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# Behavioral characterization of metrifonate-improved acquisition of spatial information in medial septum-lesioned rats

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**Abstract**

We investigated the effects of acute oral pretraining treatment with an indirect acetylcholinesterase inhibitor, metrifonate, on water maze spatial navigation in medial septum-lesioned rats. We observed that metrifonate (30 mg/kg, orally) (1) does not alter the pattern of exploration of the lesioned rats at the water maze pool or retrieval of spatial memory, (2) effectively reverses the acquisition defect, (3) enhances reversal learning, and (4) improves acquisition of water maze navigation by facilitating the encoding of the spatial representation of a specific environment. These results indicate that metrifonate does not improve escape performance to the hidden platform by modulating exploration strategy, but that metrifonate enhances the speed and accuracy of development and durability of spatial memory engrams, and facilitates learning capacity that depends on activity of the septo-hippocampal projection. 

*Keywords:* Septum lesion, medial; Metrifonate; Spatial navigation; Spatial bias; Reversal learning

## 1. Introduction

The discovery of the cholinergic deficit has created interest in cholinesterase inhibitors as therapeutic agents for Alzheimer's disease (Bartus, 1979; Whitehouse et al., 1982; Bowen et al., 1983; Bartus et al., 1985; Bowen and Davison, 1986; Hagan and Morris, 1988; Dunnett et al., 1991; Fibiger, 1991; Dunnett and Fibiger, 1993; Wenk, 1993).

Metrifonate is a second-generation cholinesterase inhibitor that has an indirect mechanism of action, broad safety/efficacy ratio and long duration of action (Becker and Giacobini, 1988; Hinz et al., 1996a,b; Blokland et al., 1995; Schmidt et al., 1996). For example, we assessed the effects of daily pretraining treatment with metrifonate on spatial navigation in the water maze test in young medial septum-lesioned and scopolamine-treated rats (Riekkinen Jr. et al., 1996). We observed that daily pretraining treatment with metrifonate in a broad dose range (10–100 mg/kg, p.o.) counteracted to some extent the deleterious effect of scopolamine treatment or medial septum-lesioning on water maze spatial navigation.

However, it is difficult to interpret the improvement of water maze navigation performance by metrifonate treatment simply in terms of enhanced memory per se, as all previous studies assessed only the actions of treatments administered before daily training trials and used a fixed training schedule (Blokland et al., 1995; Van der Staay et al., 1996a,b; Nabeshima et al., 1995; Riekkinen Jr. et al., 1996).

The present study was designed to further clarify which behavioral processes important for water maze learning are modulated by metrifonate treatment in medial septum-lesioned rats. First, we evaluated the effect of metrifonate on the pattern of spatial exploration in the water maze pool in rats with no or weak (2 days) training, moderate (5 days) training or extensive training (9 days). It was assessed whether metrifonate decreases escape distance values by decreasing swimming in the incorrect peripheral zone, subsequently increasing the probability of finding the platform. Second, it was assessed whether daily metrifonate treatment during a 9-day training period was more effective than a treatment administered only during the first 3 training days. Third, we trained a group of rats to rapidly find the location of the hidden platform, and induced the

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lesions after this training stage. Then, the platform location was reversed and the effect of metrifonate on reversal learning was studied. Fourth, the study of Bannerman et al. (1995) revealed that the effects of drug treatment and brain lesion on spatial memory vs. other factors that may affect navigation behavior can be dissociated by the use of two separate water maze systems. Bannerman et al. (1995) reported that a NMDA receptor antagonist at a dose that blocks long-term potentiation impairs spatial navigation in the water maze. However, if the rats had been trained to find the hidden water maze escape platform in another room, treatment with a similar dose of NMDA receptor antagonist has no measurable effect on spatial navigation, suggesting that the NMDA receptor antagonist impairs some process other (attention to the cues surrounding the pool, development of expectancy about the presence of the platform) than memorization of the environmental cues (i.e., spatial map) (Bannerman et al., 1995). On the contrary, lesion of the hippocampus impaired spatial navigation in the water maze, and doing spatial navigation training in another room before the induction of the brain lesions did not protect from this defect. This reveals that hippocampal function is required for the development of spatial memory trace per se (Bannerman et al., 1995). In this study we tested in medial septum-lesioned rats whether spatial training conducted in another room (pool 1) before the induction of the brain lesions protects from spatial navigation defect in a water maze system located in a different room (pool 2). Furthermore, we wanted to test whether metrifonate can alleviate the remaining spatial memory failure (pool 2) induced by medial septum lesion. Finally, to further analyze the effect of metrifonate on spatial navigation we tested whether the medial septum-lesioned rats treated with metrifonate during training in pool 1 navigate more accurately in pool 2 with no drug treatment than do those rats that received no drug treatment during training in pool 1 or 2.

## 2. Materials and methods

### 2.1. Animals

Young (3-month-old; $n = 8$/group) male Han:Wistar rats were used in the study. The rats were housed three per cage in a controlled environment ($20 \pm 2$°C, humidity at 50–60%, light period 07:00–19:00 h). Food and water were available ad libitum.

### 2.2. Drugs

Metrifonate was donated by Bayer and was dissolved in 5% sodium citrate (pH 5.5, buffered with citric acid) and given orally (p.o.) 30 min before water maze testing. Metrifonate was administered to rats at 30 mg/kg, because we previously found the compound at this dose to improve water maze navigation and to be well tolerated (Blokland et al., 1995; Riekkinen Jr. et al., 1996). During the reversal learning study, the effects of an additional dose (10 mg/kg) were assessed. Controls received metrifonate vehicle injections (p.o.) of equal volume.

### 2.3. Surgery

Medial septum (A: 0.0 mm, M: 0.0 mm, D: −7.0 mm; relative to the bregma) lesions were made by passing an anodal DC current (2 mA, 5 s) via stainless-steel electrodes (Riekkinen Jr. et al., 1990, 1991a,b, 1994, 1996). Controls were treated identically, but no current was applied (sham-lesioned). The rats were deeply anesthetized with Equithesin during the operations.

### 2.4. Water maze

The starting locations, which were labelled north, south, east and west, were located arbitrarily on the pool rim (Riekkinen Jr. et al., 1990). The temperature of the water was $23 \pm 1.5$°C. The timing of the latency to find the submerged platform was started and ended by the experimenter. The water maze pool was divided into three annuli of equal surface area. The submerged platform was located in the middle annulus, except during the reversal learning training where it was temporarily shifted to the center of the pool for two trials. The swim pattern and distance were monitored with a computerized videotracking device. The computer calculated and stored the total distance swum (in cm) and the proportion of distance swum in different quadrants or three annuli of equal surface area, and the crossings over the platform location. The rats were placed in the water at random, with their noses pointing towards the wall, at one of the starting points. Animals which failed to locate the platform within the maximum time allowed were placed on the platform by the experimenter. Two separate testing pools were used: pools 1 and 2. They were of identical size and appearance, but were located in rooms that were of different size and shape. Furthermore, the cues that were placed around the pool were different. The platform was located in the southwest (pool 1) and northeast (pool 2) quadrant of these pools. However, during one of the experiments (the platform reversal training) that was conducted solely in pool 1 the location of the platform was reversed to novel positions after the training stage had been tested.

#### 2.4.1. Pattern of spatial exploration

The effect of metrifonate on the pattern of spatial exploration was assessed using the following training schedules. First, in one experiment, water maze training was done with no platform in the pool and the rats were allowed to swim freely in the pool for 60 s. Second, separate water maze training sessions tested the effects of metrifonate in medial septum-lesioned rats after 2, 5 or 9 days of training to find a hidden escape platform located in the southwest quadrant. In all these experiments the daily

training was similar (four trials of 70 s/day, 5 s reinforcement on the platform, 30-s intertrial interval). After the last training trial, metrifonate or vehicle was administered to the rats and the spatial bias (number of crossings over the previous location of the escape platform) was measured 30 min later (a single 70 s trial, no platform at the pool). Spatial bias was measured again 7 days later in the experiments that had 5 and 9 platform training days, and metrifonate or vehicle was again administered to the rats. The rats were housed in their home cages during this break of 7 days.

*2.4.2. Long vs. short duration of metrifonate treatment*

We compared whether, during a 9-day training, metrifonate treatment that continued for all of the training days would improve water maze escape performance more effectively than a treatment administered only during the first 3 training days. Those medial septum-lesioned rats that received metrifonate during the first 3 days received vehicle during the rest of the behavioral training days. The training schedule consisted of 9 consecutive days of testing. Four platform trials of 70 s were assessed per day. The platform location was kept constant (in the southwest quadrant) during this period of training. At each trial (day 1–9), the rats were allowed to stay on the platform for 5 s. If the rats did not find the platform during the maximum duration of the trial, the experimenter placed them on it for 5 s. A 30-s recovery period was allowed between the training trials. The spatial bias was measured on days 10 and 17 during a 70-s free swim trial. The rats recovered in their home cages and no drug treatment was given during this period.

*2.4.3. Platform reversal*

Next, the effects of medial septum lesioning on platform reversal performance were measured. Rats were trained to find a hidden platform from the southwest quadrant for days 1–3 by giving them daily five 70-s training trials. Again, a 5-s reinforcement on the platform was allowed. A 30-s recovery period was used between the trials. The sham- and medial septum lesions were induced on the following day and behavioral testing was continued after a recovery of 10 days. During day 14, metrifonate was administered before the behavioral testing was started. No platform was placed in the pool during the first trial of 50 s and the retention of the previous platform location was analyzed. Next, the location of the escape platform was reversed to the northeast quadrant and two trials of 70 s were run. On day 15, metrifonate was again administered before behavioral testing was started and the platform was reversed to the southwest quadrant. Two trials of 70 s were assessed. The difference in escape distance measured during the first and second trials after the single reversal during days 14 and 15 can be used as an index of one-trial reversal learning (the greater the difference, the better the one-trial learning).

*2.4.4. Two-pool training*

Finally, we conducted two separate experiments with an identical training scheme. Training was divided into two parts and was commenced in two separate and distinct rooms that each had a water maze testing system (pools 1 and 2). In these two experiments 5 training days (four 70-s trials in a day, 5-s reinforcement on the platform, 30-s intertrial interval) were assessed and the location of the platform was kept constant during all the days. After a break of 10 days another similar training period was run in the different pool. During the break the rats were allowed to recover in their home cages and no drug treatment was given.

In the first of the studies with two pools the rats were preoperatively trained to find a hidden escape platform in pool 1. The lesions were induced after spatial training in pool 1 was finished. Following a 10-day recovery, training in pool 2 was started. Metrifonate was administered only during the training conducted in pool 2.

In the second of the studies with two pools the rats were tested postoperatively (10-day recovery) in pool 1. Metrifonate was administered during this part of training only. Following completion of pool 1 training, a 10-day break was allowed. Next, training was started in pool 2 and no drug treatments were administered during this stage of training.

*2.5. Biochemistry*

The rats were decapitated and the brains were removed and dissected on ice 3 days after the behavioral testing. The hippocampi were removed bilaterally for biochemical analysis and stored at −75°C. The hippocampi of ten randomly selected controls and 40 lesioned rats were used for biochemical analysis. The method of Fonnum (1975) was used to analyze choline acetyltransferase activity. This method is based on the formation of $^{14}$C-labelled acetylcholine from [$^{14}$C]acetyl-coenzyme A and choline. Radiolabelled acetylcholine was extracted into the organic phase as an ion pair with teraphenylboron; the radioactivity of the organic phase was then measured with a liquid scintillation counter.

*2.6. Statistics*

The multiple analysis of variance and one-way analysis of variance followed by Duncan's post-hoc multiple group comparison was used to analyze group differences for the data collected during testing.

## 3. Results

*3.1. Pattern of spatial exploration*

During the initial free swim trial, the sham-lesioned and medial septum-lesioned vehicle- or metrifonate 30 mg/kg-treated rats swam an equal amount in different quadrants and annuli. The swimming distance in the cen-

tral annulus in which the platform was placed during the training trials was nearly identical in all groups (central annulus: sham-lesioned: 6 ± 3%, medial septum-lesioned vehicle and metrifonate: 7 ± 4% and 6 ± 3%; mean ± S.D.) during the 60-s free swim trial ($F(2,21) < 0.4$, $P > 0.05$; for all comparisons). Furthermore, during this free swim trial, none of the rats crossed over the standard location of the escape platform (southwest quadrant, central annulus), showing that there was no spontaneous 'spatial bias' in sham- or medial septum-lesioned rats.

Analysis of the data collected from the separate studies showed that the escape distance values of medial septum-lesioned rats were greater during the 2-, 5- or 9-day training periods ($F(2,21) > 16.0$, $P < 0.001$ for all comparisons). The spatial bias of medial septum-lesioned rats trained for 2 and 5 days (bias measured 1 and 7 days after the last training) was lower than that of the sham-lesioned rats ($F(2,21) > 9.0$, $P < 0.001$ for both comparisons) (Fig. 1, parts A and B). On the contrary, medial septum-lesioned rats performed as well during the spatial bias test as the controls after 9 days of training ($F(2,21) = 0.2$, $P = 0.81$), but performance was impaired after a 7-day forgetting period ($F(2,21) = 3.8$, $P = 0.04$) (Fig. 1, part C). Metrifonate 30 mg/kg given before the spatial bias tests did not significantly modulate the performance of the medial septum-lesioned rats trained for 2, 5 or 9 days ($P > 0.05$). However, metrifonate-treated medial septum-lesioned rats that were trained for 9 days tended to perform better during the second bias testing measured after the 7-day break than the lesioned rats treated with vehicle ($P > 0.05$).

### 3.2. Long vs. short duration of metrifonate treatment

The vehicle-treated medial septum-lesioned rats were impaired compared with the sham-lesioned rats (Group effect: $F(3,28) = 36.4$, $P < 0.001$), but the escape distance values of this medial septum-lesioned group also decreased during the training days (Day effect: $F(8,224) = 264.1$,

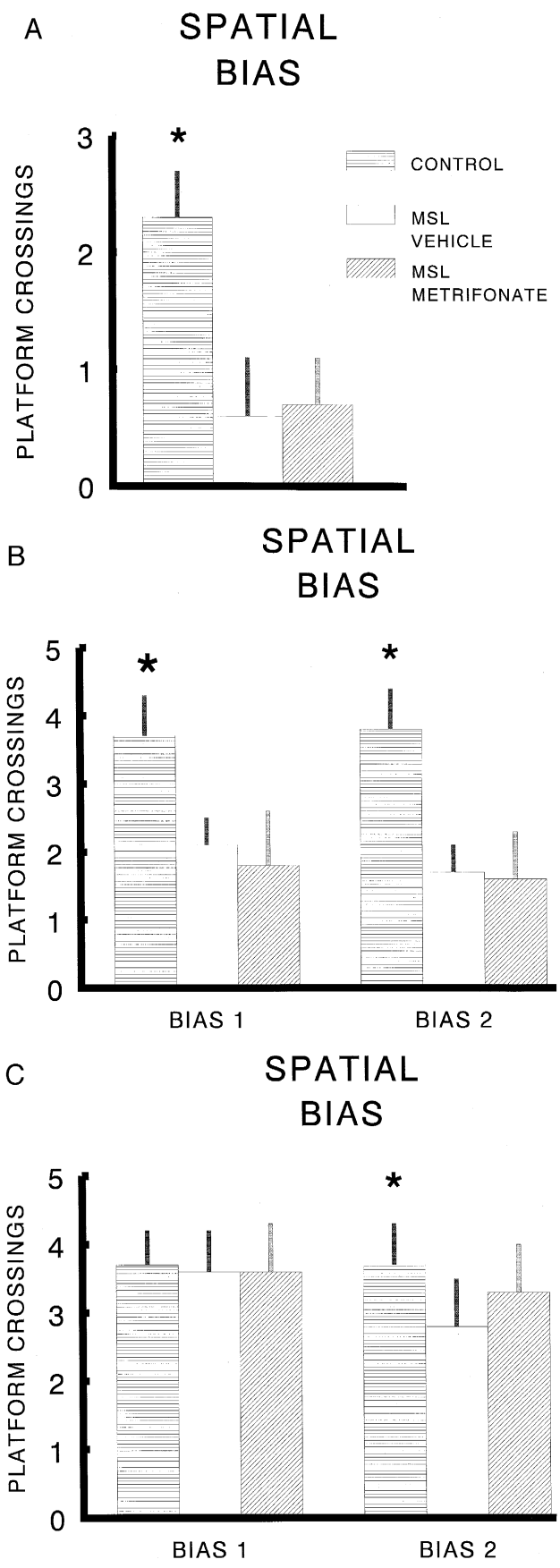


Fig. 1. Single administration of metrifonate (30 mg/kg, p.o., 30 min before testing) failed to improve spatial bias (the number of crossings over the previous location of the platform) in medial septum-lesioned rats. Groups: control = sham-lesioned + vehicle, MSL = medial septum-lesioned + vehicle, + MTF = medial septum-lesioned + metrifonate 30 mg/kg. Mean ± S.D. is shown. Part A: After a 2-day platform training the medial septum-lesioned rats had low spatial bias and the values were clearly lower than those for the sham-lesioned rats. * $P < 0.05$ vs. medial septum-lesioned rats, Duncan's post-hoc test. Part B: After a 5-day platform training the medial septum-lesioned rats showed clearly lower bias than the sham-lesioned rats after a 1-day (bias 1) or 7-day (bias 2) forgetting period. The bars represent the same groups as in part A. * $P < 0.05$ vs. medial septum-lesioned rats, Duncan's post-hoc test. Part C: After a 9-day platform training the medial septum-lesioned rats showed the same bias as the sham-lesioned rats after a 1-day (bias 1) forgetting period. However, spatial bias measured after a 7-day (bias 2) forgetting period was again decreased in medial septum-lesioned rats. The bars represent the same groups as in part A. * $P < 0.05$ vs. medial septum-lesioned vehicle-treated rats, Duncan's post-hoc test.

$P < 0.001$) (Fig. 2). Treatment with metrifonate 30 mg/kg during all 9 training days effectively decreased the escape distance of medial septum-lesioned rats (Group effect: $F(1,14) = 20.5$, $P < 0.001$). The other group of medial septum-lesioned rats received metrifonate 30 mg/kg treatment for only 3 days during the beginning of the training period. These metrifonate 30 mg/kg-treated medial septum-lesioned rats had shorter escape distance values than vehicle-treated medial septum-lesioned rats during the first 3 days (Group effect: $F(1,14) = 7.5$, $P = 0.016$). During the last 6 training days metrifonate treatment was discontinued and the performance of this group returned to the performance of the medial septum-lesioned rats treated with vehicle throughout the training period ($F(1,14) = 0.82$, $P = 0.38$). The spatial bias (number of crossings over the previous location of the platform in the probe trial) of vehicle-treated sham- and medial septum-lesioned rats was nearly identical when measured 24 h after the last training session ($F(3,28) = 1.8$, $P = 0.16$) (sham-lesioned: $3.8 \pm 0.4$, medial septum-lesioned + vehicle: $3.6 \pm 0.2$, + metrifonate for 3 days: $3.4 \pm 0.7$, + metrifonate for 9 days: $3.9 \pm 0.4$; mean $\pm$ S.D.; number of platform crossings). However, 7 days later the spatial bias of medial septum-lesioned rats was decreased ($F(3,28) = 7.8$, $P = 0.006$) (sham-lesioned: $3.7 \pm 0.3$, medial septum-lesioned + vehicle: $3.0 \pm 0.6$, + metrifonate for 3 days: $2.9 \pm 0.5$, + metrifonate for 9 days: $3.8 \pm 0.3$; mean $\pm$ S.D.; number of platform crossings). The group of medial septum-lesioned rats treated for 9 days with metrifonate 30 mg/kg was as accurate as sham-lesioned rats during the second spatial bias test ($P > 0.05$). On the contrary, the other two medial septum-lesioned groups were impaired compared with this group of medial septum-lesioned rats and the sham-lesioned group ($P < 0.05$).

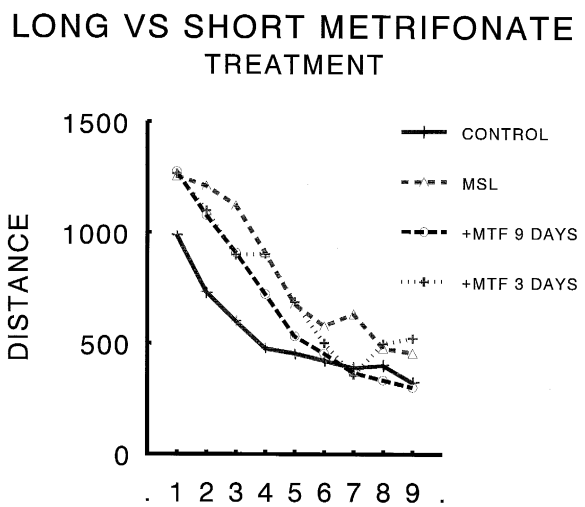


Fig. 2. Metrifonate treatment, administered 30 min before the training session on all the days, at the end of the training effectively reversed water maze failure induced by medial septum lesion. On the contrary, treatment with metrifonate for 3 days only temporarily facilitated water maze escape performance. The escape distance (cm; group daily mean) is shown on the *y*-axis and the training days on the *x*-axis. Groups: control = sham-lesioned + vehicle, MSL = medial septum-lesioned + vehicle, + MTF 9 days/3 days = medial septum-lesioned + metrifonate 30 mg/kg for all 9 training days or the first 3 training days.

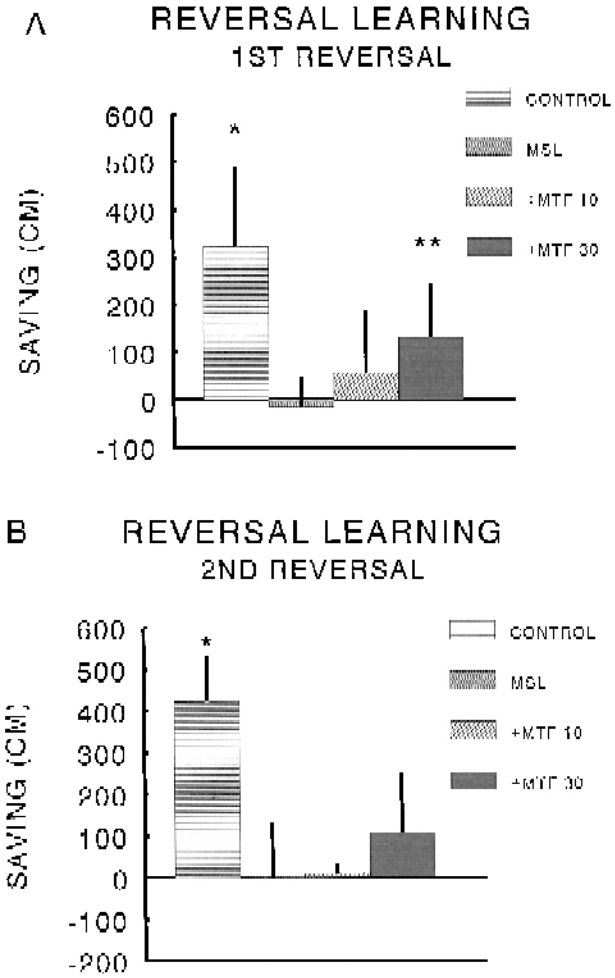


Fig. 3. Medial septum lesioning impaired performance during the platform reversal learning, and the effect was clearest after the first reversal of the platform. The index of one trial learning was calculated as the difference in escape distance between the first and the second trial (*y*-axis, cm) in the novel platform location. Metrifonate 30 mg/kg (30 min before testing, p.o.) improved reversal learning, but the smaller dose of 10 mg/kg had no effect. Group means $\pm$ S.D. are shown. Parts A and B show the reversal learning performance measured after the first and second platform reversal, respectively. Groups: control = sham-lesioned + vehicle, MSL = medial septum-lesioned + vehicle, + MTF 10 and 30 = medial septum-lesioned + metrifonate 10 and 30 mg/kg. $^{**}$ $P < 0.05$ vs. medial septum-lesioned rats; $^{*}$ $P < 0.05$ vs. vehicle-treated medial septum-lesioned rats.

### 3.3. Platform reversal

The escape performance of the rats that were sham- or medial septum-lesioned was identical during the preoperative training conducted on the first 3 days ($F(3,28) = 0.45$, $P = 0.7$) (data not shown). After this preoperative water maze training, the sham or medial septum lesions were induced on the fourth day of this study.

The spatial bias of the preoperatively learned platform location was accurate in all the sham- and medial septum-

lesioned groups during testing 10 days after sham or medial septum lesioning ($F(3,28) = 0.67$, $P = 0.8$) (platform crossings, group mean: sham-lesioned: 3.0, medial septum-lesioned + vehicle: 3.2; medial septum-lesioned + metrifonate 30 mg/kg: 3.2; medial septum-lesioned + metrifonate 10 mg/kg: 2.9). During the first reversal learning day after the recovery period, the measure for one-trial reversal learning, i.e., the difference in escape distance between the first and second platform trial was decreased by medial septum lesioning ($F(3,28) = 11.3$, $P < 0.001$), and metrifonate 30 mg/kg alleviated this defect ($P < 0.05$ vs. vehicle-treated medial septum-lesioned and sham-lesioned) (Fig. 3, part A). However, the smaller, 10 mg/kg, dose was ineffective to significantly improve the performance of medial septum-lesioned rats ($P > 0.05$).

The next day the platform was again reversed and medial septum-lesioned rats were impaired ($F(3,28) = 27.5$, $P < 0.001$) (Fig. 3, part B), as indicated by the smaller difference between the distance of the first and second trial of medial septum-lesioned than sham-lesioned rats. Again, metrifonate 30 mg/kg tended to improve the performance of medial septum-lesioned rats, but the effect was non-significant ($P > 0.05$). The smaller dose did not have any effect on the performance of the medial septum-lesioned rats ($P > 0.05$).

### 3.4. Two-pool training

The escape distance values of the different groups were equal during the training days tested in pool 1 and all the

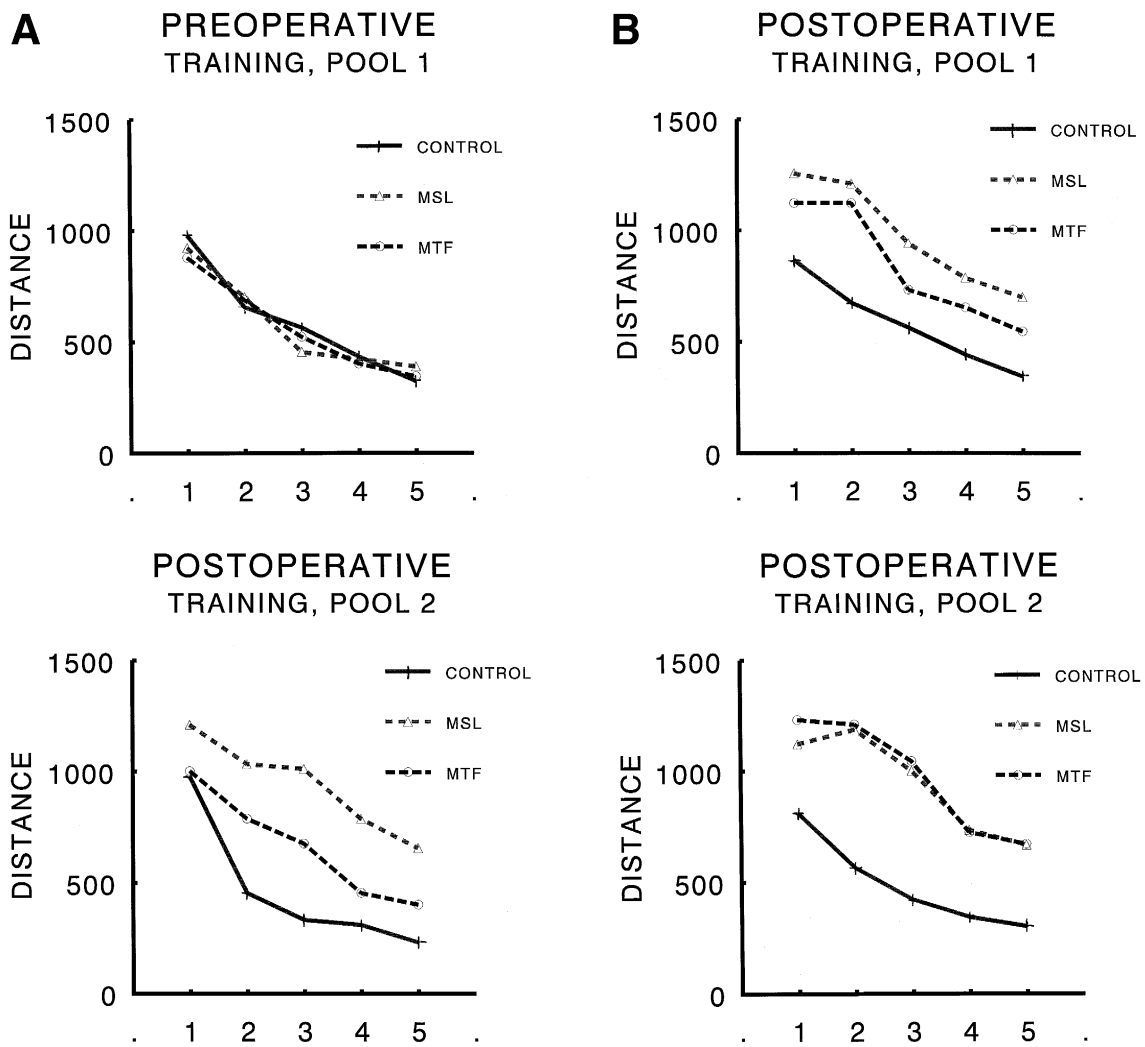


Fig. 4. The effects of metrifonate 30 mg/kg administration before the daily training on water maze spatial navigation failure induced by medial septum lesion. The escape distance (cm; group daily mean) is shown on the *Y*-axis. *X*-axis indicates training days. Groups: control = sham-lesioned + vehicle, MSL = medial septum-lesioned + vehicle, + MTF = medial septum-lesioned + metrifonate 30 mg/kg. Part A: Preoperative spatial training in pool 1 (top). The groups were sham- or medial septum-lesioned after this training stage. Training was continued 10 days later in pool 2 (bottom) and medial septum lesion impaired the water maze spatial navigation. Treatment with metrifonate 30 mg/kg during this stage of training facilitated spatial navigation by medial septum-lesioned rats. Part B: Postoperative spatial training in pool 1 showed that daily treatment with metrifonate 30 mg/kg before the training sessions improved acquisition performance of medial septum-lesioned rats (top). Training was continued in pool 2, but neither of the groups of medial septum-lesioned rats received metrifonate. The two groups of medial septum-lesioned rats were equally impaired during this stage of training (bottom).

groups showed similar learning curves ($F(2,21) = 0.5$, $P = 0.7$) (Fig. 4, part A). After this training period, sham and medial septum lesions were induced, and training in pool 2 was started 10 days later. During this stage sham-lesioned and half the medial septum-lesioned rats were treated with vehicle, and the escape distances of these groups differed significantly ($F(1,14) = 21.1$, $P < 0.001$). The remaining medial septum-lesioned rats that were treated with metrifonate 30 mg/kg had shorter escape distance values than did vehicle-treated medial septum-lesioned rats ($F(1,14) = 23.2$, $P < 0.001$).

In a separate experiment, another group of rats were sham- or medial septum-lesioned before behavioral training. In this experiment half the medial septum-lesioned rats received daily metrifonate 30 mg/kg or vehicle before water maze hidden platform training in pool 1 (Fig. 4, part B). The metrifonate 30 mg/kg-treated group performed better than the vehicle-treated medial septum-lesioned group in testing conducted in pool 1 ($F(1,14) = 20.1$, $P < 0.001$). Following the completion of testing in pool 1, testing continued in pool 2, but the metrifonate treatment was not continued during this stage of water maze training. The two groups of medial septum-lesioned rats were similarly impaired during this stage of training ($F(1,14) > 32.1$, $P < 0.001$ vs. sham-lesioned, for both comparisons) and no difference was found between the two groups ($F(1,14) = 0.4$, $P = 0.7$).

### 3.5. Biochemistry

Medial septum lesions decreased choline acetyltransferase activity in the hippocampi (control-lesioned: $1.19 \pm 0.10$, medial septum-lesioned + vehicle: $0.42 \pm 0.09$ nmol/mg protein per min; $P < 0.05$).

## 4. Discussion

In the present study we evaluated the effect of medial septum lesioning on spatial navigation, using different training schemes to analyze the effect of metrifonate treatment on the various mnemonic and non-mnemonic performance factors that can modulate spatial navigation.

We observed that metrifonate given to medial septum-lesioned rats (1) does not alter the spatial pattern of exploration at the water maze pool or retrieval of spatial memory, (2) effectively reverses the acquisition defect, (3) enhances reversal learning and (4) improves the acquisition of water maze navigation by facilitating the encoding of the spatial representation of a specific environment.

We tested if metrifonate can modulate retrieval of spatial memories. It is relevant to first discuss the effect of medial septum lesion on the development of spatial bias in rats. In our control experiment, we found that a medial septum lesion did not affect the swimming pattern during a free swim trial (no earlier training trials) and metrifonate did not alter the swimming pattern of rats that had not been trained to find the platform. On the contrary, a medial septum lesion disrupted acquisition of spatial memory that was reflected as a decrease of spatial bias measure. The spatial bias test carried out with the postoperatively tested sham- and medial septum-lesioned rats, trained for 2, 5, or 9 days to find the submerged platform, revealed that the sham-lesioned rats developed spatial bias more rapidly than did medial septum-lesioned rats (Fig. 1A–C). Comparison of the rats trained for 9 days revealed no lesion-induced defect of spatial bias and also the escape distance values during the end of the training period were nearly as low as those of the controls. However, the spatial memory trace of medial septum-lesioned rats trained for 9 days was more labile, as after a break of 7 days the bias values were lower than those of the controls. On the contrary, rats that were preoperatively trained to find a hidden platform were as accurate as sham-lesioned rats during the spatial bias test (see Section 3.3; values in parentheses). Therefore, accurate acquisition and use of previously well learned spatial memory is possible for the medial septum-lesioned rats, but disruption of the septohippocampal system retards the speed of learning and weakens the strength of memory engrams. It is possible that the lack of an effect of a medial septum lesion on the retention of a preoperatively learned spatial engram is due to partial dysfunction of the hippocampus, that is less deleterious to spatial navigation than a complete lesion of the hippocampus.

The present results showed that metrifonate did not enhance retrieval of the platform location learned before or after induction of the lesion, as indicated by unchanged spatial bias values. The use of medial septum-lesioned rats that were trained for 2, 5 or 9 days shows that the failure to observe any improving action of metrifonate on spatial bias was not due to a ceiling or floor effect contributed by too good or inaccurate memory retention. Therefore, the present results suggest that the enhanced acquisition performance induced by metrifonate that had been observed in medial septum-lesioned rats (Riekkinen Jr. et al., 1996) and confirmed in the present set of experiments is not related to alternation of swim pattern, such as an increased tendency to swim in the middle sector containing the platform. Furthermore, metrifonate treatment may improve the acquisition of reference memory more effectively than retrieval function.

It is noteworthy that we found in the present study that metrifonate not only increased the speed of learning during the early days of training, but also improved the accuracy of escape performance to the control level at the end of the training period. Furthermore, the spatial bias of medial septum-lesioned rats that were treated with metrifonate did not decline during the forgetting period as it had in the other groups of medial septum-lesioned rats. On the contrary, the 3-day treatment with metrifonate at the beginning of the escape training stimulated performance only during the treatment period, and the navigation accuracy was

similar to that of the vehicle-treated group during the rest of the training period. Interestingly, Blokland et al. (1995) showed that metrifonate treatment enhanced water maze escape performance of aged rats and the effect was more pronounced during the second week of the training period. Therefore, the present and previous data of Blokland et al. (1995) show that no tolerance develops to the effect of metrifonate during repeated administration and that metrifonate may effectively enhance the maximum performance accuracy in a spatial navigation task.

We observed that the reversal learning (measured after the platform reversal) was impaired by medial septum lesions in rats that were trained preoperatively to find the hidden platform from one fixed location in the pool. On the contrary, retention of the platform location that was preoperatively trained was unaffected, suggesting that the medial septum lesion did not impair the reference memory component of the water maze performance. Therefore, the defect during the platform reversal sessions induced by the lesion can be related to impaired working memory or extinction of a previously reinforced response.

We found that 30 mg/kg metrifonate enhanced reversal learning performance, but that the lower 10 mg/kg metrifonate dose was less effective. The dose-response relation is in good agreement with that in our earlier study investigating the effect of metrifonate to improve water maze spatial reference memory in different rat models (Riekkinen Jr. et al., 1996). The similarity of the metrifonate doses that improved the initial acquisition and reversal learning in medial septum-lesioned rats raises the possibility that metrifonate may produce these cognition-enhancing actions via the same neurochemical mechanism of action. However, two distinct possible behavioral processes exist, that could be modulated by the treatment and that could mediate the effect of metrifonate on reversal learning. First, metrifonate may facilitate working memory function and enhance the induction of a memory trace about the novel relationship between the specific environment cues surrounding the pool and the new location of the platform. Second, metrifonate may restore the behavioral flexibility of the medial septum-lesioned rats and normalize the speed of extinction of the previously learned escape strategy that has become irrelevant. Our results possibly favour the latter theory, as metrifonate significantly improved reversal learning measured only immediately after the spatial bias test. In contrast, only a non-significant trend was observed after the second platform reversal. Therefore, it is possible that metrifonate facilitated extinction of the previous platform location during or immediately after the spatial bias test, resulting in improved reversal learning performance.

The present study also showed that metrifonate improves water maze navigation by enhancing the memorization of spatial representation of a specific environment in medial septum-lesioned rats. Bannerman et al. (1995) reported that a hippocampal lesion impaired spatial navigation despite spatial pretraining in a different pool located in a room different from that used for postoperative water maze testing. They (Bannerman et al., 1995) suggested that the hippocampus is involved in the processing of spatial memory about the specific environment surrounding the water maze pool and that the hippocampus-lesioned rats were impaired by their inability to learn the relationship between the escape platform and extra-maze cues surrounding the pool. Our results suggest that proper functioning of the septohippocampal projection is important for the activity of the hippocampus in this case. We observed that despite preoperative spatial navigation training in pool 1, medial septal-lesioned rats were impaired compared with the sham-lesioned rats for navigating to the platform in pool 2. Therefore, this result indicates that similarly to a hippocampal lesion (Morris et al., 1990; Bannerman et al., 1995) the encoding of the specific extra maze cues, and the relationship between the escape platform and these cues surrounding the water maze pool are hampered by medial septum lesioning.

More interestingly, the present data suggest that metrifonate facilitates spatial memory and does not modulate the development of escape strategies that could be used to guide navigation independently of the knowledge about the relationship between the platform and the extra maze cues. In one experiment rats were preoperatively trained in pool 1. In that study, during postoperative training at pool 2, metrifonate administration was started and the treatment enhanced the navigation performance of medial septum-lesioned rats. On the contrary, the other study conducted with two water maze systems revealed that metrifonate treatment improved postoperative navigation performance in medial septum-lesioned rats tested in pool 1. However, metrifonate treatment was not continued during further testing in pool 2 and the two groups of medial septum-lesioned rats (treated with vehicle or metrifonate during training in pool 1) were equally impaired. Thus, metrifonate treatment may alleviate the impairment of short-term spatial memory function, resulting in defective memorization of the relationship between the escape platform and the extra maze cues in medial septum-lesioned rats.

In conclusion, the present results showed that, in medial septum-lesioned rats, treatment with metrifonate improved spatial navigation without altering the swim patterns, had a negligible effect on retrieval of spatial information but increased the speed, accuracy and stability of induction of spatial memory traces. It is interesting to compare the effect of medial septum lesion and previously published data about the effects of hippocampus lesion on water maze spatial navigation. Hippocampal damage disrupts postoperative acquisition and retention of spatial memories acquired earlier, and also despite the fact that preoperative training severely impairs postoperative acquisition of spatial navigation (Morris et al., 1990; Bannerman et al., 1995). Thus, the qualitative profile of impairment of spatial navigation produced by medial septum lesion is quite

similar to that produced by hippocampal damage, but the severity of performance defect is considerably less. Indeed, it is likely that the medial septum lesion may disrupt the control by the septohippocampal projection over the atropine-sensitive hippocampal rhythmical slow wave (Whishaw and Petrie, 1988) activity that is normally generated by exploratory activity, resulting in impaired processing of spatial memory traces. It is, therefore, tempting to speculate that metrifonate treatment may enhance memory that depends on the functioning of the hippocampus. It is noteworthy that metrifonate improved the performance of medial septum-lesioned rats as assessed with several different training schemes that test separate aspects of spatial memory (speed of induction and durability of reference memory trace, reversal learning) and are sensitive to detect hippocampal dysfunction (Morris et al., 1990; Bannerman et al., 1995).

The present results are also relevant for the development of treatment strategies for Alzheimer's disease that is associated with hippocampal dysfunction and memory loss early in the course of the disease. Our present data suggest that metrifonate may be a potential cognitive enhancer that could alleviate the impairment of memory function in Alzheimer's disease.

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