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Characterization of learning and memory behaviors and the effects of metrifonate in the C57BL strain of mice

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Abstract

In the near future, a number of transgenic mouse models with neuropathological characteristics of Alzheimer's disease are expected to become widely available. It will be important to characterize their behavior in models for learning and memory. As a first step, we have characterized normal, medial septal-lesioned and hippocampal-lesioned C57BL mice, in different behavioral tests, i.e., water maze spatial navigation, Y-maze and passive avoidance behavior. These experiments were complemented by an investigation of the effects of acute treatment with an acetylcholinesterase inhibitor, metrifonate, in these behavioral tests. Normal C75BL mice perform very well in the water maze and the Y-maze, but suboptimally in the passive avoidance task. Lesioning of the medial septum or the dorsal hippocampus clearly impaired the performance of the mice. In medial septal-lesioned mice, metrifonate stimulated spatial navigation and alleviated the loss of activity in the Y-maze and passive avoidance. In hippocampal-lesioned mice, metrifonate had no effect on spatial navigation. It is concluded that C75BL mice are useful for testing in classical models for learning and memory, and that septohippocampal pathology is very likely to induce cognitive deficits in some of these models. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Medial septum; Hippocampus lesion; Metrifonate; Spatial navigation; C57BL; Alzheimer's disease; (Mouse)

1. Introduction

The development of transgenic animal models for Alzheimer's disease has opened a new era in the study of the neurobiology and treatment of memory dysfunctions that result from pathology of the medial temporal lobe memory systems. There are now transgenic mouse models that mimic at least to some extent, the pathology of Alzheimer's disease (Games et al., 1995; Hsiao et al., 1996). The pathological changes in these models are initially restricted to the temporal lobe area but spread to other brain areas later. The affected areas contain diffuse plaques and later neuritic plaques also. A cognitive defect is also present (Hsiao et al., 1996; Nalbantoglu et al., 1997). These transgenic animals are expected to be far better models for Alzheimer's disease research than the previous animal models which were based on ageing, basal forebrain lesioning or acetylcholine receptor block. These models failed to reproduce the neuropathology (cell and synapse loss, accumulation of plaques and tangles) observed in the hippocampus and the surrounding medial temporal lobe cortical areas of Alzheimer patients.

The best evidence supporting the role of cholinergic pathology in the development of cognitive and noncognitive dysfunction is provided by studies showing that acetylcholinesterase inhibitors can diminish the severity of dementia, improve cognitive functioning and decrease neuropsychiatric problems in Alzheimer's disease (Eagger et al., 1992; Morris et al., 1998). However, even though cholinesterase inhibitors can to some extent diminish the severity of dementia in Alzheimer's disease, their effect on memory function is still unspectacular. It is possible that the atrophy of the hippocampus and surrounding cortical areas which starts during the early stage of Alzheimer's disease can block the memory-improving effect of cholinesterase inhibitors (Riekkinen et al., 1995, 1998).

Metrifonate is a second generation cholinesterase inhibitor that acts as prodrug for a long-acting metabolite and appears to have a broad therapeutic index (Schmidt et al., 1998). There are earlier experimental reports that metrifonate treatment increases acetylcholine levels (Mori et al., 1995; Scali et al., 1997) and facilitates cognitive

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functioning in rats and rabbits (Schmidt et al., 1997). For example, a medial septal lesion can remove most of the cholinergic input to the hippocampus and disrupt spatial memory in rats (Hagan and Morris, 1988). Metrifonate can dose dependently completely reverse the spatial memory failure in medial septal-lesioned and aged rats (Riekkinen et al., 1996).

A commonly used species for Alzheimer's transgenic manipulations is the C57BL-mouse. However, few studies have examined the effects of lesions of those brain areas involved in memory on cognition in this mouse strain. We designed the present study to characterize the behavior of neurologically intact, medial septal or hippocampal-lesioned C57BL mice in models of learning and memory. Furthermore, we wanted to elucidate whether the C57BL mouse is sensitive to the cognition-enhancing actions of metrifonate.

2. Materials and methods

2.1. Animals

Young (3 to 4 months old; $n = 184$) female C57BL/6J//Kuo mice were used in the present study. The mice were housed five per cage, except for the lesioned mice, which were housed one per cage. The environmental conditions were controlled and constant ($21 + 1$°C, humidity at $50 + 10$%, light period 0700–1900). Food and water were available ad libitum. The study plan was approved by the municipal government of Kuopio county.

2.2. Drugs

Metrifonate (a donation from Bayer) was dissolved in NaCl 0.9% and injected intraperitoneally (i.p.) at 5, 15 and 50 mg/kg (10 ml/kg). Controls received vehicle injections of an equal volume. The groups received two injections: 30 min before and immediately after the daily water maze, Y-maze or passive avoidance training. Some groups received metrifonate before the training and vehicle after the training, and separate groups received vehicle before the training and metrifonate after the training. No drug or vehicle treatment was given during the passive avoidance testing day.

2.3. Surgery

Medial septal (A: 0.9 mm, M: 0.0 mm, D: −4.7 mm; relative to the bregma) lesions were made by passage of an anodal DC current (1 mA, 15 s) via tungsten electrodes (diameter 0.0625 mm, 1.0 mm tip uninsulated). Sham-lesioned mice were treated identically, but no current was applied. Hippocampal (A: −2.3 mm, M: +1.0 mm, D: −2.4 mm; A: −2.9 mm, M: +1.8 mm, D: −2.2 mm relative to the bregma) lesions were made by passage of an anodal DC current (1 mA, 10 s) via tungsten electrodes (diameter 0.0625 mm, 0.5 mm tip uninsulated). Sham-lesioned mice were treated identically but no current was applied. The mice were deeply anaesthetised with a 1:1 mixture of Dormicum (Roche) and Hypnorm (Janssen Pharmaceutica) (s.c.) during the operations and for analgesia were given a 0.1 mg/kg injection of buprenorfine (Temgesic; Reckitt & Colman) (s.c.) after the surgery. The mice were allowed to recover from the surgery for 2 weeks before the start of the first experiments.

2.4. Water maze

We used a black plastic circular pool, diameter 120 cm, and a black painted stainless steel square platform; 14×14 cm, 1.0 cm below the water line. The pool was divided into three rings of equal surface area, and the escape platform was always in the middle ring. The starting locations, which were labelled North, South, East and West, were located arbitrarily on the pool rim. The timing of the latency to find the submerged platform was started and ended by the experimenter. A computer connected to an image analyser (HVS Image, Hampton, UK) monitored the swimming pattern, escape distance and escape latency. The mouse was placed in the water with its nose pointing towards the wall at one of the starting points in a random manner. If the mouse failed to find the platform in the maximum time, it was placed there by the experimenter. The mice were allowed to stay on the platform for 5 s. A 30-s recovery period was allowed between training trials. The temperature of the water was kept constant throughout the experiment (20.5 ± 0.5°C).

The training schedule for intact and medial septal-lesioned mice consisted of 8 consecutive days of testing. Four platform trials of 60 s were assessed per day during the first 5 training days. The platform location was kept constant (the Southwest quadrant) during this period of training. On the sixth day, the platform was removed from the pool and the mice were allowed to swim for 50 s. Immediately after this spatial bias test, the platform was placed in the Northeast quadrant and five 50-s platform trials were assessed. The schedule on the seventh day also consisted of five 50-s platform trials (platform in the Northeast quadrant). The water maze experiment was finished on the eighth day with a spatial bias test (a 50-s trial without the platform).

The training schedule for hippocampal-lesioned mice consisted of 15 consecutive days of testing. Four platform trials of 60 s were assessed per day during the first 10 training days. The platform location was kept constant (the Southwest quadrant) during this period of training. For the last 5 days, the location of the pool was changed to another room with clearly different visual cues, and no drug treatments were administered. The location of the platform was

changed to the Southeast quadrant and four platform trials of 60 s were assessed per day.

During the platform training trials, the following parameters were measured: escape latency, escape distance, percentage of animals that found the platform and swimming speed. During the spatial bias test, the number of counter-crossings was measured. Counter-crossing was defined as crossing a circular area in which the platform had previously been located and which was three times larger than the platform (radius = 12.1 cm).

2.5. Y-maze

The Y-maze experiment was started 24 h after the water maze testing sessions. The Y-maze used in this experiment had black plastic walls 10 cm high. Its arms consisted of three compartments (10 cm × 10 cm) connected with 4 cm × 5 cm passages. The mouse was placed in one of the arm compartments and was allowed to move freely for 6 min without reinforcers. An arm entry was defined as complete entry of the body of a mouse, except for its tail, in an arm compartment. The sequence of arm entries was recorded manually. An alternation was defined as the entry into all three arms on consecutive choices. The number of maximum alternations was then the total number of arms entered minus 2, and percent alternation was calculated as (actual alternations/maximum alternations) × 100. The test was run on 2 consecutive days. Spontaneous alternation behavior is considered to reflect a primitive form of spatial working memory. This test was not performed with hippocampal-lesioned mice.

2.6. Passive avoidance

The passive avoidance training trial was performed immediately after the Y-maze trial. The passive avoidance box consisted of a lit and a dark compartment. During the training trial, the mice were placed in the lit compartment and 30 s later the sliding guillotine door was opened. After the mice entered the dark compartment (the latency was measured), the door was closed and a foot shock of 0.1 mA (0.5 s) was given. Then the mice were returned to their home cage and 24 h later they were again placed in the lit compartment and the latency to enter the dark compartment was measured (900 s maximum latency). The final latency was defined as the difference between the latencies on the first and the second day.

2.7. Histology

After the last day of testing, the lesioned mice were decapitated. The brains of the mice were removed and immersed for 1–2 days in phosphate-buffered 4% formalin solution. 50 μm sections were cut with a vibrating microtome and the sections were stained. The lesioned areas were stained with cresyl fast violet to identify the position of the lesion. The hippocampal sections of medial septal-lesioned mice were stained with acetylcholinesterase staining (Hedreen et al., 1985; a butyrylcholinesterase inhibitor was included in the assay mixture) in order to confirm the decrease in acetylcholine-containing fibers in the hippocampus.

2.8. Statistics

We evaluated the effect of the drugs on escape latency and swimming speed, using an analysis of variance (ANOVA) for repeated measurements. We also assessed the effect of the drugs on the ability of the mice to find the platform, using the Mann–Whitney *U*-test for two independent samples. The Bonferroni correction was used with the ANOVA and Mann–Whitney *U*-test. For analysis of

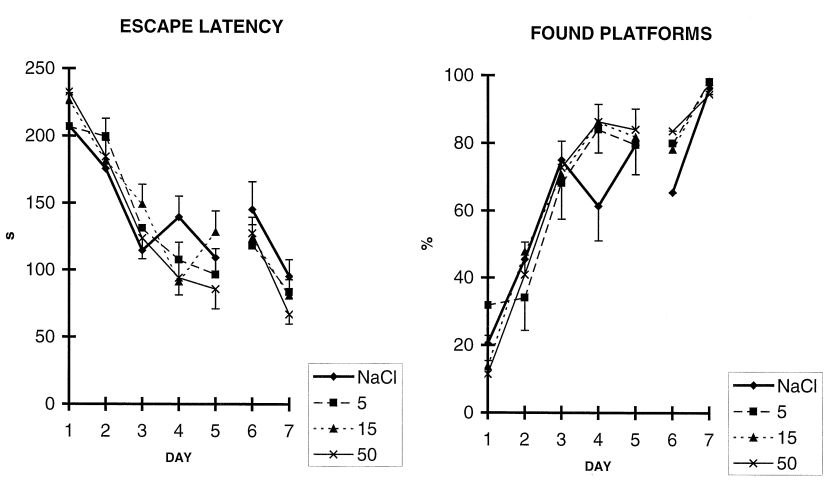

Fig. 1. The effect of administration of metrifonate 5, 15 and 50 mg/kg 30 min before the daily training on intact mice. The *X*-axis indicates the reference memory training days 1–5 (fixed platform location) and reversal learning days 6–7 (reversal of the platform location). The values are daily group means + S.E.M. There were no significant differences between the groups. The number of mice in each treatment group: vehicle: $n = 11$, 5 mg/kg: $n = 11$, 15 mg/kg: $n = 11$, 50 mg/kg: $n = 11$.

Table 1
The effect of metrifonate on swimming speed and bias performance of intact, medial septal- and hippocampal-lesioned mice

		Speed		Counter crossings	
		Days 1–5	Days 6–7/ 10–15[a]	Day 6	Day 8
	vehicle	17.6±2.8	14.9±2.5	2.3±1.7	3.7±1.6
Intact	5	18.9±2.9	16.1±4.0	2.1±1.7	3.5±1.9
Pre	15	18.9±2.7	15.5±5.6	1.6±1.2	5.4±3.0
	50	16.0±2.8	14.8±4.4	3.2±3.1	5.4±2.4
Intact	vehicle	16.8±1.6	16.0±3.3	2.8±1.4	3.9±2.8
Post 1	5	18.9±3.7	17.3±5.5	1.3±1.2	2.3±2.0
	15	18.4±4.9	14.4±7.6	1.6±1.5	1.7±2.0
Intact	vehicle	17.6±2.8	14.9±2.5	2.3±1.7	3.7±1.6
Post 2	50	20.3±1.8	11.8±4.1	1.0±1.4	4.1±1.9
	SHAM	21.3±2.0	21.2±3.3	5.4±3.7	7.7±3.0
MS-lesion	vehicle	17.7±3.5	18.2±2.3	3.6±1.7	4.0±2.4[b]
Pre	15	22.5±2.4	22.6±3.8	5.0±2.6	3.6±2.6[b]
	50	21.1±2.2	23.3±2.4	4.6±2.3	3.5±2.2[b]
MS-lesion	vehicle	17.0±3.1	–	5.0±2.2	–
Post	50	15.9±4.6	–	4.4±1.7	–
	SHAM	16.1±4.7	14.2±6.8	–	–
HC-lesion	vehicle	17.9±2.8	14.6±6.0	–	–
Pre	15	17.4±1.1	14.3±6.6[c]	–	–
	50	17.6±2.0	14.4±3.9[c]	–	–

Intact pre = Intact mice, metrifonate administered before the training; Intact post 1 = Intact mice, metrifonate administered after the training I; Intact post 2 = Intact mice, metrifonate administered after the training II; MS-lesion pre = medial septal-lesioned mice, metrifonate administered before the training; MS-lesion post = medial septal-lesioned mice, metrifonate administered after the training; HC-lesion pre = hippocampal-lesioned mice, metrifonate administered before the training.
[a] Days 10–15 for hippocampal-lesioned mice, days 6–7 for other groups.
[b] $P < 0.05$ vs. sham-lesioned group.
[c] Metrifonate 15 and 50 mg/kg—pretreated group.
The speed values are cm/s. All values are expressed as means ± S.D.

the activity in Y-maze, we used a one-way analysis of variance followed by Scheffe's post-hoc multiple group comparison, and for analysis of the behavior in passive avoidance testing we used the Kruskall–Wallis test and Mann–Whitney *U*-test.

3. Results

3.1. Intact mice, metrifonate administered before and after the training

3.1.1. Water maze

During the first 5 training days, metrifonate administered before (5, 15 and 50 mg/kg) the daily training had no effect on the number of platforms found ($P > 0.05$), escape latency or swimming speed (group: $F(3,40) < 2.65$, $P > 0.05$; Fig. 1, Table 1). Metrifonate administered after (I: 5 and 15 mg/kg (Fig. 2); II: 50 mg/kg (Fig. 3)) the daily training had no effect on escape latency or swimming speed (group: $F(2,19)/(1,17) < 3.13$, $P > 0.05$; Table 3), but it decreased the probability of platform finding at the dosages of 5 and 15 mg/kg ($P < 0.027$; Fig. 2). There were no group differences in counter-crossings during the first bias assessment ($F(3,40)/(2,19)/(1,17) = 3.024$, $P > 0.05$; Table 3).

During the platform reversal stage on days 6 and 7, metrifonate treatment before or after the training had no effect on escape latency (Figs. 1–3) or swimming speed (group: $F(3,40)/(2,19)/(1,17) = 4.07$, $P > 0.05$; Table 3). However, metrifonate 15 mg/kg administered after the training did decrease the number of mice that found the platform ($P < 0.001$; Fig. 2). During the second bias assessment measured on the eighth testing day, there were no

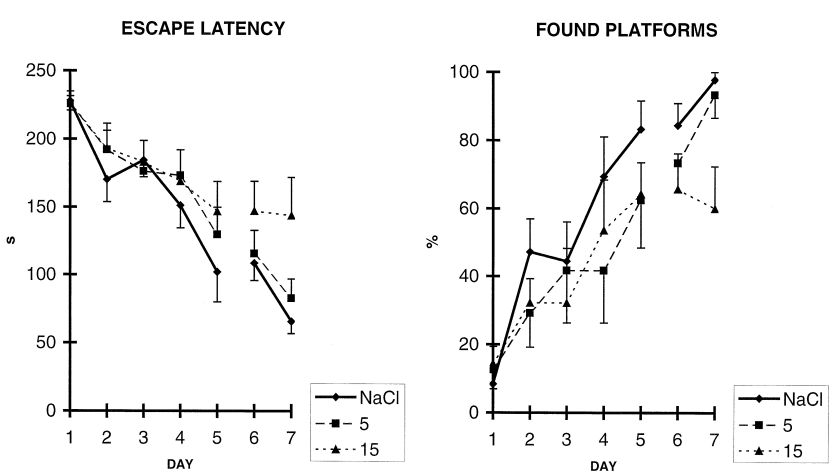

Fig. 2. The effect of administration of metrifonate 5 and 15 mg/kg immediately after the daily training on intact mice. The *X*-axis indicates the reference memory training days 1–5 (fixed platform location) and reversal learning days 6–7 (reversal of the platform location). The values are daily group means + S.E.M. There were no differences in escape latency, but the probability of platform finding was decreased by doses 5 and 15 mg/kg during days 1–5 ($P < 0.05$) and by dose 5 mg/kg during days 6–7 ($P < 0.001$). The number of mice in each treatment group: vehicle: $n = 9$, 5 mg/kg: $n = 6$, 15 mg/kg: $n = 7$.

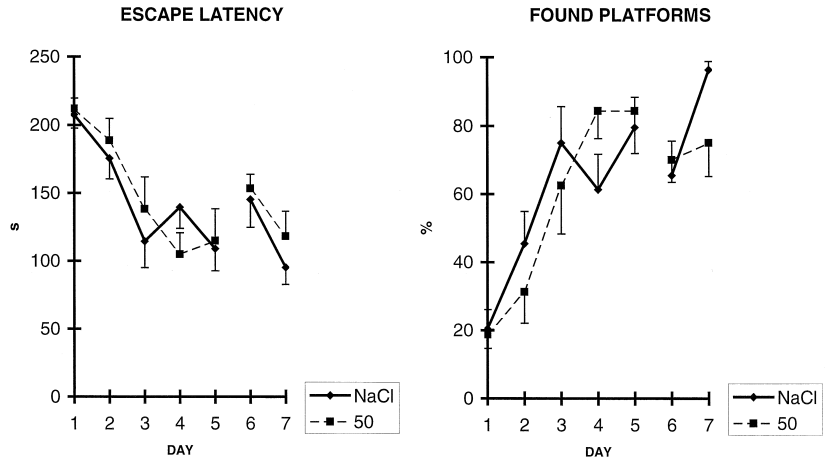

Fig. 3. The effect of administration of metrifonate 50 mg/kg immediately after the daily training on intact mice. The *X*-axis indicates the reference memory training days 1–5 (fixed platform location) and reversal learning days 6–7 (reversal of the platform location). The values are daily group means + S.E.M. There were no significant differences between the groups. The number of mice in each treatment group: vehicle: $n = 11$, 50 mg/kg: $n = 8$.

differences in counter crossings ($F(3,40)/(2,19)/(1,17) < 1.853$, $P > 0.05$; Table 3).

3.1.2. Y-maze and passive avoidance

There were no group differences in total moves or percent alternation between any of the groups in the Y-maze ($F(3,40) < 3.278$, $P > 0.05$, Table 2). For passive avoidance, there were no group differences in the latency to enter the dark compartment during the training day when metrifonate was administered before the training ($P > 0.05$). When metrifonate was administered after the training, there were no group differences in the latency to enter the dark compartment during the training day ($P > 0.05$) or any difference between entry latencies during the testing day and the training day ($P > 0.05$; Table 2).

Table 2
The effect of metrifonate on Y-maze and passive avoidance behavior of intact and medial septal-lesioned mice

		Y-maze		PA	
		Tot	%	Day 1 (s)	Diff. (s)
	vehicle	19.3 ± 11.3	61.5 ± 13.6	12.7 ± 9.05	176.4 ± 104.9
Intact	5	17.9 ± 4.4	65.7 ± 18.7	32.7 ± 69.0	311.8 ± 342.7
Pre	15	16.0 ± 12.2	60.0 ± 25.0	12.7 ± 6.5	404.3 ± 354.3
	50	14.8 ± 4.9	66.1 ± 12.0	9.6 ± 1.5	269.1 ± 147.9
Intact	vehicle	17.3 ± 4.9	55.2 ± 8.7	26.7 ± 37.3	453.3 ± 222.7
Post 1	5	18.7 ± 5.5	66.7 ± 21.0	9.2 ± 3.8	723.3 ± 183.0
	15	17.7 ± 5.9	66.3 ± 12.6	9.3 ± 3.5	445.0 ± 308.4
Intact	vehicle	19.3 ± 11.3	61.5 ± 13.6	12.7 ± 9.1	176.4 ± 104.9
Post 2	50	22.8 ± 5.9	67.6 ± 12.0	56.3 ± 86.0	423.8 ± 302.9
	SHAM	22.7 ± 8.8	61.1 ± 8.9	39.0 ± 45.8	146.5 ± 109.2
MS-lesion	vehicle	3.8 ± 4.2[a]	30.0 ± 28.3	247.7 ± 116.3[a]	449.6 ± 257.9[a]
Pre	15	10.2 ± 12.0	43.8 ± 6.3	248.6 ± 90.4[a]	388.2 ± 231.2[a]
	50	19.3 ± 15.2[b]	50.0 ± 15.6	169.0 ± 124.7[a]	358.5 ± 286.8

The effects of metrifonate on Y-maze and passive avoidance tests with intact animals (metrifonate 5, 15 and 50 mg/kg administered before and after the daily training) and medial septal-lesioned animals (metrifonate 15 and 50 mg/kg administered before the daily training). These tests were not performed with other groups. Intact pre = Intact mice, metrifonate administered before the training; Intact post 1 = Intact mice, metrifonate administered after the training I; Intact post 2 = Intact mice, metrifonate administered after the training II; MS-lesion pre = medial septal-lesioned mice, metrifonate administered before the training; Y-maze: tot = number of total alternations; % = percent alternations. Passive avoidance: day 1 = entry latency of the training day; diff. = the difference between the latencies of the testing day and the training day.

[a] $P < 0.015$ vs. sham-lesioned group.

[b] $P = 0.002$ vs. medial septal-lesioned vehicle group.

The values are expressed as means ± S.D.

3.2. Medial septal-lesioned mice, metrifonate administered before the training

3.2.1. Water maze

During the first 5 days there were group differences in the number of mice that found the platform ($P < 0.05$), for escape latency, Fig. 4; and swimming speed (group: $F(3,33) > 5.70$, $P < 0.003$; Table 1)). Comparison of the sham-lesioned group and the medial septal-lesioned vehicle group showed a clear impairment in escape latency ($F(1,17) = 35.39$, $P < 0.001$) and platform finding ($P < 0.001$) during the first 5 days. The 50-mg/kg dose of metrifonate decreased the escape latency of medial septal-lesioned mice ($F(1,15) = 8.08$, $P = 0.036$), and increased the probability of platform finding ($P = 0.03$). The only group difference in swimming speed was the decreased swimming speed of the medial septal-lesioned vehicle group compared to that of the sham-lesioned group ($F(1,17) = 7.57$, $P = 0.042$; Table 3). On the sixth day, during the spatial bias test, no group differences in counter-crossings were observed ($F(3,33) = 0.792$, $P > 0.05$; Table 3).

During the platform reversal stage, the medial septal-lesioned vehicle-treated group was not impaired as compared to the sham-lesioned group for escape latency ($F(1,17) < 4.94$, $P > 0.05$), but the probability of platform finding was decreased ($P = 0.03$; Fig. 4). Metrifonate 15 or 50 mg/kg did not restore the navigation failure of medial septal-lesioned mice during the platform reversal stage ($P > 0.05$ for all comparisons). However, metrifonate stimulated both groups of medial septal-lesioned mice to swim faster than the vehicle-treated medial septal-lesioned group ($F(1,15) > 9.12$, $P < 0.024$, for both comparisons; Table 1). On the eighth day, during the spatial bias test, the performance of all the medial septal-lesioned groups was impaired as compared to that of the sham-lesioned group for counter crossings ($F(3,33) = 5.822$, $P = 0.003$) and metrifonate failed to improve the bias of medial septal-lesioned mice (Table 1).

3.2.2. Y-maze and passive avoidance

In the Y-maze, during the first day, the medial septal-lesioned vehicle group made significantly fewer total moves than the sham-lesioned group, and this failure was reversed by the 50-mg/kg dose of metrifonate ($F(3,33) = 6.029$, $P = 0.002$; Table 2). There were no group differences for the percentage alternation ($F(3,18) = 4.403$, $P > 0.05$). In passive avoidance testing, a large proportion of medial septal-lesioned mice refused to enter the dark compartment even on the training day. All the medial septal-lesioned groups had significantly greater latencies than did the sham-lesioned group (vehicle vs. sham-lesioned group: ($P = 0.01$); metrifonate 15 mg/kg vs. sham-lesioned group: ($P = 0.0012$); metrifonate 50 mg/kg vs. sham-lesioned group: ($P = 0.033$)). It was observed that the difference between the latencies to enter the dark compartment on the testing day and the training day was greater in the vehicle ($P = 0.028$) and metrifonate 15 mg/kg groups ($P = 0.044$) than in the sham-lesioned group. The metrifonate 50 mg/kg group did not differ from the sham-lesioned group ($P > 0.05$) or the vehicle group ($P > 0.05$; Table 2).

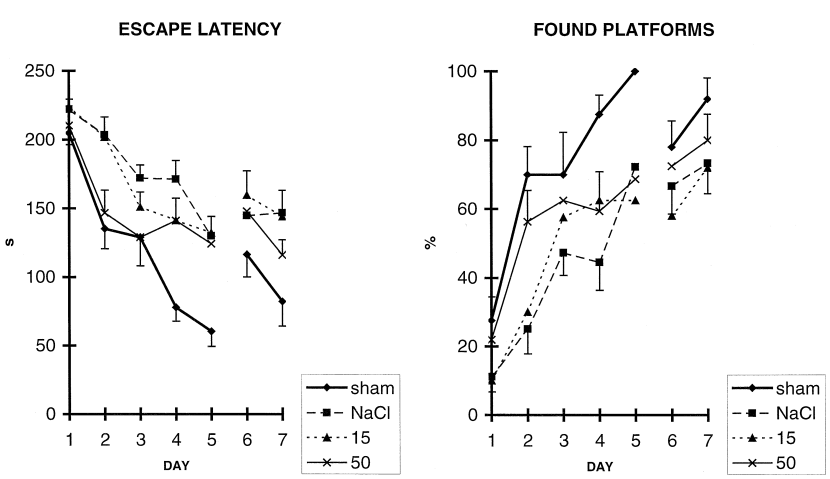

Fig. 4. The effect of administration of metrifonate 15 and 50 mg/kg 30 min before the daily training on medial septal-lesioned mice. The *X*-axis indicates the reference memory training days 1–5 (fixed platform location) and reversal learning days 6–7 (reversal of the platform location). The values are daily group means + S.E.M. During days 1–5, medial septal lesioning caused a defect for latency and platforms found ($P < 0.001$) and it was alleviated by metrifonate 50 mg/kg ($P < 0.05$). The number of mice in each treatment group: sham-lesioned: $n = 10$, vehicle: $n = 9$, 15 mg/kg: $n = 10$, 50 mg/kg: $n = 8$.

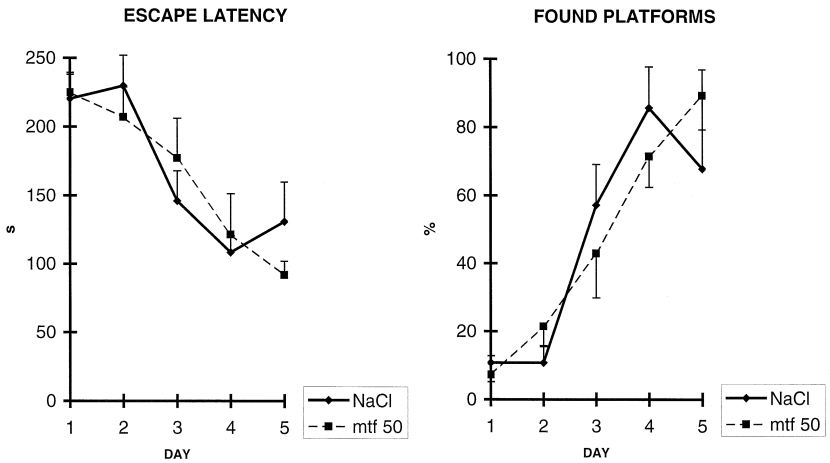

Fig. 5. The effect of administration of metrifonate 50 mg/kg immediately after the daily training on medial septal-lesioned mice. The *X*-axis indicates the reference memory training days 1–5 (fixed platform location). The values are daily group means + S.E.M. There were no significant differences between the groups. The number of mice in each treatment group: vehicle: $n = 11$, 50 mg/kg: $n = 8$.

3.3. Medial septal-lesioned mice, metrifonate administered after the training

3.3.1. Water maze

No group differences were observed in the number of platforms found ($P > 0.05$), the escape latency (Fig. 5) or swimming speed (Table 1) during the first 5 training days (group: $F(1,12) < 0.41$, $P > 0.05$). There were no group differences for counter-crossings ($F(1,12) = 0.287$, $P > 0.05$) during the measurement of bias assessment on the sixth day of the water maze training (Table 1).

3.4. Hippocampal-lesioned mice

3.4.1. Water maze

During the first 10 training days, all the lesioned groups showed an impaired performance compared to that of the sham-lesioned group (escape latency: (group: $F(1,18) > 6.10$, $P < 0.024$, for all comparisons); found platforms: ($P = 0.000$, for all comparisons; Fig. 6)), but no group differences were found when the hippocampal-lesioned metrifonate groups were compared to the hippocampal-lesioned vehicle group (escape latency: (group: $F(1,18) <$

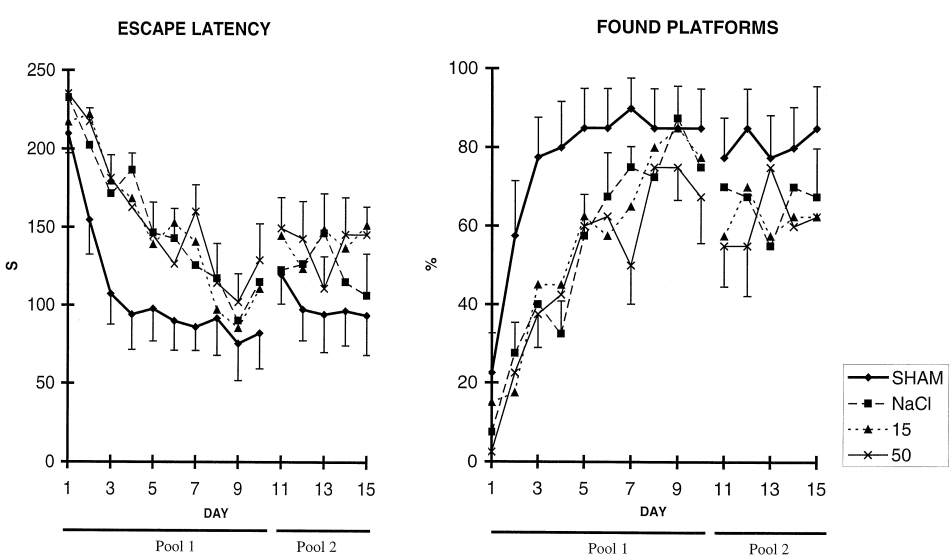

Fig. 6. Acquisition of reference memory performance by hippocampal-lesioned mice treated with vehicle and metrifonate 30 min before the training. Metrifonate 15 and 50 mg/kg was administered during days 1–10. During days 11–15, all the groups received vehicle injections. Metrifonate or vehicle injections were administered 30 min before the daily training. The *X*-axis indicates the reference memory training days 1–10 in the first environment and days 11–15 in the second environment. The values are daily group means + S.E.M. Hippocampal lesioning caused a defect for escape latency and platforms found ($P < 0.05$) during the testing in both environments, but metrifonate did not alleviate this defect ($P > 0.05$). The number of mice in each treatment group: sham-lesioned: $n = 10$, vehicle: $n = 10$, 15 mg/kg: $n = 10$, 50 mg/kg: $n = 10$.

0.12, $P > 0.05$, for both comparisons); platforms found: ($P > 0.05$, for both comparisons; Fig. 6)). There were no group differences in swimming speed during the first 10 days (group: $F(3,36) = 0.73$, $P > 0.05$; Table 1). The platform reversal training was not done in this part of the study.

During days 11–15 (after the change of environment), both previously metrifonate-treated lesioned groups showed

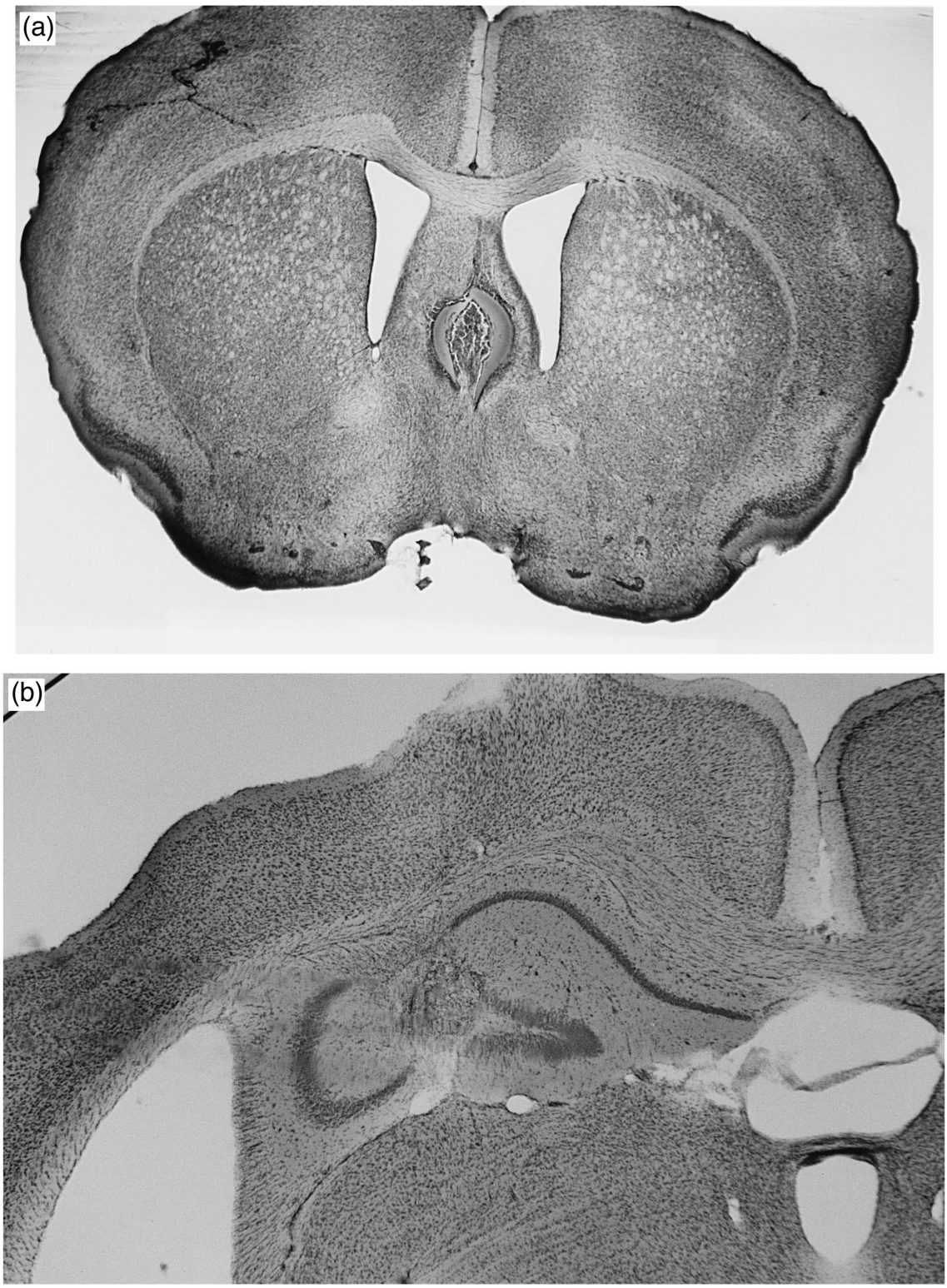

Fig. 7. Part A. Cresyl violet staining of a coronal section containing a medial septal-lesion site. Part B. Cresyl violet staining of a coronal section containing a hippocampal lesion. site.

an impaired performance for escape latency (group: $F(1,18) > 6.98$, $P < 0.017$, for all comparisons) compared to that of the sham-lesioned group (Fig. 6). There were no group differences when the hippocampal-lesioned metrifonate groups were compared to the hippocampal-lesioned vehicle group for escape latency: (group: $F(1,18) < 2.03$, $P > 0.05$, for both comparisons). The ability to find the platforms was impaired in the lesioned groups when compared to that of the sham-lesioned group ($P < 0.002$, for all comparisons), but there were no differences when the metrifonate-treated groups were compared to the vehicle group ($P > 0.05$, for both comparisons). There were no group differences for swimming speed during the last 5 days (group: $F(3,36) = 0.01$, $P > 0.05$; Table 1).

3.5. Histology

Correct location of the lesions in medial septal-lesioned mice (Fig. 7, Part A) and hippocampal-lesioned mice (Fig. 7, Part B) was confirmed in cresyl fast violet-stained sections. The decrease of acetylcholine-containing fibers in the hippocampus of medial septal-lesioned mice was confirmed by acetylcholinesterase staining. A visual examination of the sections revealed that the lesion removed most of the cholinergic fibers in the hippocampus.

4. Discussion

The cognitive testing performed in the present study revealed that the wild type of C75BL mice perform rather well in the water maze and the Y-maze, and suboptimally in the passive avoidance task.

Lesion of the medial septum or the dorsal hippocampus induced a deficit in cognitive performance of the mice for finding the platform in the water maze. The effect of the two lesions was qualitatively similar: escape distance and escape latency were increased and the ability to find the platform was decreased. Swimming speed, however, was not affected. Therefore, both lesions disrupt spatial memory in mice in the same way as in rats (Morris et al., 1982; Riekkinen et al., 1997). These results suggest that the septo-hippocampal axis is crucial for spatial navigation in C75BL mice also.

On the other hand, medial septal lesioning appeared to have no effect on 'cognitive' components of the Y-maze and passive avoidance tests. Medial septal lesioning only reduced motor activity in the Y-maze and increased the latency of movement during training and testing trials for passive avoidance, suggesting that its effects were primarily to decrease motor activity and induce a state reminiscent of apathy. Apathy is an important behavioral problem in Alzheimer's disease, and metrifonate treatment significantly attenuates the incidence and severity of this distressing psychiatric and behavioral disturbance in Alzheimer patients (Cummings et al., 1998; Morris et al., 1998). Interestingly, we observed that metrifonate treatment restored normal motor activity in the Y-maze and also alleviated the immobility of medial septal-lesioned mice during passive avoidance testing.

Moreover, metrifonate 50 mg/kg administered 30 min before the daily training could markedly stimulate the water maze spatial navigation behaviour of medial septal-lesioned mice. This is the first report of the cognition-enhancing effect of metrifonate in a mouse model and it therefore supplements evidence from rat and rabbit studies (Schmidt et al., 1997). However, administration of metrifonate after the training was also ineffective in both initial and reversal learning. This may be related to the slow onset of the action of metrifonate, due to the necessity to first release the active metabolite from the inactive prodrug (Schmidt et al., 1997). This slow onset in turn may prevent the drug from stimulating the short-lasting consolidation phase of learning when the injection is given immediately after the training. Alternatively, the neurobiological mechanisms involved in acquisition may be sensitive to metrifonate treatment, but those that mediate consolidation are insensitive to this drug. Previously, we have reported that metrifonate improves reversal learning in rats (Riekkinen et al., 1997), but the present results indicate that there is no such effect in mice. Since the doses now used were effective on the initial learning, we suggest that there may be some fundamental differences between the cognitive functions of rats and mice.

It is relevant to note that the beneficial effect of the cholinesterase inhibitor, tacrine, to improve memory in Alzheimer's patients decreases along with the development of atrophy in the hippocampus, which becomes increasingly prominent as Alzheimer's disease progresses (Riekkinen et al., 1995). In the present study, the atrophy of the hippocampus observed in Alzheimer's disease was mimicked by partial dorsal hippocampal lesions and indeed, a strong navigation failure was seen in hippocampal-lesioned mice. In this experiment, however, all the hippocampal-lesioned mice were able to learn to locate the platform position, but were clearly impaired as compared to sham-lesioned animals. The test was thus only more difficult for the hippocampal-lesioned mice than for their unlesioned littermates. In contrast to the situation with medial septal-lesioned mice, metrifonate failed to reverse the cognitive deficit in dorsal hippocampus-lesioned mice. This leaves two explanations: either the dorsal hippocampus is the target area of metrifonate to improve spatial reference memory, or metrifonate acts primarily via other brain areas, and these cannot compensate for the effects of hippocampal lesion on spatial navigation.

Results of this study and a previous report (Ikonen et al., 1998) suggest that, unlike rats, young control mice did not develop a spatial bias in the water maze. This is evident from the fact that despite the impairment in escape distance, latency and number of platforms found, the medial septal-lesioned mice were not impaired for bias assess-

ment. Therefore, it is impossible to see any beneficial effects of metrifonate and, most likely, that of any other test compounds in this kind of bias test with medial septal-lesioned mice.

In conclusion, wild type C57BL-mice are a useful strain for testing cognitive performance in models previously used for testing rats. One of the most appropriate and flexible models to test learning and memory performance, the spatial water escape task, particularly shows the septo-hippocampal axis to be crucial for spatial navigation in mice, as lesioning of this system disrupts the accuracy of platform localization. Therefore, neuropathological defects in the medial septal nucleus and the hippocampus, as induced in experimental transgenic Alzheimer's disease model mice, may be expected to translate into an impaired water maze spatial navigation. Such an effect has been shown in one mouse model that carries Alzheimer's disease transgenes. (Hsiao et al., 1996). The water maze spatial memory defect induced by a medial septal lesion of the wild type of these mice was alleviated by metrifonate treatment, whereas the spatial memory defect induced by a partial lesion of the dorsal hippocampus was not altered. This is in line with the view that cholinesterase inhibitors may be more effective on memory in the mild to moderate stages of Alzheimer's disease than in later, severe stages where there is already considerable degeneration of the hippocampus. The clinically effective cholinesterase inhibitor, metrifonate, can alleviate some of the cognitive (spatial navigation) and noncognitive (apathy) deficits in medial septal-lesioned mice. Given these encouraging findings about cognitive deficits in mice with septohippocampal pathology, it could be anticipated that transgenic Alzheimer's disease mouse models, which have some form of hippocampal pathology might also show cognitive deficits and hence may be suitable models both regarding the behavioral and neuropathological aspects of the disease and for the identification of therapeutic drugs which can alleviate these deficits.

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