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Learning Deficits After Unilateral AF64A Lesions in the Rat Basal Forebrain: Role of Cholinergic and Noncholinergic Systems

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NAKAMURA, S., Y. TANI, Y. MAEZONO, T. ISHIHARA AND T. OHNO. *Learning deficits after unilateral AF64A lesions in the rat basal forebrain: Role of cholinergic and noncholinergic systems.* PHARMACOL BIOCHEM BEHAV 42(1) 119–130, 1992.—Rats were given unilateral infusions of ethylcholine aziridinium ion (AF64A) into the basal forebrain (BF). BF-lesioned rats had significant acquisition and retention deficits in two different types of learning tasks (water maze and active avoidance). Choline acetyltransferase activity was lower than control in the frontal cortex but not in the hippocampus or striatum. AF64A markedly reduced the levels of norepinephrine, dopamine, and serotonin in all brain regions studied. However, L-glutamic acid decarboxylase activity was not altered by AF64A injection. Cholinergic agents (physostigmine and arecoline) ameliorated the AF64A-induced learning deficits in the water maze task but not in the active avoidance task. Noncholinergic agents (desipramine and L-dopa) ameliorated the AF64A-induced avoidance deficits in the active avoidance task but not in the water maze task. 5-Methoxy-*N*,*N*-dimethyltryptamine did not improve either active avoidance or water maze learning. These results suggest that intra-BF injection of AF64A produces extensive brain dysfunction and that different neuronal systems are involved in associative and spatial learning.

AF64A Active avoidance task Morris water maze task Basal forebrain Choline acetyltransferase
L-glutamic acid decarboxylase Monoamine contents Cholinergic agents Noncholinergic agents

SENILE dementia of the Alzheimer type (SDAT) is often characterized as cholinergic dysfunction in the basal forebrain (BF), including the nucleus basalis magnocellularis (NBM), which normally provides cholinergic input to the neocortex and amygdala, although noncholinergic neurochemical variables are also changed (7,8,17,26,48). Lesions of the rat BF cause not only a marked decrease in choline acetyltransferase (ChAT) activity in the cerebral cortex, but also learning deficits in the passive and active avoidance, T-maze, radial arm maze, and Morris water maze tasks (10,13,29,35,46).

Because cholinergic hypofunction might contribute to memory loss in patients with SDAT, the effects of cholinergic drugs on learning in rats with BF lesions have been studied. Dokla and Thal (9) reported that acetylcholinesterase inhibitors improved performance in the Morris water maze in BF-lesioned rats. Furthermore, Miyamoto et al. (30) found that continuous subcutaneous administration of cholinergic drugs markedly improves acquisition and retention in rats with BF lesions.

Excitotoxins such as ibotenate, kainate, or quisqualate have been used in neurochemical and behavioral studies of rats to lesion the BF. In contrast, ethylcholine aziridinium ion (AF64A) has also been used to damage cholinergic neurons in the brain (25), although there are only a few reports concerning BF lesions caused by AF64A. In a previous study, we observed that unilateral or bilateral infusion of a small dose (1 nm/side) of AF64A into the BF caused severe learning impairment and a marked decrease in ChAT activity in the cerebral cortex (34,35). Kozlowski and Arbogast (22) and McGurk et al. (27) have shown that AF64A damages noncholinergic neurons when administered into the rat BF at high doses. Nonetheless, there have been no detailed studies of both behavior and neurochemistry after intracerebral AF64A. Therefore, we studied the relationship between behavioral and neurochemical changes in rats with BF lesions caused by AF64A.

We unilaterally lesioned the BF with AF64A and measured the acquisition and retention of two different learning tasks. We also measured neurochemical markers, that is, ChAT and glutamic acid decarboxylase (GAD) activities, and mono-

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amine contents, and examined the effects of cholinergic and noncholinergic drugs on learning deficits in BF-lesioned rats.

METHOD

Subjects

A total of 258 male Fischer 344 rats (Charles River, Japan Breeding Laboratories) were used (body weight 280–310 g). Rats were housed in a climate-controlled room (room temperature 23 ± 1°C and humidity 55 ± 5%) with food and water available ad lib. Fifty-nine rats were used for behavioral tests to assess the effect of BF lesion by AF64A. After completion of behavioral testing, the same rats were used for neurochemical and histological analysis. The remaining 199 rats were used for pharmacological effects on the lesion-induced behavioral deficits.

Surgery

Rats were anesthetized with sodium pentobarbital (40 mg/kg, IP). To make a unilateral lesion in the BF, a cannula was stereotaxically inserted into the right side of the BF (1.0 mm posterior to the bregma, 2.6 mm lateral to the midline, 7.9 mm ventral to the surface of the skull) according to the coordinates in the atlas of Paxinos and Watson (38). The cannula was connected with a polyethylene tube to a 10-μl Hamilton microsyringe. The AF64A solution (1 μl) or saline was injected into the BF (1 nm/μl) at the rate of about 0.5 μl/min. The cannula was removed 3 min after completing injection. The AF64A solution was prepared as described by Fisher et al. (12).

Drugs

The following drugs were used: acetylcholinesterase inhibitors; physostigmine salicylate (PHY; 0.1 and 0.2 mg/kg, IP, Sigma Chemical Co., St. Louis, MO), muscarinic agonists; arecoline hydrobromide (ARE; 0.5 and 1.0 mg/kg, IP, Sigma), selective norepinephrine (NE) uptake inhibitor; desipramine hydrochloride (DMI; 7.5 mg/kg, IP, Sigma), precursor of dopamine (DA); L-dopa (10 and 20 mg/kg, IP, Sigma); and serotonin 5-hydroxytryptamine-1a (5-HT_{1A}) agonist 5-methoxy-*N*,*N*-dimethyltryptamine (5-methoxy DMT; 0.025 and 0.05 mg/kg, IP, Sigma). PHY, ARE, DMI, L-dopa, and

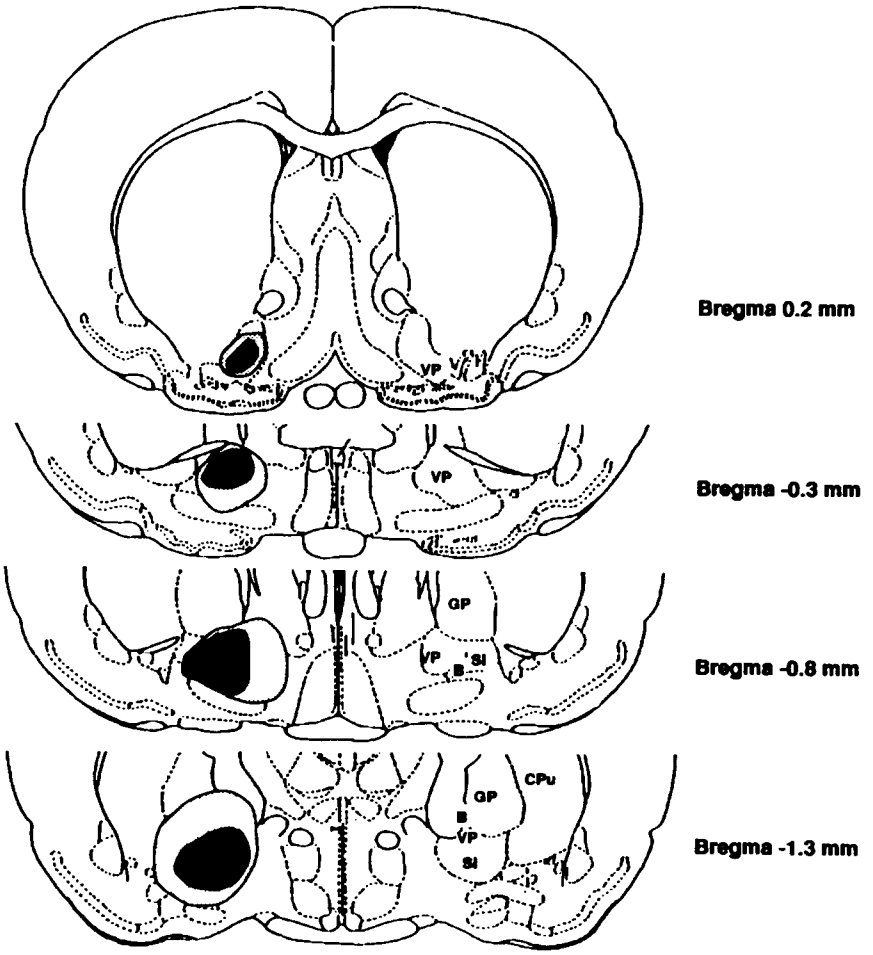

FIG. 1. Schematic drawings of the unilateral lesion of the BF according to the atlas of Paxinos and Watson (38). CPu, caudate putamen; GP, globus pallidus; VP, ventral pallidum; SI, substantia innominata; B, nucleus basalis of Meynert. (■), minimum lesion area; (□), maximum lesion area.

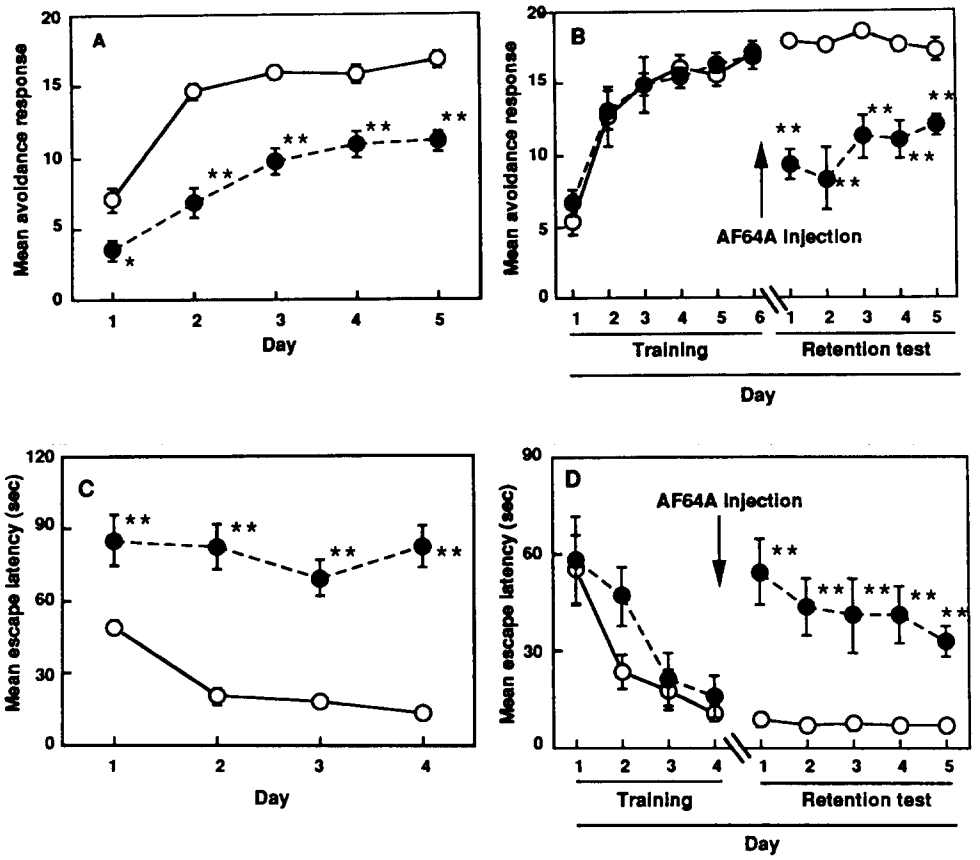

FIG. 2. Acquisition and retention of active avoidance response and Morris water maze learning in BF-lesioned rats. (A) Acquisition of the active avoidance response: (—○—), saline (n = 11); (--●--), AF64A (n = 10). (B) Retention of the active avoidance response: (—○—), saline (n = 7); (--●--), AF64A (n = 6). (C) Acquisition of the Morris water maze learning: (—○—), saline (n = 7); (--●--), AF64A (n = 6). (D) Rentention of the Morris water maze learning: (—○—), saline (n = 6); (--●--), AF64A (n = 6). $^*p < 0.05$, $^{**}p < 0.01$ compared with the saline control group (Tukey's test).

5-methoxy DMT have reportedly been shown to improve the hemicholinium- or scopolamine-induced amnesia that were studied in spatial discrimination learning or radial arm maze learning (15,19). We preliminarily examined the same doses in the present study. However, we could not find the marked efficacies at these doses; therefore, we used the larger doses.

PHY, ARE, and DMI were dissolved in saline and L-dopa was suspended in 1% gum arabic. 5-Methoxy DMT was dissolved in 1% alcohol. Drugs (1 ml/kg, IP) were administered 20 min prior to each training session once a day for 5 days. Experiments began 1 week after the BF lesion.

Active Avoidance Task

The active avoidance task has been previously described (33). In brief, the apparatus used consisted of a shuttle box (66 × 25 × 30 cm) with two identical compartments separated by a stainless steel plate (4 cm in height, Biomedica, Japan). A buzzer (2.8 kHz, 70 dB) attached to the ceiling of the shuttle box was turned on for 5 s as the conditioned stimulus (CS). If the rat crossed over the hurdle into the other compartment during the CS or within 5 s after it, an avoidance response was recorded. Otherwise, a foot-shock (1.0 mA, maximum 5 s) was delivered through the grid floor as the unconditioned stimulus. Each rat was given 20 trials daily for 5 days with a fixed intertrial interval of 20 s.

We followed the criterion that rats that avoided the CS in at least 16 of 20 trials in the sixth training session were used to make it easier to measure retention of the active avoidance response. However, all rats examined satisfied this criterion. Experiments began 1 week after BF lesion.

Morris Water Maze

Apparatus. The apparatus was a circular water tank 132 cm in diameter and 60 cm deep that was manufactured strictly according to Morris (31). The interior surface of the tank was covered with white tape. During the experiment, the tank was filled to a height of 42 cm with water (23 ± 1°C) that was clouded with 0.8 kg of powdered milk. A colorless acrylic platform (9 cm in diameter) was used for training and its top was submerged 1-2 cm below the water surface in the center of one of the four quadrants of the maze. The tank was located in a large room with many extramaze cues (an experimenter, ceiling lights, animals cages, a calendar, etc.). The positions of the extramaze cues were constant throughout the experiment. The movement of the animal in the tank was

monitored with a video tracking system (VIOS-88, Biomedica, Japan) and analyzed with a computer (PC-9801, NEC, Japan).

Protocol. On the first day, each rat was placed into the Morris water maze without escape platform and allowed to swim for 60 s. The animal was then returned to its home cage. Maze training began on the next day. In each trial, the rat was put into the water from one of four starting points (north, east, south, and west) on the edge of the tank. The starting point was changed in each trial but the escape platform was fixed. In each trial, the time required to escape onto the hidden platform was recorded. If the rat found the platform, it was allowed to remain there for 10 s. If it did not find the platform within 120 s, the trial was terminated and the experimenter placed the rat onto the platform and allowed it to stay there for 10 s. Rats were trained for 4 consecutive days with four trials each day and 60-s intertrial intervals.

We followed the criterion that rats that escaped within 20 s on day 4 were used to make it easier to measure the retention of the water maze performance. *However, all rats examined satisfied this criterion.*

After the final training session, a single transfer test was conducted. The escape platform was removed and each rat was allowed to swim for 60 s in the maze. The number of times the rat crossed the annulus where the platform had been located was recorded. Experiments began 1 week after BF lesion.

Neurochemical Analysis

Rats were decapitated, brains were rapidly excised, and the following brain regions were dissected out according to the method of Glowinski and Iversen (16): frontal cortex (FC), hippocampus (HC), and striatum (ST). Tissue samples were stored at −80°C until assay.

ChAT activity was assayed by the method of Fonnum (14). In brief, tissue samples were homogenized in 20 vol ice-cold 10 mM EDTA (pH 7.4) containing 0.5% Triton X-100. The reaction mixture contained 20 μM [^{14}C]acetyl coenzyme A (acetyl CoA; 45.1 mCi/mmol, New England Nuclear Corp., Newton, MA), 25 mM sodium phosphate buffer (pH 7.4), 600 mM sodium chloride, 40 mM EDTA, 100 μM PHY, 8 mM choline bromide, 200 μM acetyl CoA, and the tissue homogenate (about 70 μg protein) in a total volume of 100 μl. The sample was incubated for 30 min at 37°C and then washed with 5 ml sodium phosphate buffer. Radioactivity was counted with a liquid scintillation counter.

GAD activity was assayed by the method of Kimura and Kuriyama (21). In brief tissue samples were homogenized in 20 vol nitrogen-gassed distilled water. The reaction mixture contained 20 μM [1-^{14}C]glutamic acid (52.6 mCi/mmol, New England Nuclear Corp.), 10 mM L-glutamic acid, 10 μM pyridoxal phosphate, 2 mM aminoethylisothiouronium bromide, 40 mM potassium phosphate buffer (pH 6.4), and the tissue homogenate in a total volume of 70 μl. The incubation vessel was capped with a glass stopper containing a folded piece of filter paper immersed in 0.1 ml hydroxide of hyamine base to absorb the [$^{14}CO_2$] evolved from L-[1-^{14}C]glutamic acid. After exposure to purified nitrogen gas, the vessel was incubated for 60 min at 37°C. After terminating the reaction by injecting 50 μl 5 N H_2SO_4, the vessel was incubated for an additional 60 min. Radioactivity was counted with a liquid scintillation counter. Protein was determined by the method of Smith et al. (42).

Biogenic amines were measured by the method of Tani and Ishihara (43). In brief, the chromatographic system used to measure the biogenic amines consisted of an electrochemical detector (LC-4B, Bioanalytical System, Stockholm) with a glossy carbon electrode coupled to a Bio phase II ODS analytical column maintained at a constant temperature of 35°C with

TABLE 1

EFFECT OF UNILATERAL BF LESION CAUSED BY AF64A ON MONOAMINE CONTENTS IN RAT BRAIN

	% Control					
	Frontal Cortex		Hippocampus		Striatum	
Region	Saline	Lesion	Saline	Lesion	Saline	Lesion
NE						
Ipsilateral	100 ± 7.0 (163.3 ± 11.5)	37.4 ± 4.9*	100 ± 1.6 (238.2 ± 3.8)	36.0 ± 2.5*	100 ± 5.0 (28.1 ± 1.4)	42.0 ± 3.6*
Contralateral	100 ± 5.2 (147.0 ± 7.7)	76.4 ± 9.0	100 ± 3.3 (205.4 ± 6.7)	95.0 ± 3.1	100 ± 6.1 (36.1 ± 2.2)	114.7 ± 8.6
DA						
Ipsilateral	100 ± 8.5 (61.2 ± 5.2)	34.8 ± 6.9*	100 ± 12.9 (6.2 ± 0.8)	38.7 ± 9.7*	100 ± 3.6 (5710.9 ± 5.3)	39.6 ± 8.2*
Contralateral	100 ± 6.8 (76.7 ± 5.2)	77.2 ± 16.3	100 ± 5.8 (5.2 ± 0.3)	80.8 ± 5.8	100 ± 11.6 (5404.9 ± 62.7)	93.3 ± 2.5
5-HT						
Ipsilateral	100 ± 5.7 (275.4 ± 15.7)	24.8 ± 3.8*	100 ± 9.7 (263.2 ± 25.6)	38.4 ± 8.6*	100 ± 2.8 (217.2 ± 6.1)	18.5 ± 2.3*
Contralateral	100 ± 8.7 (226.9 ± 19.8)	74.8 ± 11.2	100 ± 6.0 (239.5 ± 14.4)	72.5 ± 8.3	100 ± 9.7 (229.8 ± 22.4)	93.6 ± 9.7

Each value is a mean ± SEM. Numbers in parentheses are the absolute value (ng/g tissue weight).
*$p < 0.01$ compared to the saline group.

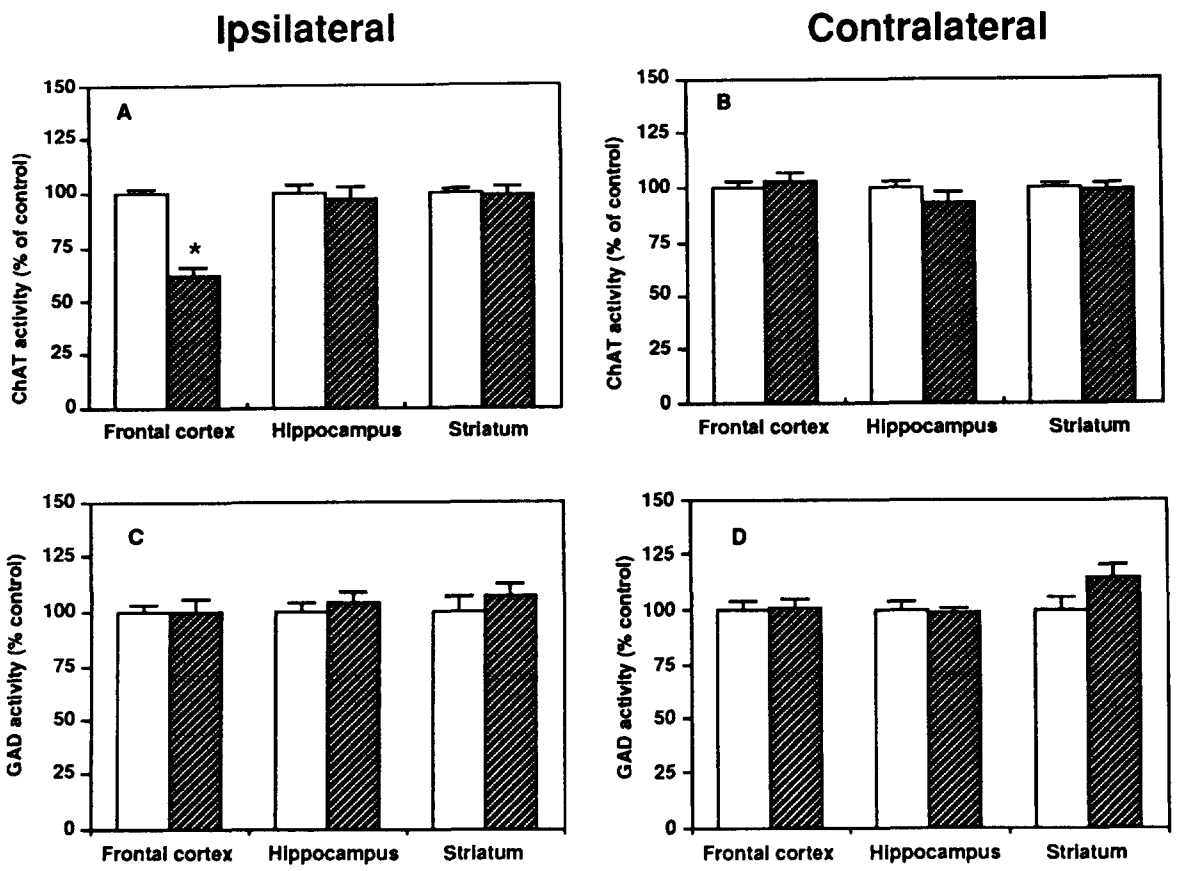

FIG. 3. Effect of unilateral BF lesions caused by AF64A on ChAT and GAD activity in rat brains. (A,B) ChAT activity: (□), saline (n = 11); (▨), AF64A (n = 9). (C,D) GAD activity: (□), saline (n = 5); (▨), AF64A (n = 8). (A,C) Ipsilateral side. (B,D) Contralateral side. *$p < 0.01$ compared to the saline control.

a BAS (LC-22A) column heater. The detector was operated at a potential of 750 mV vs. AG/AgCl. The mobile phase consisted of 0.6% v/v triethylamine, 8% v/v acetonitrile, 0.1 mM EDTA·2Na, and 10 mM heptane-sulphonate·Na. pH was adjusted to 2.7 with orthophosphoric acid. Brain levels of the biogenic amines (NE, dopamine, and serotonin) were quantified by calculating the areas under the curves using an integrator (C-R4AX, Shimadzu, Japan). The neurochemical parameters were assayed 2 weeks after BF lesion.

Histology

Rats were anesthetized with pentobarbital sodium (40 mg/kg, IP) and then brains were perfused with saline and 10% formalin in 0.1 M phosphate buffer (PB, pH 7.4). Thereafter, brains were excised and immersed in PB containing 30% sucrose for 48–72 h at 4°C. Sections were cut 20–30 μm in thickness and stained with Cresyl violet. The location and extent of the lesion were verified.

Statistics

Analysis of variance (ANOVA) followed by the Tukey multiple-range test was used for the data obtained in the behavioral tests. The data from the biochemical studies were analyzed by Student's t-test.

RESULTS

Histology

The lesion site of the BF is shown schematically in Fig. 1. AF64A lesion of the BF involved the substantia innominate, medial forebrain bundle, ventral pallidum, and globus pallidus.

Effects of BF Lesion on Behavior and Neurochemical Markers

Active avoidance task.

Acquisition. The mean avoidance response (MAR), measured with the shuttle box, increased with training in both groups, but the MAR in the BF-lesioned group was lower than that in the control group on all 5 days (Fig.1A). There was a significant group effect, $F(1, 19) = 72.2, p < 0.01$.

Retention. Figure 1B shows the effect of the unilateral BF lesion on the retention of the active avoidance task. There was a significant group effect, $F(1, 11) = 59.1, p < 0.01$. Tukey's test indicated that MAR in the BF-lesioned group was lower than that in the control group.

Morris water maze.

Acquisition. The mean escape latency (MEL) decreased with training in the control group. In BF-lesioned rats, MEL

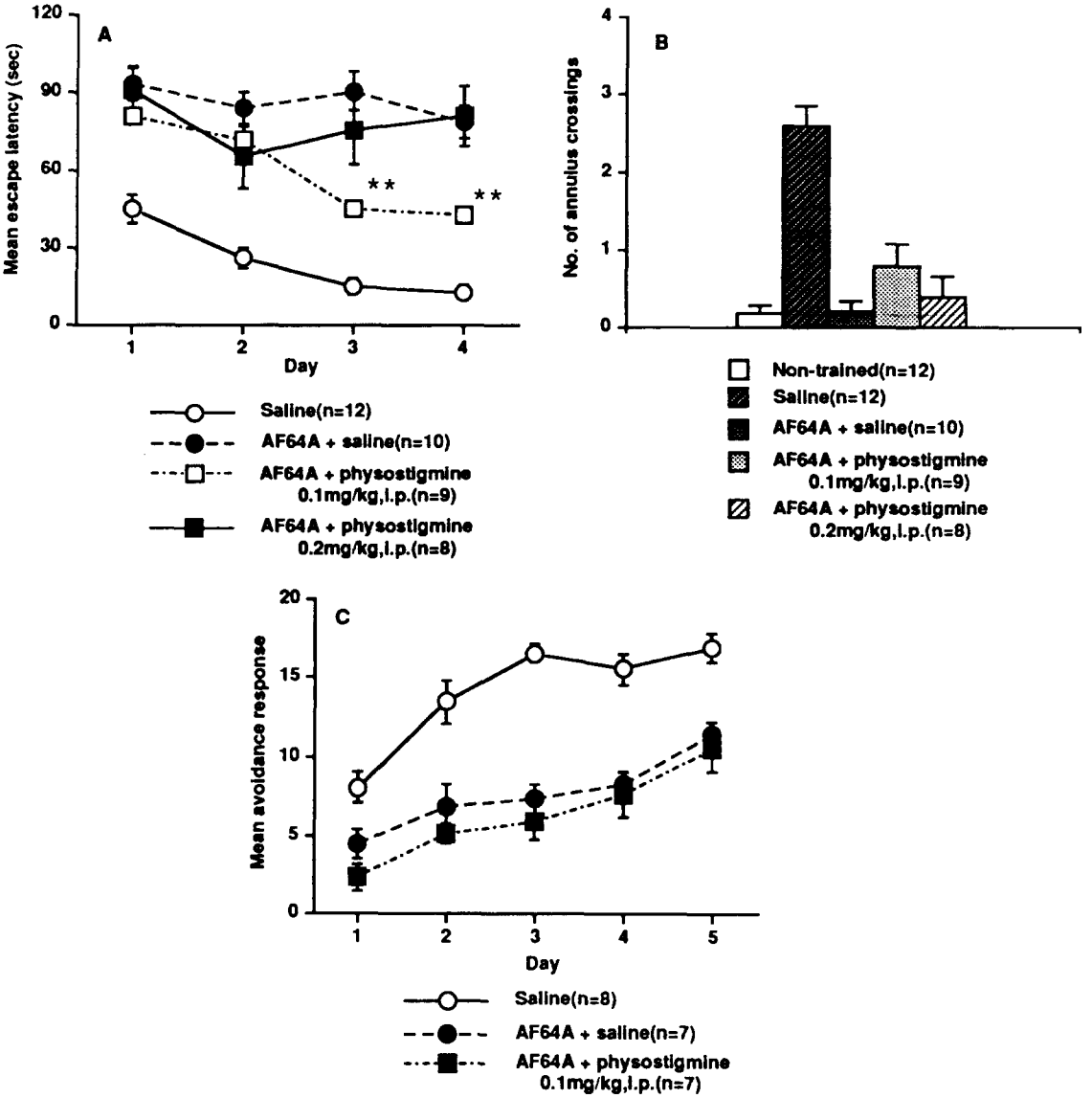

FIG. 4. Effect of physostigmine on the active avoidance and water maze performance in BF-lesioned rats. (A) Acquisition test for the water maze task. Each point represents a mean latency to escape onto the hidden platform. (B) Transfer test. The number of times the rat crossed the annulus where the platform had been during training. (C) Acquisition test for the active avoidance task. Each point represents a mean avoidance response. $^{**}p < 0.01$ compared with the lesioned control.

remained long throughout the experiment. The difference in MEL between the two groups was statistically significant, $F(1, 11) = 53.3, p < 0.01$ (Fig. 1C).

Retention. The BF lesion caused an increase in MEL as shown in Fig. 1D. There was a significant group effect, $F(1, 10) = 33.6, p < 0.01$. Tukey's test indicated that the MEL in the BF-lesioned group was longer than that in the control group.

ChAT activity. ChAT activity in BF-lesioned rats was 40% lower only in the ipsilateral FC as compared with that in the ipsilateral FC of the sham-operated control rats, but was unchanged in the HC and ST (Fig. 2A). In contrast, ChAT activity on the contralateral side was unchanged in all regions tested (Fig. 2B).

GAD activity. As shown in Figs. 2C and D, the unilateral BF lesions caused no significant change in the GAD activity in any of the brain regions tested.

Biogenic amines. The amounts of biogenic amines (noradrenaline (NA), DA, 5-HT) are summarized in Table 1. The contents of NA, DA, and 5-HT in the ipsilateral FC of BF-lesioned rats were significantly lower than in the control group: 37, 35, and 25%, respectively. Similar changes were observed in the HC and ST. In contrast, the amounts of biogenic amines on the contralateral side were slightly but not significantly lower than control.

Effects of Cholinergic and Noncholinergic Drugs on Learning Deficits in BF-Lesioned Rats

Cholinergic drugs. The effects of PHY and ARE on the

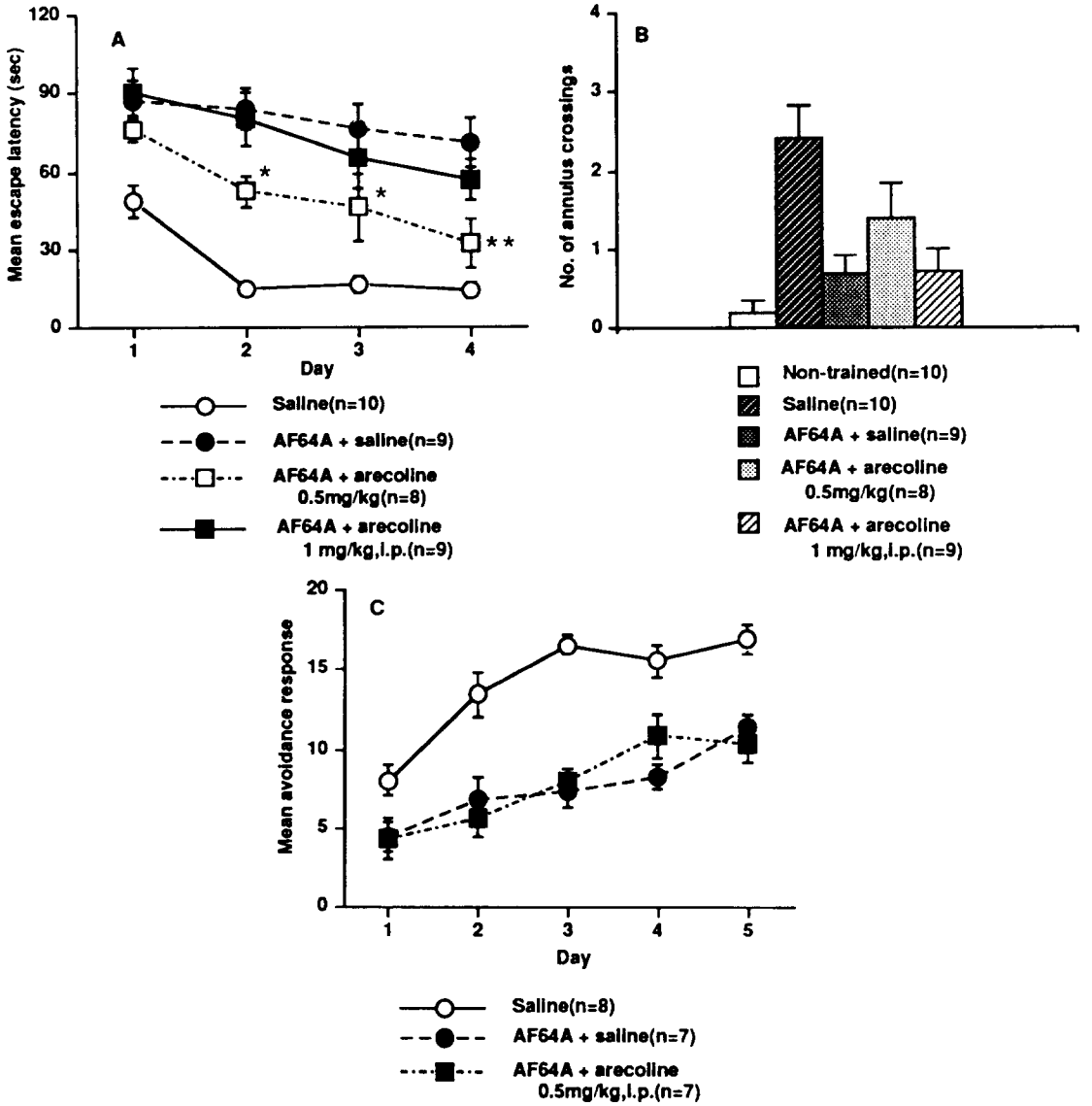

FIG. 5. Effect of ARE on the active avoidance and water maze performance in BF-lesioned rats. (A,B,C) See the legend for Fig. 3. $*p < 0.05$, $**p < 0.01$ compared with the lesioned control.

acquisition deficits in BF-lesioned rats were evaluated using the water maze and active avoidance tasks. The MEL in the PHY- and ARE-treated groups decreased with training, but MEL in the nontreated BF-lesioned group did not change. Significant effects were observed on days 3 and 4 in the group treated with the lower dose of PHY (0.1 mg/kg) and on days 2, 3, and 4 in the group treated with the lower dose of ARE (0.5 mg/kg; Figs. 3A and 4A). However, the higher doses of PHY (0.2 mg/kg) and ARE (1 mg/kg) had no significant effects. The number of times the rat crossed the annulus in the platform quadrant was slightly but not significantly higher in both the PHY- and ARE-treated groups (Figs. 3 and 4B).

In the active avoidance test, the learning deficits in BF-lesioned rats were not alleviated by treatment with PHY (0.1 mg/kg) or ARE (0.5 mg/kg), even though these doses were effective in the Morris water task (Figs. 3C and 4C).

Noncholinergic drugs. Figures 5-7 show the effects of noncholinergic drugs on learning deficits in the active avoidance and water maze tasks in rats with unilateral lesions of the BF. DMI 7.5 mg/kg significantly increased MAR on days 3, 4, and 5, while saline treatment had no effect on MAR. The avoidance response in the DMI-treated group was significantly different from that in the lesioned control group on days 3 and 4 after the higher dose of L-dopa (20 mg/kg). However, 5-methoxy DMT did not affect the avoidance deficit at the doses tested (0.025 and 0.05 mg/kg, IP) (Fig. 8).

In the Morris water maze test, the learning deficits in BF-lesioned rats were not affected by DMI (7.5 mg/kg) or L-dopa (20 mg/kg), although these drugs were effective in the active avoidance tests. 5-Methoxy DMT (0.025 and 0.05 mg/kg) had less effect on performance in the water maze than it had on avoidance.

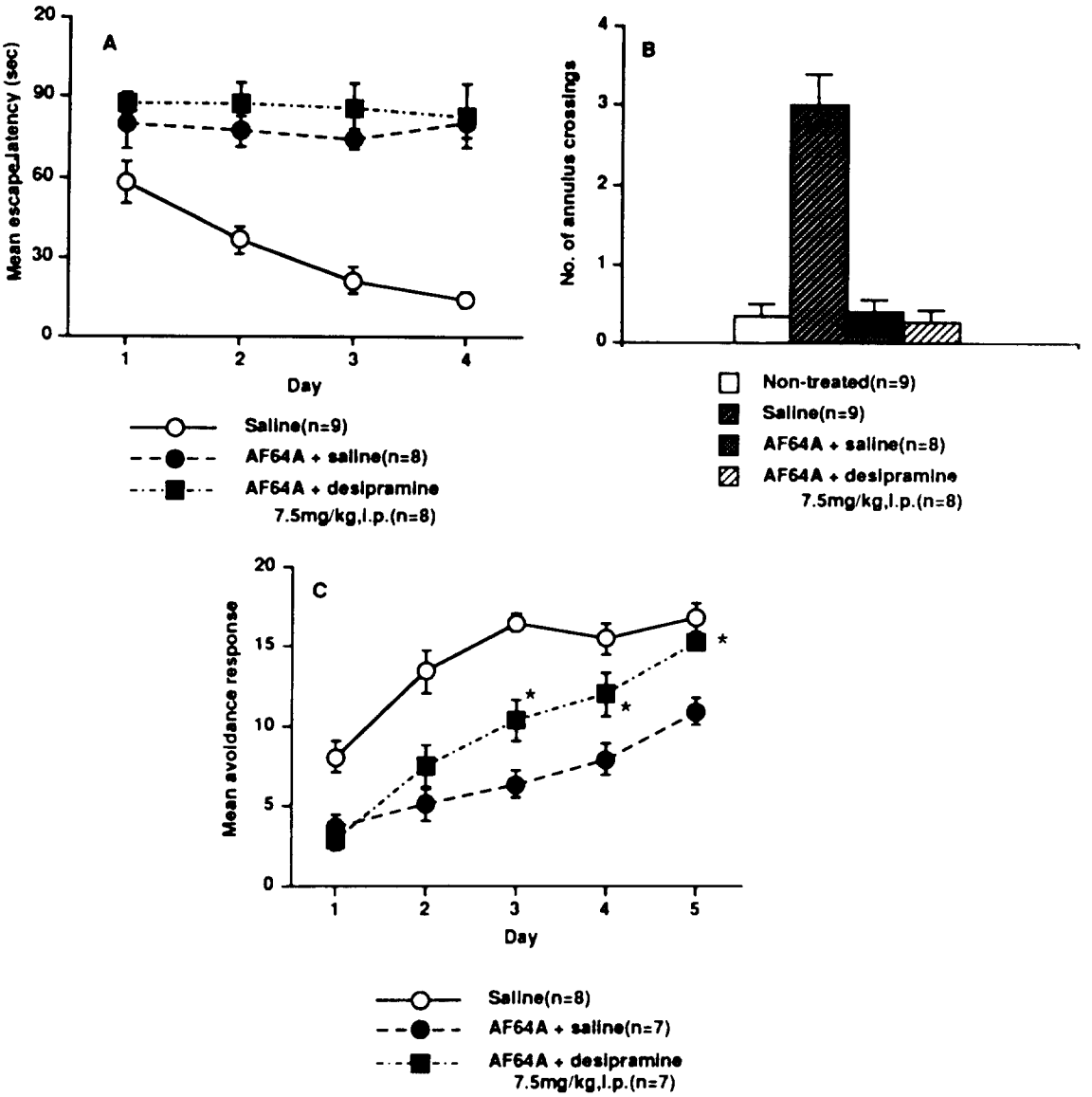

FIG. 6. Effect of DMI on the active avoidance and water maze learning in NBM-lesioned rats. (A,B,C) See the legend for Fig. 3. *$p < 0.05$ compared with the lesioned control.

DISCUSSION

Unilateral infusion of AF64A into the BF significantly decreased ChAT activity only in the ipsilateral FC, and did not cause changes in the enzyme activity in the HC and ST. This cholinergic dysfunction was associated with severe impairment in acquisition and retention of both the active avoidance and Morris water maze tasks (Fig. 2). Disruptions of cholinergic markers and of learning have also been observed in animals whose nucleus basalis was damaged by excitotoxins or electrolysis (2,13,23,28,44). However, the degree of these deficits varied depending on the methods used to lesion the BF. Wenk et al. showed that the behavioral deficit caused by an ibotenate-induced BF lesion is more severe than that caused by a quisqualate lesion, although the quisqualate lesion results in a more marked decrease in cortical ChAT activity (47). In addition, Dunnett et al. reported that ibotenate and quisqualate lesions had different effects on the place navigation task (11). Furthermore, Robbins et al. (40) and Riekkinen et al. (39) demonstrated that the NBM cortical cholinergic system is not significantly involved in spatial navigation. It is possible that AF64A-induced behavioral deficits result from damage to some other neurochemical system.

The present data show that injection of AF64A into the unilateral BF has deleterious effects on noncholinergic markers. There were marked decreases in levels of NE, DA, and 5-HT in all brain regions tested in rats with BF lesions. Similarly, Tilson et al. (44) reported that bilateral injection of 2.5 μg colchicine into the BF significantly decreases levels of NE, DA, and 5-HT in the FC but not in the HC or ST. Furthermore, Araki et al. (2) demonstrated that electrolysis of the posterior BF decreases the levels of NE, DA, and 5-HT in the

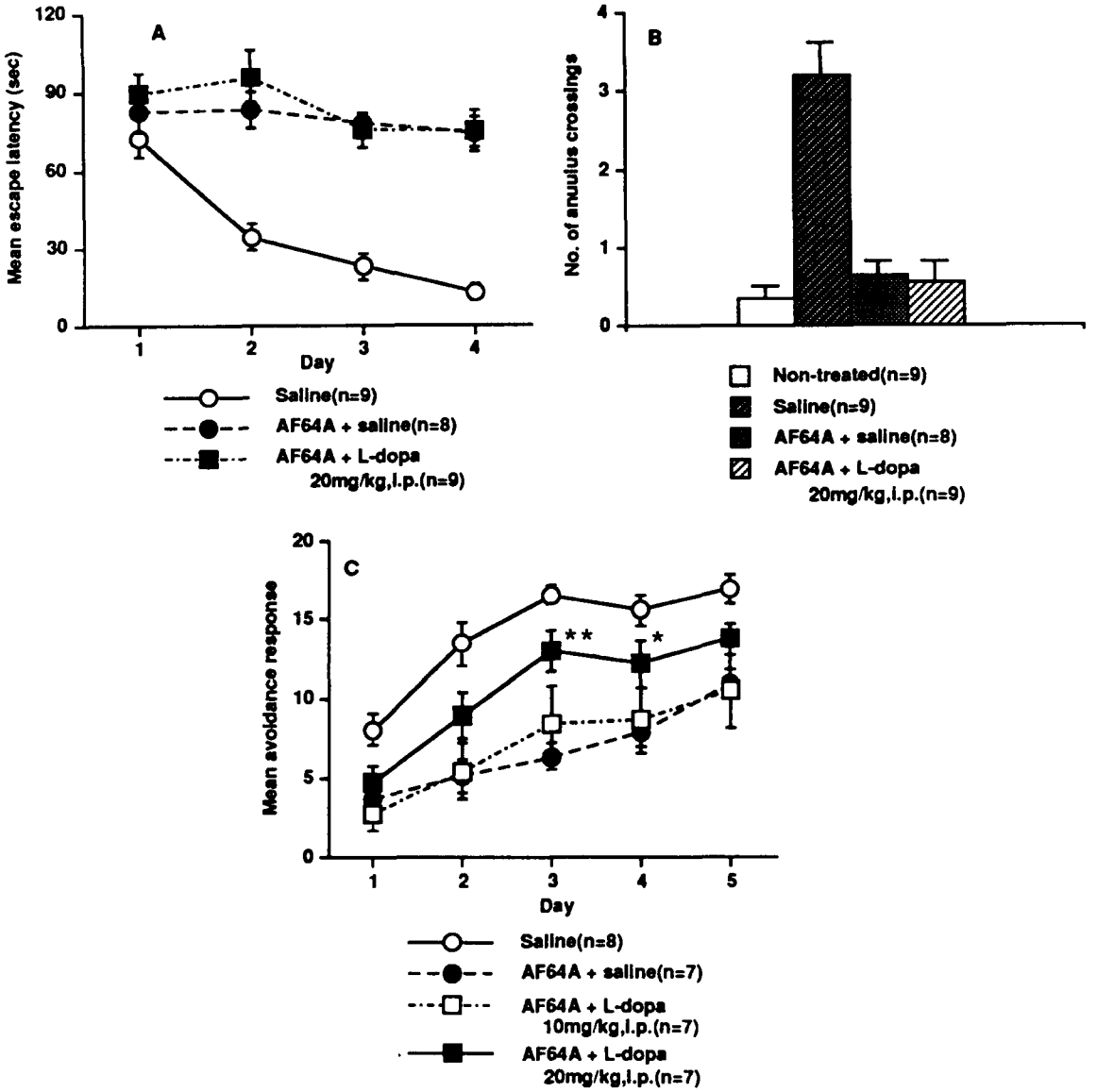

FIG. 7. Effect of L-dopa on the active avoidance and water maze performance in NBM-lesioned rats. (A,B,C) See the legend for Fig. 3. $*p < 0.05$, $**p < 0.01$ compared with the lesion control.

occipital cortex, HC, and ST. Lerer et al. (24) also reported that kainic acid lesions of the BF decrease the 5-HT level in the FC.

In the present study, GAD activity was not affected by AF64A. This result is compatible with those reported by La-Marca and Fibiger (23) and by McKinney and Coyle (28). Therefore, it is very likely that intra-BF injection of AF64A causes extensive brain dysfunction by damaging both cholinergic and monoaminergic systems and that AF64A-induced behavioral deficits are produced as a result of these neuronal losses. Histological analysis also indicated that the areas damaged by AF64A spread, centering around the NBM.

The unilateral BF lesion caused by AF64A significantly impaired the acquisition and retention of both associative and spatial learning tasks in the present study. Previously, we reported that behavioral recovery in rats with unilateral BF lesions varied with the task. Specifically, the impairing effect on active avoidance almost vanished within 4 or 5 weeks after BF lesion, but that on the Morris water maze task persisted for more than 19 weeks (34). This difference may be due to a difference in the neuronal systems involved in these two memory tasks.

The deficit in acquisition of the Morris water maze task was ameliorated both by a cholinesterase inhibitor (PHY) and a muscarinic agonist (ARE).

On the contrary, the deficit in acquisition of the active avoidance task was not affected by the cholinergic drugs at doses effective in the Morris water maze task. Dokla and Thal (9) and Murray and Fibiger (32) demonstrated that some cholinesterase inhibitors attenuated the BF lesion-induced impairment in the acquisition of water maze and radial maze tasks. Hagan et al. (19) reported that HC-3-induced spatial discrimination learning deficits were reversed by cholinergic drugs. Therefore, cholinergic drugs may stimulate the hippocampal

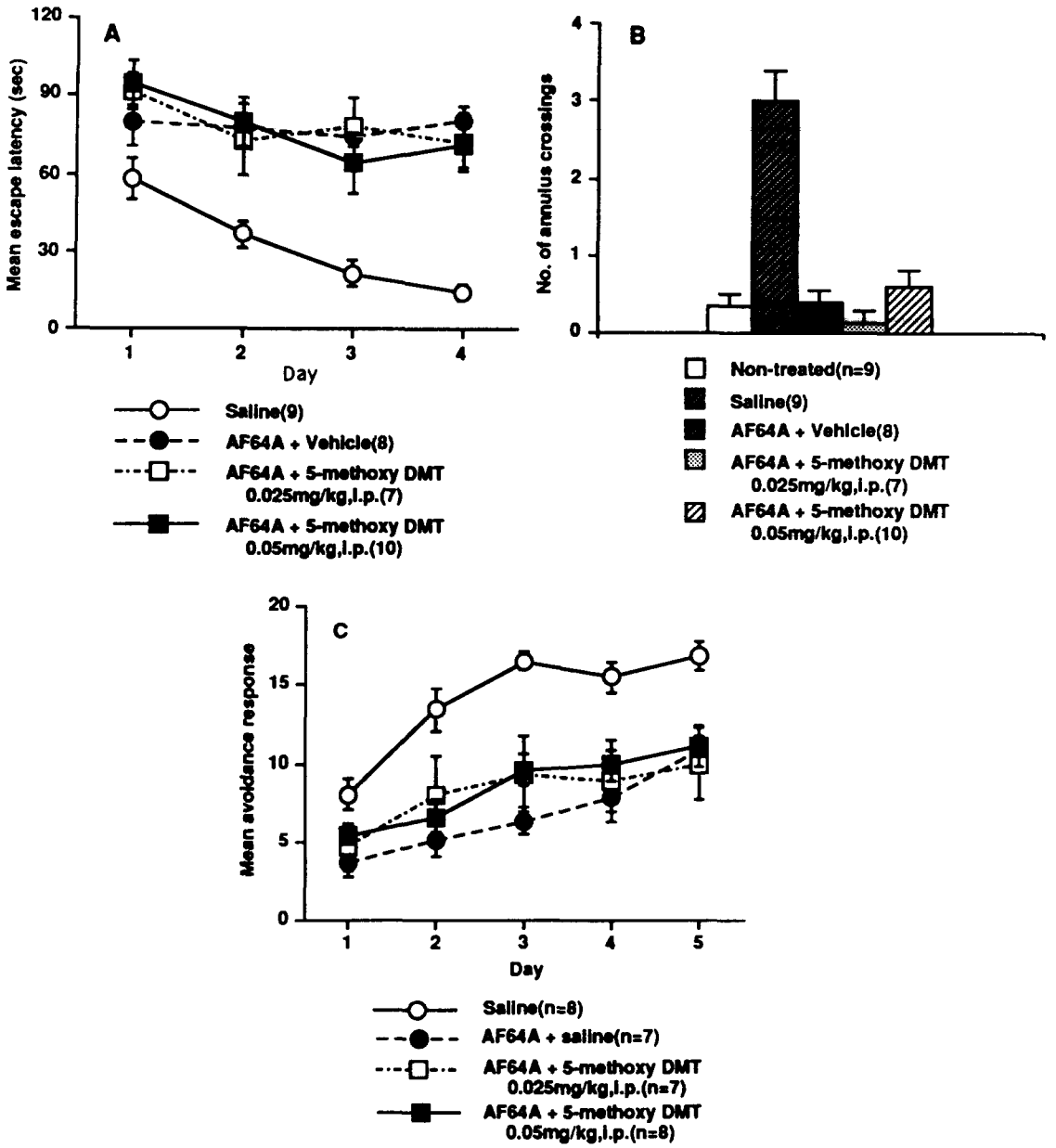

FIG. 8. Effect of 5-methoxy DMT on the active avoidance and water maze performance in NBM-lesioned rats. (A,B,C) See the legend for Fig. 3.

cholinergic system, which is centrally involved in spatial learning. However, Miyamoto et al. (30) demonstrated that a continuous subcutaneous infusion of PHY or oxotremorine completely reversed the learning deficits in both the water maze and active avoidance tasks in BF-lesioned rats. In the present study, however, cholinergic drugs improved the performance in the water maze learning task but not in the active avoidance learning task in rats with BF lesions. This discrepancy may be due to differences in the mode of drug administration and in the learning tasks used. In the study reported by Miyamoto et al. (30), the same animals sequentially performed three types of learning tasks (passive avoidance, active avoidance, and water maze), but in the present study different rats were given two different learning tasks (active avoidance and water maze).

DMI, a selective NE uptake inhibitor, and L-dopa, a precursor of DA, did not affect the impairment in Morris water maze learning, although these agents did ameliorate the deficit in active avoidance learning in BF-lesioned rats. Ögren et al. (36) demonstrated that desipramine reverses the active avoidance learning deficit produced by DSP-4, an NE depletor. In addition, 5-methoxy DMT, a 5-HT_{1A} agonist (41), also did not affect the learning deficit in either the active avoidance or the Morris water maze task.

On the contrary, central catecholamine depletion due to local brain lesions caused by administration of 6-hydroxydopamine or peripheral DSP-4 do not impair water and radial arm maze learning in rats (4,6,18,20). However, the data regarding central 5-HT depletion produced by *p*-chloroamphetamine are contradictory. This drug has been reported to facili-

tate (1) and impair (3) complex spatial discrimination learning and to enhance (45) and have no effect (5,37) on avoidance behavior.

The results of the present study indicate that rats with lesions in the BF caused by AF64A develop severe spatial and associative memory impairments, and suggest that these deficits result from damage to both cholinergic and noncholinergic neuronal systems. The present pharmacological findings strongly support the notion that diverse brain neuronal systems are involved in associative and spatial learning. Therefore, it is suggested that cortical and subcortical monoamine levels in addition to acetylcholine/choline levels should be assessed after all purported lesions of the NBM.

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REFERENCES

1. Altman, H. J.; Ögren, S.-O.; Berman, R. F.; Normile, H. J. The effects of *p*-chloroamphetamine, a depletor of brain serotonin, on the performance of rats in two types of positively reinforced complex spatial discrimination tasks. Behav. Neural Biol. 52:131–144; 1989.
2. Araki, H.; Uchiyama, Y.; Kawashima, K.; Aihara, H. Impairment of memory and changes in neurotransmitters induced by basal forebrain lesion in rats. Jpn. J. Pharmacol. 41:497–504; 1986.
3. Asin, K. E.; Wirtshafter, D.; Fibiger, H. C. Electrolytic, but not 5,7-dihydroxytryptamine, lesions of the nucleus medianus raphe impair acquisition of a radial maze task. Behav. Neural Biol. 44: 415–424; 1985.
4. Becker, T. J.; Walker, J. A.; Olton, S. D. Neuroanatomical bases of spatial memory. Brain Res. 200:307–320; 1980.
5. Brody, J. F. Behavioral effects of serotonin depletion and of *p*-chlorophenylalanine (a serotonin depletor) in rats. Psychopharmacologia 17:14–33; 1970.
6. Chrobak, J. J.; Dehaven, D. L.; Walsh, T. J. Depletion of brain norepinephrine with DSP-4 does not alter acquisition or performance of a radial-arm maze task. Behav. Neural Biol. 44:144–150; 1985.
7. Coyle, J. T.; Price, D. L.; Delong, M. R. Alzheimer's disease: A disorder of cortical cholinergic innervation. Science 219:1184–1190; 1983.
8. Davies, P.; Maloney, A. J. F. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 2:1403; 1976.
9. Dokla, C. P.; Thal, L. J. Effect of cholinesterase inhibitors on Morris water task behavior following lesions of the nucleus basalis magnocellularis. Behav. Neurosci. 102:861–871; 1988.
10. Dubois, B.; Mayo, W.; Agid, Y.; Lemoal, M.; Simon, H. Profound disturbances of spontaneous and learned behaviors following lesions of the nucleus basalis magnocellularis in the rat. Brain Res. 338:249–258; 1985.
11. Dunnett, S. B.; Whishaw, I. Q.; Jones, G. H.; Bunch, S. T. Behavioral, biochemical and histological effects of different neurotoxic amino acids injected into nucleus basalis magnocellularis of rats. Neuroscience 20:653–669; 1987.
12. Fisher, A.; Mantione, C. R.; Abraham, D. L.; Hanin, I. Long-term central cholinergic hypofunction induced in mice by ethylcholine aziridinium ion (AF64A) in vivo. J. Pharmacol. Exp. Ther. 222:140–145; 1982.
13. Flicker, C.; Dean R. L.; Watkins, D. L.; Fisher, S. K.; Bartus, R. T. Behavioral and neurochemical effects following neurotoxic lesions of a major cholinergic input to the central cortex in rat. Pharmacol. Biochem. Behav. 18:973–981; 1983.
14. Fonnum, F. A rapid radiochemical method for the determination of choline acetyltransferase. J. Neurochem. 18:407–409; 1975.
15. Fujiwara, M.; Iwasaki, K. Neurotransmitter abnormalities in Alzheimer's disease and nootropic candidate. Jpn. J. Neuropsychopharmacol. 11:793–805; 1989 (in Japanese).
16. Glowinski, J.; Iversen, L. L. Regional studies of catecholamine in the rat brain. I: The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]dopa in various regions of the brain. J. Neurochem. 13:655–669; 1966.
17. Gottfries, C. G. Alzheimer's disease and senile dementia: Biochemical characteristics and aspects of treatment. Psychopharmacology (Berl.) 86:245–252; 1985.
18. Hagan, J. J.; Alpert, J. E.; Morris, R. G. M.; Iversen, S. D. The effects of central catecholamine depletion on spatial learning in rats. Behav. Brain Res. 9:83–104; 1983.
19. Hagan, J. J.; Jansen, J. H. M.; Broekkamp, C. L. E. Hemicholinium-3 impairs spatial learning and the deficit is reversed by cholino-mimetics. Psychopharmacology (Berl.) 98:347–356; 1989.
20. Kessler, J.; Markowitsch, H. J. Delayed alteration performance after kainic acid lesions of the thalamic mediodorsal nucleus and the ventral tegmental area in the rat. Behav. Brain Res. 3:125–130; 1981.
21. Kimura, H.; Kuriyama, K. A new microassay method for L-glutamic acid decarboxylase (GAD) activity. Jpn. J. Pharmacol. 25: 189–195; 1975.
22. Kozlowski, M.; Arbogast, R. Specific toxic effects of ethylcholine nitrogen mustard on cholinergic neurons of the nucleus basalis. Brain Res. 372:45–54; 1986.
23. LaMarca, M.; Fibiger H. Deoxyglucose uptake and choline acetyltransferase activity in cerebral cortex following lesions of the nucleus basalis magnocellularis. Brain Res. 307:366–369; 1984.
24. Lerer, B.; Warner, J.; Friedman, E.; Vincent, G.; Gamzu, E. Cortical cholinergic impairment and behavioral deficits produced by kainic acid lesion of rat magnocellularis basal forebrain. Behav. Neurosci. 99:661–677; 1985.
25. Mantione, C. R.; Fisher, A.; Hanin, I. The AF64A-treated mouse: Possible model for central cholinergic hypofunction. Science 213:579–580; 1981.
26. Marchbanks, R. M. Biochemistry of Alzheimer's dementia. J. Neurochem. 39:9–15; 1982.
27. McGurk, S. R.; Hartgraves, S. L.; Kelly, P. H.; Gordon, M. N.; Butcher, L. L. Is ethylcholine mustard aziridinium ion a specific cholinergic neurotoxin? Neuroscience 22:215–224; 1987.
28. McKinney, M.; Coyle, J. Regulation of neocortical muscarinic receptors: Effects of drug treatments and lesions. J. Neurosci. 2: 97–105; 1982.
29. Miyamoto, M.; Kato, J.; Narumi, S.; Nagaoka, A. Characteristics of memory impairment following lesioning of the basal forebrain and medial septal nucleus in rats. Brain Res. 419:19–31; 1987.
30. Miyamoto, M.; Narumi, S.; Nagaoka, A.; Coyle, J. T. Effects of continuous infusion of cholinergic drugs on memory impairment in rats with basal forebrain lesions. J. Pharmacol. Exp. Ther. 248:825–835; 1989.
31. Morris, R. G. M. Spatial localization does not depend upon the presence of local cues. Learn. Motiv. 12:239–260; 1981.
32. Murray, C. L.; Fibiger, A. Learning and memory deficits after lesions of the nucleus basalis magnocellularis: Reversal by physostigmine. Neuroscience 19:1025–1032; 1985.
33. Nakamura, S.; Ishihara, T. Region selective increase in activities of CNS cholinergic marker enzymes during learning of memory tasks in aged rats. Pharmacol. Biochem. Behav. 34:805–810; 1989.
34. Nakamura, S.; Ishihara, T. Task-dependent memory loss and recovery following unilateral nucleus basalis lesion: Behavioral and neurochemical correlation. Behav. Brain Res. 39:113–122; 1990.
35. Nakamura, S.; Nakagawa, Y.; Kawai, M.; Tohyama, M.; Ishihara, T. AF64A (ethylcholine aziridinium ion)-induced basal forebrain lesion impairs maze performance. Behav. Brain Res. 29:119–126; 1988.

36. Ögren, S.-O.; Archer, T.; Ross, S. B. Evidence of a role for the locus coeruleus noradrenaline system in learning. Neurosci. Lett. 20:351–356; 1980.
37. Ögren, S.-O.; Fuxe, K.; Archer, T.; hall, H.; Holm, A. C.; Kohler, C. Studies of the role of central 5-HT neurons in avoidance learning: A behavioral and biochemical analysis. In: Haber, B.; Gabay, S.; Issidorides, M. R.; Alivisatos, S. G. A., eds. Serotonin: Current aspect of neurochemistry and function. New York: Plenum Press; 1981:681–705.
38. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1982.
39. Riekkinen, P., Jr.; Sirvio, J.; Riekkinen, P. J. Similar memory impairments found in medial septal-vertical diagonal band of Broca and nucleus basalis lesioned rats: Are memory defects induced by nucleus basalis lesions related to the degree of nonspecific subcortical cell loss? Behav. Brain Res. 37:81–88; 1990.
40. Robbins, T. W.; Everitt, B. J.; Marston, H. M.; Wilkinson, J.; Jones, G. H.; Page, K. J. Comparative effects of iboteic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: Further implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. Behav. Brain Res. 35:221–240; 1989.
41. Smith, L. M.; Peroutka, S. J. Differential effects of 5-hydroxytryptamine-1A selective drugs on the 5-HT behavioral syndrome. Pharmacol. Biochem. Behav. 24:1513–1519; 1986.
42. Smith, P. K.; Krohn, R. I.; Hermanson, G. T.; Mallia, A. K.; Gartner, F. H.; Provenzano, M. D.; Fujimoto, E. K.; Goeke, N. M.; Olson, B. J.; Klenk, D. C. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76–85; 1985.
43. Tani, Y.; Ishihara, T. Simultaneous measurement of tetrahydrobiopterin (THBP) and biogenic amines by liquid chromatography with electrochemical detection. Life Sci. 46:373–378; 1990.
44. Tilson, H. A.; Mclamb, R. L.; Shaw, S.; Rogers, B. C.; Pediaditakis, P.; Cook, L. Radial-arm maze deficits produced by colchicine administered into the area of the nucleus basalis are ameliorated by cholinergic agents. Brain Res. 438:83–94; 1988.
45. Valzelli, L.; Pawlowski, L. Effect of *p*-chlorphenylalamine on avoidance learning of two differentially housed mouse strains. Neuropsychobiology 5:121–128; 1979.
46. Wenk, G. L.; Cribbs, B.; McCall, L. Nucleus basalis magnocellularis: Optimal coordinates for selective reduction of choline acetyltransferase in frontal neocortex by ibotenic acid injection. Exp. Brain Res. 56:335–340; 1984.
47. Wenk, G. L.; Markowska, A. L.; Olton, S. S. Basal forebrain lesions and memory: Alterations in neurotensin, not acetylcholine, may cause amnesia. Behav. Neurosci. 103:765–769; 1989.
48. Whitehouse, P.; Price, D.; Struble, R.; Clark, A.; Coyle, J.; Delong, M. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. Science 215:1237–1239; 1983.