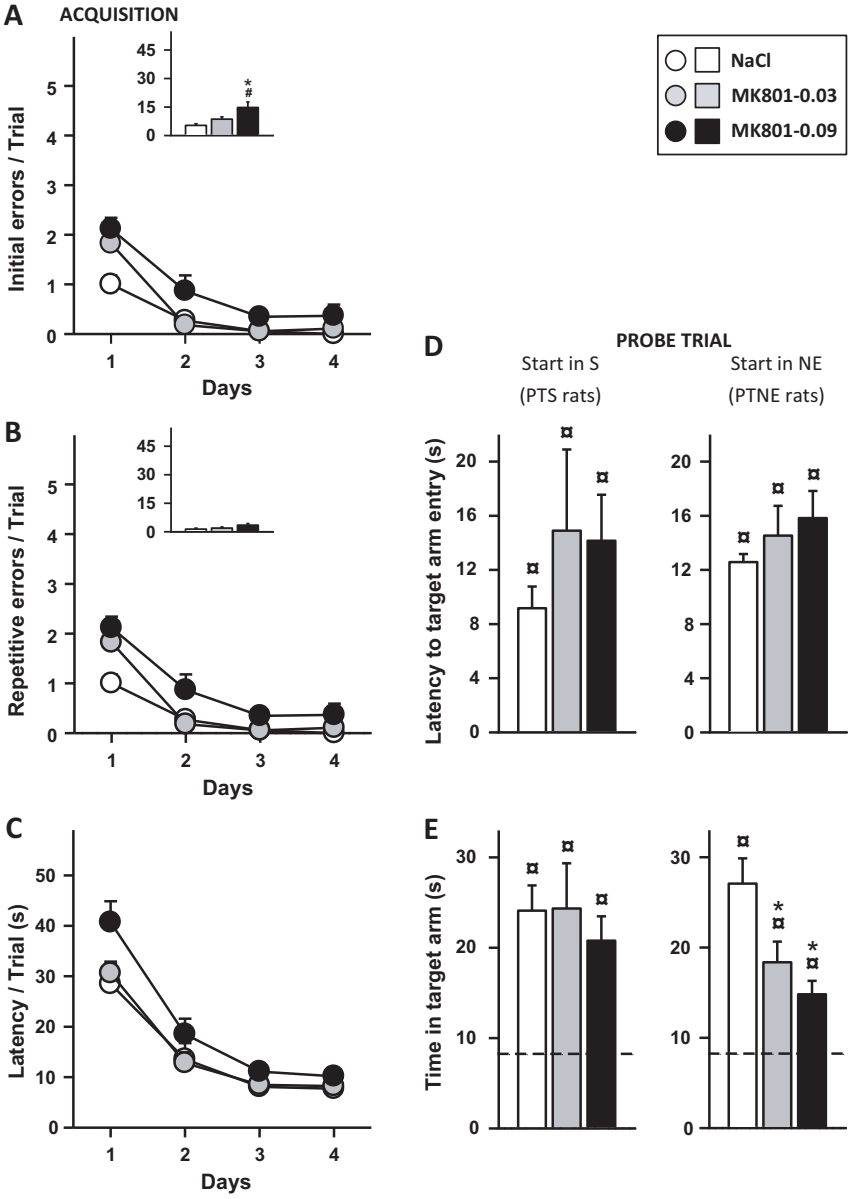

Fig. 10. Acquisition (left panel) and retention (right panel) of the platform location (in the NW arm) in experiment 4. Rats were given 4 trials/day over 4 days, for which they were released from the N arm, and then a probe trial 1 day after the last training trial. For this probe trial, half of them were released from the S arm, the other half being released from the NE arm (see Fig. 11). Ten minutes before each training session, the rats were injected i.p. with a saline solution (NaCl), or with 0.03 mg/kg or 0.09 mg/kg dizocilpine (MK801-0.03 and MK801-0.09, respectively). The figure shows the average (+s.e.m.) number of initial errors (A), repetitive errors (B) and latencies to the platform (C). The inserts in A and B show the average number of errors in each group cumulated over all days. The probe trial performance is shown as the latency to reach the target arm (D) and the time spent in this arm (E) in PTS (start in S) and PTNE (start in NE) rats. In D, the average first trial latency is of 42.8 s. In E, the chance level (8.25 s) is indicated by the interrupted line. Statistics: ¤ indicates a significant difference from average first trial latencies or chance, $p < 0.001$.

doses ($p < 0.05$, in each case). We also compared the first choice made by PTS and PTNE rats (Fig. 11), as done for the scopolamine treatment. When given NaCl, 4 out of 6 PTS rats but none of the 7 PTNE rats made an allocentric choice ($\chi^2 = 6.7$, $p < 0.01$), the others making an egocentric one. When given MK801-0.03, 5 out of 7 PTS rats but only one of the 6 PTNE rats made an allocentric choice ($\chi^2 = 4.7$, $p < 0.05$), the others making an egocentric one. Finally, in the rats given MK801-0.09, 4 out of 6 PTS rats but only one of the 6 PTNE rats made an allocentric choice ($\chi^2 = 3.8$, $p = 0.05$), all others making an egocentric one.

4. Discussion

The currently reported data validate the double-H maze test as a simple and rapidly acquired memory task requiring no prior motivation-inducing manipulations such as food or water deprivation. Our results show that (i) under conditions of sustained, drug-free training, performance accounting for a vivid remote memory (18 days post-acquisition) is not significantly degraded in comparison with recent memory (e.g., after 1 or 5 days post-acquisition); moreover (ii) only 2 days of training (2 × 3 trials) are sufficient to obtain a memory trace lasting for at least 1 day; (iii) under such training conditions, the administration of scopolamine or that of MK801, two classical amnestic drugs, weaken overall performance during training, and alter probe trial performance in a dose-dependent manner; (iv) the different variables recorded and analyzed do not appear equivalently reliable to demonstrate a learning deficit, (v) under conditions of more sustained training (4 days, 12 trials), however, a memory is established despite scopolamine or MK801 treatment; (vi) the type of training pro-

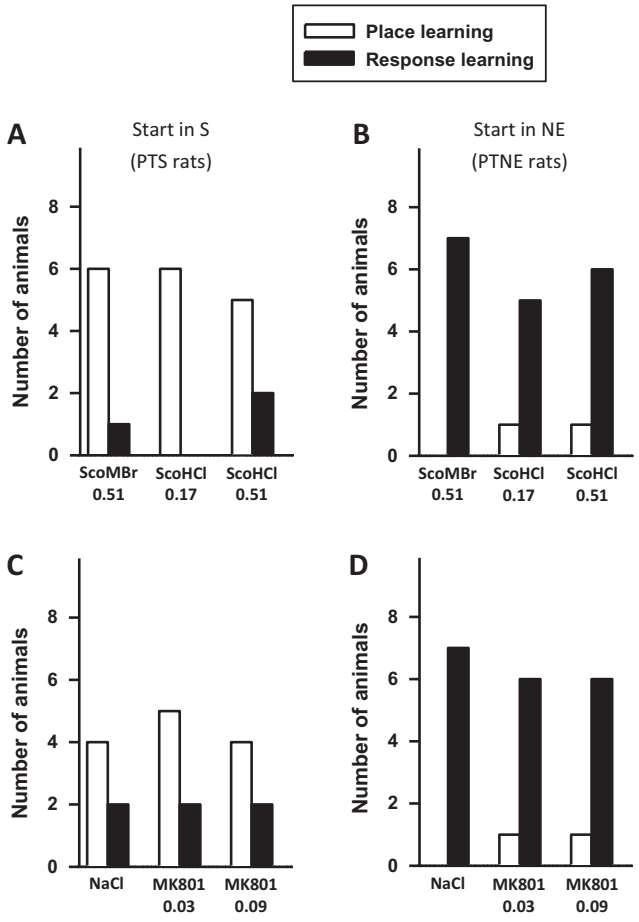

Fig. 11. Number of rats in each drug condition (as in the other figure legends) that exhibited a first swim path accounting for a behaviour based on either response learning (egocentric) or on place learning (allocentric) in the probe trial. PTS rats were released from the S arm and PTNE ones from the NE one, but all rats were trained with a platform in the NW arm and were systematically released from the N one. In PTS rats, a response learning-based first choice would lead them to the SE arm, in PTNE rats such a behaviour would lead them to the N arm.

tocol can orient the rats behaviour towards response learning or place learning, but both memory systems co-exist, and the immediate engagement of one or the other is clearly depending on the environmental context at the start of the probe trial.

These observations demonstrate that the task can be acquired over very short period of training (2 days, 6 trials); compared to other maze tasks, this is a clear-cut advantage for preclinical approaches or/and drug screening, in which time-consuming tasks are not given priority. While being sensitive to amnestic drugs (under conditions of a short training period), the task is also simple enough to prevent the disruptive effects of scopolamine and MK801 under conditions of more sustained training (4 days, 12 trials). Finally, by adaptations of the training protocol, it enables some control over an animal's strategy, and seems particularly appropriate to investigate the dynamic balance between memory systems such as the declarative-like and the procedural ones.

4.1. Acquisition and trace duration

The basic principle of the double-H maze is identical to that of the water maze: during task acquisition, rats have to escape from cold water by climbing on a platform hidden underneath the water surface; for the probe trial, this platform is removed. Thus, the usual drawbacks of the water maze (e.g. [25]), especially regarding stress, still apply to the double-H maze. A major difference between both tasks, however, is that there is a limited number of navigation possibilities in the double-H maze, and that the task, with extended practice, is apparently simple enough for being learned under the influence of amnestic drugs (see below). It is noteworthy that because the rats were released from two different start points (namely N or S) in unpredictable sequences, it was not possible for them to solve the tasks of experiments 1–3 by a simple routine consisting in repeating a pair of angular choices such as a two successive right turns. In order to materialize performance, we have recorded several variables during acquisition. Using the 6-day long training protocol, learning could be evidenced by a reduction of the number of initial (from about 2 to almost 0) and repetitive errors (from about 0.5 to 0), as well as by the progressive shortening of the latency to reach the platform (from about 35 s to about 10 s); the distances to the platform followed a picture strictly similar to that found for the latencies. The fact that the rats had learned and could retrieve the position of the platform was also evidenced in the probe trial, during which, even at the longest post-acquisition delay, the mean latency to enter the target arm was always below 8 s (average first trial latency = 42.8 s) and the time in the target arm was slightly above 20 s (chance level = 8.25 s). Interestingly, although longer latencies and shorter times in target arm were found at the longest post-acquisition delay (18 days), there was no statistical evidence for performance degradation vs. shorter delays (1 and 5 days). This absence of statistical significance, however, does not mean that performance level would resist delays much longer than 18 days; we have evidence for nondegraded performance after 25 post-acquisition days (unpublished), but we did not assess trace persistence beyond this time. Furthermore, it is not because the double-H maze test has a relatively weak cognitive demand that the experimental manipulations known to produce heavy deficits in classical spatial navigation tasks (radial, water and other mazes) will not induce alterations in the double-H. Indeed, we recently established that rats subjected to neonatal ventral hippocampal lesions – the so-called Lipska model of schizophrenia (e.g. [26]) – and tested when they were adults were unable to acquire the location of the platform in the double-H maze: there was no improvement at all over three consecutive learning days (4 trials/day; Lecourtier et al., in preparation) and probe trial performance was at chance. In another still running experiment, we found that, despite sustained training, rats which had developed status epilepticus following lithium + pilocarpine treatment were unable to acquire the location of the platform in the double-H, as was also the case in a standard version of the water-maze test (Faure et al., in preparation).

These arguments, along with our yet unpublished observations (see above) and our findings with scopolamine and MK801 (see below), lead us to believe that the double-H is a reliable and efficient device to assess place learning in rats, and that it could be particularly adapted for rapid preclinical evaluations of drug effects or, given the weakness of performance degradation over about 3 post-acquisition weeks, for studies aiming at identifying the spatio-temporal dynamics of remote memories at the system level.

4.2. Effects of systemic muscarinic receptor blockade by scopolamine

In spatial memory tasks such as the radial maze or the water maze, scopolamine treatment at doses comparable to the ones used in the current series of experiments (range of efficacy 0.3–0.7 or more mg/kg) usually induces deficits (e.g. [27]), which can be marked enough to result not only in a significant overall Drug effect, but also in a significant Drug × Trial, Drug × Trial-block or Drug × Day interaction. These deficits are not always interpreted as reflecting cognitive dysfunctions (e.g. [28]). With the 2-day acquisition protocol, we found a significant overall Drug effect on all

variables, but could evidence a Drug × Day interaction for none of them. Using the 4-day acquisition protocol, at the end of which the drug and control groups had reached similar performance levels, we also found a significant overall Drug effect on all variables, but no interaction with the other factor. Clearly, this outcome of our statistical analyses, as also suggested by an examination of Figs. 4 and 6, is due to the fact that the rats given the high dose of scopolamine showed impaired task acquisition, but were able to improve performance over days despite the action of the drug, and did so regardless of the acquisition protocol. Based on our probe trial data, there was nevertheless a clear difference between the 2-day and the 4-day training protocol in terms of drug effects on memory formation. With the short protocol, the rats given the high dose, as those given the low one failed to remember the location of the platform, their time in the target arm being not significantly different from chance. The fact that their time to reach the target arm was not different from controls might indicate that motivational, sensory and motor aspects of the task had been integrated to an extent that was sufficient to permit a fast engagement into a behavioural response (swim to reach an escape point). It could be argued that the poor performance level in rats given the centrally active form of scopolamine is in fact the consequence of a state-dependent effect, rats being under the drug's influence during the acquisition sessions but not during the probe trial. This possibility, however, can be discarded on the basis of the data obtained in experiment 3. Indeed, rats were also given the drug only before the training trials, but, as a result of extended training, exhibited drug-free probe trial performance accounting for retrieval of the platform location. There is also literature indicating that in some tasks at least, scopolamine given during learning does not produce state-dependent effects on subsequent retrieval ([29]; but see [30]). Therefore, the most appropriate explanation for our drug effects with the short training protocol is that the rats given the centrally active scopolamine experienced a drug-induced alteration of encoding/consolidation processes, which an extended practice was able to overcome. This conclusion is compatible with the findings of von Linstow Roloff et al. [31] in the Morris water maze. Their data indicate that despite scopolamine treatment during training, rats may show evidence for recall of the platform location after three days of training (6 trials/day), but not after one or two days of training. Based on our observations after scopolamine administrations under both training conditions, it seems that the most informative variable to account for whether a memory has or not been established is the time spent in the target arm during the probe trial. The latency to the target arm entry could therefore be regarded as a relevant motivation index.

4.3. Effects of systemic NMDA receptor blockade by MK801

NMDA receptor blockade by MK801 has been shown to prevent acquisition of a variety of learning and memory tasks. As was the case with scopolamine, rats given MK801 were able to improve their performance over days, and this was true for both training protocols used. At the end of the 4-day training period, performance of the rats subjected to the high dose of MK801 was close to that found in the control groups given saline or the low dose of the drug. With the 2-day training protocol, this improvement was less marked in the rats given the high dose of MK801, but the difference with both other groups was not sufficient to be evidenced statistically by a significant Group × Day interaction. We propose to interpret this improvement as we did for scopolamine: the formation of a consolidated memory for the platform location under the influence of NMDA receptor blockade was made possible by a longer training duration. With the low dose of MK801 and the short training protocol, rats were able to acquire the task and to retrieve the location of the platform. This is not surprising as the same dose of MK801 (0.03 mg/kg) was also found to be ineffective in a more complex task, namely a reference memory task in the water maze [27]. Regarding the preventive effects of increased training against the consequences of the high dose of MK801, it is worth mentioning that, in a T-maze, a dose of 0.06 mg/kg MK801 did not prevent acquisition of a reinforced alternation task [32] and did not disrupt the capability to perform a genuine place response in 7 out of 10 tested rats. The same has been observed in a water maze task [33]. The fact that training duration is a determinant factor as to whether MK801 has disruptive consequences or not on memory function is also consistent with the report by Caramanos and Shapiro [34]. Finally, along this line, although using a different experimental approach and another test, Saucier and Cain [35] reported that task familiarization by non-spatial pretraining protected against the disruptive effects of subsequent NMDA receptor blockade on water maze acquisition. MK801 making recall state-dependent for some authors ([36,41]; but see [37]), it could be argued, as for scopolamine, that the deficit found with the short training period was due to state-dependency. This possibility can be discarded on the basis of our observations after the lengthened training: rats given the drug before each of the four acquisition sessions but not before the probe trial were able to recall the platform location. It is also noteworthy that the effects of MK 801 confirm that the most informative variable to account for an established memory is the time spent in the target arm during the probe trial.

4.4. Procedural vs. declarative-like memory-based responses and drug effects

In an elegant experiment on the neuroanatomical substrates of place vs. response learning, Packard and McGaugh [38] demonstrated in rats, which were consistently released from the same start-arm and had to gain food consistently placed in the same goal-arm of a cross maze, that a majority of these animals first mastered the task with a place learning strategy engaging the hippocampus. Over further training, however, most of them shifted to a motor response strategy engaging the caudate nucleus. In this study, using reversible inactivations, the authors also demonstrated that, after extended training, the brain of the rats showing a caudate-dependent response responded so while the hippocampus-dependent place representation was still intact and could be retrieved upon request (e.g., following inactivation of the caudate nucleus). In fact, both types of memories cohabited but, after extensive training, only the expression of response learning was *driving* the rats' behaviour. The protocol which we used in our fourth experiment was partly inspired by Packard and McGaugh's experiment. We consistently released our rats from the same start arm (N) and the animals had to swim to a constant place where the platform was located (NW). Reaching the platform could thus be done by a hippocampus-dependent process (swim to the place) or by a process depending upon the caudate nucleus (make a response consisting in turning right and then again right). To test whether rats had learned a place or a response, we gave them a probe trial in which they were misled. In part of them, the misleading manipulation was marked, as these rats were released from an arm in which they had never entered before (S), and from where they had an initial perspective on the room cues never encountered earlier at a trials' start. In the other part, the misleading manipulation was much more subtle, as the rats were released from an arm in which they could enter during their training (NE). More importantly, however, this start arm proposed a perspective on the room cues, which, although different, was relatively similar to the one they have had during training. Indeed, the start point for the probe trial was displaced over only 60 cm aside from the start point used for all training trials, but remained on the same side of the maze. When the first response of the control rats (no centrally active

drug given during training) was taken into consideration, it clearly appeared that all rats released from the NE displayed an expression of response learning (100%; SCOMBr and NaCl control conditions collapsed), whereas a large majority of rats released from the S displayed an expression of place learning (77%). These observations clearly point to the fact that rats trained to acquire a motor response-based procedure can express both response learning and place learning in a misleading probe trial, but their initial approach of the task is strongly dependent on the view these animals have on their testing environment from the release point. Our results also demonstrate that the switch from one to the other memory system can be immediate when the view of the environment is very different from that of training, but does not occur when this view is less contrasted with that from the release point used for all training trials. Thus, it clearly appears from these findings that rats do not forget the hippocampus-dependent, allocentric representation of the location of this place, despite the development of a caudate-dependent, egocentric motor routine. In fact, the engagement of one or the other memory system is driven by the view these animals have on their environment on the start of a trial. When this view is relatively comparable from training trials to the probe trial, the rats behave on the basis of response learning. When this view is clearly different, most rats immediately switch to a place learning-based behaviour.

After having shown that under conditions of sustained training in the double-H maze, neither scopolamine nor MK801 prevented learning of the platform location (experiment 3 vs. experiment 2), we also found that, under training conditions using an identical number of trials with constant start- and goal-arms (i.e., 16), these drugs did not affect place learning (which confirms our findings in experiment 3), did not prevent response learning, and had no effect at all on the capability of rats to appropriately use one or the other memory systems, and even to appropriately switch from one to the other (response to place) in a misleading probe trial. These data further emphasize that the double-H maze test, in its currently used training and testing protocol versions, proposes a task which, in fact, may be considered relatively simple. Our data from the probe trials of the fourth experiment also indicate that within a single misleading trial, it is possible to know if a rat, of which the very first swim pattern indicates expression of response learning, is also able to switch to a memory for a place. Interestingly, in the rats given scopolamine during training, we observed a shorter time spent in the target arm during the probe trial as compared with the control condition, but only when the rats were released from the NE arm (see Fig. 10E). This observation can be paralleled with a former report by Poucet and Buhot [39], who demonstrated that scopolamine treatment was able to alter response-to-change capabilities, indicating impaired processing of distal information.

5. Conclusions

The current series of experiments are the first ones which we carried out to validate a novel spatial memory test termed the double-H maze test. We believe that because this test enables the formation of a relatively stable memory trace and enables some control over an animal's strategy, it could be particularly adapted to study the neurobiological substrates of (and the dynamic interplay between) procedural, caudate-dependent and declarative-like, hippocampus-dependent memory functions.

Conflict of interest

The authors have no conflict of interest to declare.

Role of funding sources

Our funding sources had no involvement in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

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