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The Allocentric Place Discrimination Task Is Selectively and Highly Dependent on the Central Muscarinic System in Rats

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KIKUSUI, T., T. TONOHIRO AND T. KANEKO. *Allollocentric place discrimination task is selectively and highly dependent on the central muscarinic system in rats.* PHARMACOL BIOCHEM BEHAV **65**(1) 131–139, 2000.—The allocentric place discrimination task (APDT) is useful in evaluating working memory separately from and simultaneously with motivation, motor and sensory ability. Muscarinic acetylcholine receptor antagonist scopolamine has been shown to selectively impair the accuracy of APDT without changing swimming speed, distance, and still time. For further evaluation of other neurotransmitters' roles in the APDT, pharmacological manipulations were performed. Neither diazepam 3.0 mg/kg, mecamylamine 10 mg/kg, haloperidol 0.5 mg/kg, nor 8-OH DPAT 1.0 mg/kg affected accuracy of place discrimination. Two kinds of responses were observed following the administration of MK-801 0.3 mg/kg: the accuracy of rats for longer swimming distance tended to decrease, and the accuracy of rats for normal swimming distance did not change. Therefore, NM-801 did not seem to affect the working memory selectively. In addition, neither flumazenil 10 mg/kg, ondansetron 0.3 mg/kg nor R(−)-α-metylhistamine 10 mg/kg attenuated the scopolamine-induced deficits. These results suggest that the central muscarinic receptors are selectively and highly important in the APDT. © 1999 Elsevier Science Inc.

Allocentric place discrimination task Central muscarinic system Rats

THE Morris water maze is a particularly useful tool for assessment of ability for spatial memory (37,39), which is reliably sensitive to hippocampal function (40,43), the cholinergic system (3,15–17,19,27,33,55,58,59), and aging (9,11–14). Impairments of spatial memory following administrations of centrally acting drugs were reported for the place navigation task using the Morris water maze (30). Most previous studies performed sensorimotor assessments in combination with place training—typically by using a visible platform procedure. But it is still uncertain that poor performing animals maintain their sensorimotor and motivational function normally because these two assessments were conducted individually. In other words, it is difficult to detect memory deficits independently of influences of other processes just when animals perform poorly in the common Morris water maze. We have modified the Morris's two-platform test (38), and established the allocentric place discrimination task (APDT), in which ability for spatial memory can be evaluated separately from and simultaneously with motivational, motor, and sensory processes in individual animals (21). In this task, accuracy of place discrimination is a good index of ability for allocentric spatial working memory. A selective decrease of swimming speed reflects motor deficits, and an increase of still time accompanied by a decrease of swimming speed reflects decreases in motivation (21). Therefore, processes that drugs act on can be detected more accurately with this task than with the place-navigation task in the Morris water maze. To verify the roles of neurotransmitters in the spatial learning and memory processes, we performed two experiments. In Experiment 1, the following pharmacological manipulations, which have been reported to impair the place-navigation task in the Morris water maze, were performed in the APDT: diazepam (DZP) as a BZP-GABA receptor agonist (1,2,24,25,28,29,31, 32,60), mecamylamine (MCML) as a nicotinic acetylcholine

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receptor antagonist (8,48,49), haloperidol (HAL) as a D_2 receptor antagonist (44,45), (±)-8-OH-DPAT (DPAT) as a 5-HT_{1A} receptor agonist (4–6,46,47), and MK-801 as an NMMA receptor noncompetitive antagonist (18,20,36,56).

We previously showed that the muscarinic acetylcholine antagonist scopolamine impairs the accuracy in the APDT (21); therefore, interaction of the muscarinic system and other neurotransmitters' systems were investigated in Experiment 2.

EXPERIMENT 1

Method

Subjects. Male Fisher-344 rats ($n = 39$) were obtained from Japan Charles River Co., Ltd. At the beginning of training, the animals were 9 to 10 weeks old. They were housed three to five per cage, in a room with a 12 L:12 D cycle (lights on: 0700–1900 h), and the environment was kept at constant temperature (24 ± 1°C) and humidity (45 ± 5%). Food and water were provided ad lib. All experiments were performed from 0900 to 1800 h.

Apparatus. A circular water tank, 1.5 m in diameter and 0.5 m in height, was located in the center of a small room and was surrounded by numerous extramaze cues on the walls of the room. The tank was divided into four quadrants (N, E, W, and S) by two imaginary perpendicular lines crossing the center of the tank, and the tank was filled with clear water to a depth of 40 cm, and was maintained at 23.5 ± 1.0°C. The experimenter stood in the southwest corner of the room.

Round disk platforms 12 cm in diameter were used. A transparent platform was used for place-navigation task training, and was located 1.5 cm beneath the water surface. Two visible platforms, both made of white acrylate, were used for the APDT tests, and were located 0.5 cm above the water surface. These visible platforms were the same in appearance; one was fixed to the pool bottom with a plastic bar so that a rat was able to climb onto the platform from the water, whereas the other was a float connected to the pool bottom with a thread, and would sink when a rat tried to climb onto it.

An automated color tracking system (CAT-10, Muromachi Kikai, Co., Ltd., Tokyo) recorded the position of a rat in the tank. The camera was mounted 1.5 m above the surface of the water.

Training procedure: place navigation task. The procedure was the same as previously reported (21). The rats received one daily handling for 3 days, after which they were trained in the place-navigation task of the working memory. One session, consisting of six trials, was given each day for 12 days. For these sessions, the transparent platform was located 1.5 cm beneath the water surface in one of the four quadrants (N, E, W, or S) and at one of three distances from the edge of the tank (20, 40, or 60 cm). The platform location remained the same throughout a session of six trials. Between the sessions, the platform location was varied in a pseudorandom manner. Rats were released into the water at one of the three quadrants not containing the platform. The sequence of start location was chosen from the three quadrants in a pseudorandom manner. A trial began by releasing a rat, facing the wall of the tank, into the water, and ended when the rat found the platform or in 90 s, whichever came first. If the rat could not reach the platform within 90 s, the experimenter led the rat to the platform. The rat remained on the platform for 60 s, and was then rereleased into the water from the next start location. The criterion for the acquisition of this navigation task was that a rat could reach the platform within 300 cm of swimming distance from the second to the sixth trials for three consecutive sessions. All animals tested fulfilled the criterion within 12 sessions and were, therefore, used in the APDT tests.

Test procedure: allocentric place discrimination task. The APDT was performed to evaluate the ability for working memory. Two visible platforms were located in the same tank simultaneously. One test consisted of two sessions, one session per day for 2-consecutive days. One session consisting of six trials was given each day. In the first session, the solid platform was placed at the center of the N or S quadrant in a pseudorandom manner, and the float was placed in the opposite quadrant. The platform locations were kept in the same positions during the first session, and reversed in the second session. A trial began by releasing the rat, facing the wall of the tank, into the water while from one of the remaining two quadrants (E or W). The sequence of start location was chosen in a pseudorandom manner such that the same start location was not employed for more than three consecutive trials; each location was used three times during a session. The trial ended when the rat reached either the solid platform or the float, or at 90 s after the start, whichever came first. If the rat could not reach the solid platform, the experimenter led the rat to the solid platform. The rat remained on the solid platform for 45 s, and was then rerealeased into the water. The accuracy was calculated as the ratio (%) between the number of times of choice of solid platform to floating platform in five trials for each session. The first trial in each session was excluded from the calculation because it served as an informational trial of the location of the solid platform for each session. Animals were used repeatedly for the APDT tests, and a refresher session consisting of one session of the place navigation task was inserted between tests.

The criterion of the APDT was that the mean accuracy of the first and second session was above 80% for three consecutive tests. The animals that fulfilled the criterion were used for drug tests. All drug administrations were conducted on the second session of each test. Three to 5 days after each drug test, all animals performed one refresher session to minimized the interference of the location of the solid platform of proceeding session, and there were at least 6 days between each drug test to ensure the withdrawal of the effects of the previous drug. One APDT without drug manipulation was conducted to confirm the stability of accuracy between each drug test. The data of animals whose accuracy of no-drug APDT tests was below 80% was omitted.

Drugs. Mecamylamine hydrochloride, MK-801 hydrogen maleate (Sigma Chemical Co., St. Louis, MO) and (±)-8-OH-DPAT hydrobromide (Research Biochemical International, Natick, MA) were each dissolved in 0.9% saline. Haloperidol (Janssen, Beerse, Belgium) and diazepam (Sankyo, Co., Ltd., Tokyo, Japan) were each suspended in 0.5% tragacanth solution of saline. All injections were conducted intraperitoneally at a volume of 1 ml/kg in the home cage. DZP 3.0 mg/kg ($n = 11$) or vehicle ($n = 10$) was administrated 30 min, MCML 0. 5 ($n = 12$), 10 mg/kg ($n = 12$), or vehicle ($n = 12$) was administrated 30 min, HAL 0.1 ($n = 10$), 0.5 mg/kg ($n = 10$) or vehicle ($n = 10$) was administrated 60 min, DPAT 0.5 ($n = 12$), 1.0 mg/kg ($n = 12$), or vehicle ($n = 12$) was administrated 30 min, and MK-801 0.1 ($n = 12$), 0.3 mg/kg ($n = 12$), or vehicle ($n = 11$) was administrated 45 min prior to the test. Maximal number of drug or vehicle tests performed on a single animal was five times, and same drug was not administrated to the same animal at different dosages.

Data analysis. Data analyses were performed with StatView + Graphics 4.1J (Abacus Concepts, Inc., Berkeley,

CA). The significance level for all statistical tests was set at 0.05. The swimming distance, the swimming speed, the still time, and the swimming time were measured for each trial independently of accurate performance trial. A two-way ANOVA with repeated-measures was performed for these four parameters, with trials as a repeated measure. When there was a significant difference, post hoc analysis for comparison of each group was performed by Tukey's WSD test. The Kruskal–Wallis test was performed for comparison of the accuracy, and post hoc analyses was performed by Mann–Whittney test adopted by Ryan's procedure for multiple comparison.

Results

Neither DZP, MCML, HAL, DPAT, nor MK-801 impaired accuracy in the APDT (Table 1: DZP, $H = 3.21$, $p = 0.20$; MCML, $H = 0.59$, $p = 0.97$; HAL, $H = 1.33$ $p = 0.97$; DPAT, $H = 0.73$, $p = 0.69$; MK-801, $H = 2.67$, $p = 0.26$). These drugs, however, affected swimming distance, swimming speed, swimming time, and/or still time.

DZP 3.0 mg/kg decreased swimming speed and increased swimming time, but did not affect swimming distance and still time (Fig. 1). In swimming speed, ANOVA showed significant effects by the groups, $F(1, 19)$ 199.0, $p < 0.0001$, and trial, $F(5, 19) = 2.70$, $p < 0.05$, but not a significant interaction between groups and trial, $F(5, 19) = 0.44$. In swimming time, ANOVA showed significant effects by groups, $F(1, 19) = 48.1$, $p < 0.0001$, but not by trial, $F(5, 19) = 0.99$, not a significant interaction between group and trial, $F(5, 19) = 0.78$.

An overall ANOVA showed significant effects of MCML on swimming speed, $F(2, 33) = 9.11$, $p < 0.001$, and still time, $F(2, 33) = 7.57$, $p < 0.005$, but did not affect swimming distance and swimming time (Fig. 2). Post hoc analysis revealed that MCML 10 mg/kg, $F(1, 22) = 20.64$, $p < 0.05$, but not 5 mg/kg, $F(1, 22) = 0.33$, decrease swimming speed compared to vehicle controls. There is no significant trial difference and interaction between group and trial in swimming speed ($F < 1$). In addition, post hoc analysis showed that MCML 10 mg/kg, $F(1, 22) = 14.00$ $p < 0.05$, but not 5 mg/kg, $F(1, 22) = 3.61$, increased still time compared to vehicle controls. There is a significant trial difference, $F(2, 33) = 4.69$, $p < 0.0005$, but no interaction between group and trial in still time, $F(2, 33) = 0.90$.

An overall ANOVA showed significant effects of HAL on swimming speed, $F(2, 27) = 4.55$, $p < 0.05$, and still time, $F(2, 27) = 4.57$, $p < 0.05$, but did not affect swimming distance and swimming time (Fig. 3). Post hoc analysis revealed that HAL 0.5 mg/kg, $F(1, 18) = 8.12$, $p < 0.05$, and 0.1 mg/kg, $F(1, 18) = 6.62$, $p < 0.05$, increase swimming speed compared to vehicle controls. There is a significant trial difference, $F(5, 27) = 3.27$, $p < 0.01$, but no interaction between group and trial in swimming speed, $F(10, 27) = 1.6$. In addition, post hoc analysis showed that HAL 0.5 mg/kg, $F(1, 18) = 5.52$, $p < 0.05$, but not 0.1 mg/kg, $F(1, 18) = 4.80$, decreased still time compared to vehicle controls. There is no significant trial difference and no interaction between group and trial in still time ($F < 1.5$).

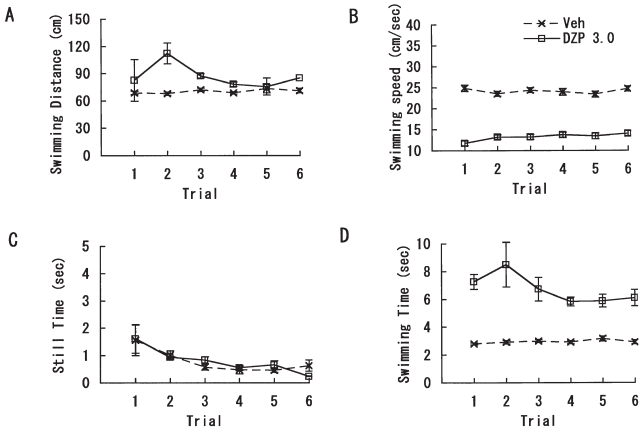

FIG. 1. The effects of DZP on the APDT. DZP 3.0 mg/kg IP ($n = 11$) decreased swimming speed (B) and increased swimming time (D) compared to the vehicle control (Veh, $n = 10$), but still time (C) and swimming distance (A) were not affected. Mean ± SEM.

TABLE 1

EFFECTS OF CENTRALLY ACTING DRUGS ON THE ACCURACY OF THE ALLOCENTRIC PALCE DISCRIMINATION TASK

Drugs	Dose (mg/kg)	Accuracy
DZP	0	78.0 ± 8.7
	3.0	66.0 ± 9.0
MCML	0	80.0 ± 5.4
	0.5	80.0 ± 3.8
	10	78.3 ± 6.9
HAL	0	78.0 ± 8.7
	0.1	74.0 ± 4.3
	0.5	76.0 ± 6.5
DPAT	0	80.0 ± 6.0
	0.5	80.0 ± 5.4
	1.0	86.7 ± 4.9
MK-801	0	81.8 ± 6.0
	0.1	76.9 ± 5.7
	0.3	66.7 ± 7.8

Mean ± SEM ($n = 10$–13).

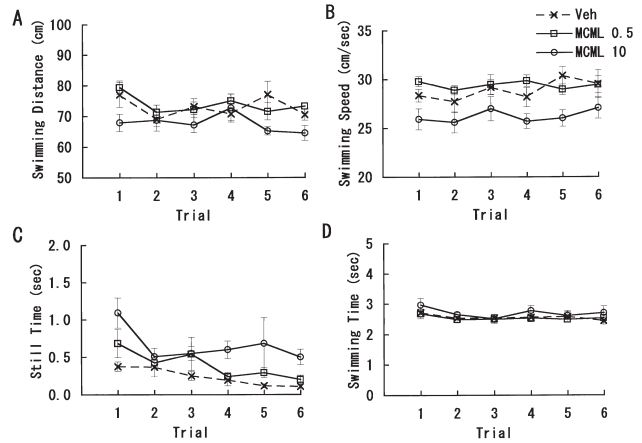

FIG. 2. The effects of MCML on the APDT. MCML 10 mg/kg IP ($n = 12$), not 0.5 mg/kg IP ($n = 12$), decreased swimming speed (B) and still time (C) compared to the vehicle control (Veh, $n = 12$), but swimming time (D) and swimming distance (A) were not affected. Mean ± SEM.

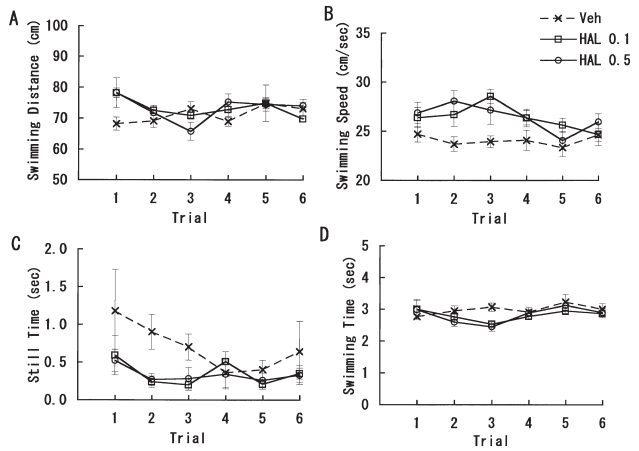

FIG. 3. The effects of HAL on the APDT. HAL 0.1 (n = 10), 0.5 mg/kg IP (n = 10) increased swimming speed (B) and decreased still time (C) compared to the vehicle control (Veh, n = 10), but swimming time (D) and swimming distance (A) were not affected. Mean ± SEM.

An overall ANOVA showed significant effects of DPAT on swimming distance, $F(2, 33) = 9.04$, $p < 0.001$, and swimming speed, $F(2, 33) = 13.2$, $p < 0.0001$, but did not affect still time and swimming time (Fig. 4). Post hoc analysis revealed that DPAT 10 mg/kg, $F(1, 22) = 16.91$, $p < 0.05$, and 0.5 mg/kg, $F(1, 22) = 7.39$, $p < 0.05$, increase swimming distance compared to vehicle controls. There is a significant trial difference, $F(5, 33) = 5.63$, $p < 0.0001$, and the interaction between group and trial, $F(10, 33) = 2.90$, $p < 0.005$, in swimming distance. In addition post hoc analysis showed that DPAT 1.0 mg/kg, $F(1, 22) = 18.91$, $p < 0.05$, and 0.5 mg/kg, $F(1, 22) = 19.4$, increased swimming speed compared to vehicle controls. There is no significant trial deference ($F < 1$), but a significant interaction between group and trial in swimming speed, $F(10, 33) = 2.16$, $p < 0.05$.

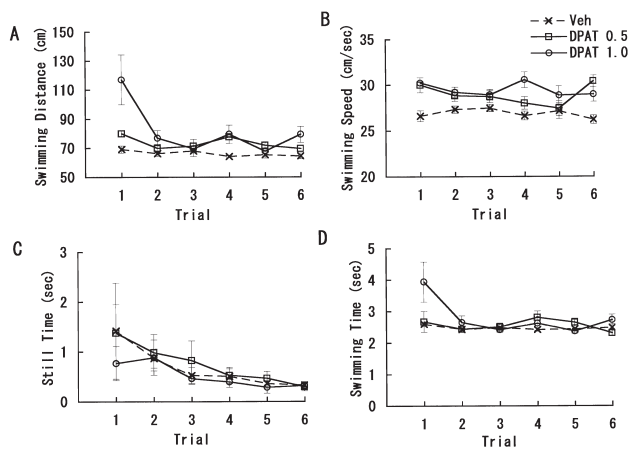

FIG. 4. The effects of DPAT on the APDT. DPAT 0.5 (n = 12), 1.0 mg/kg IP (n = 12) increased swimming speed (B) and swimming distance (A) compared to the vehicle control (Veh, n = 12), but still time (C) and swimming time (D) were not affected. Mean ± SEM.

An overall ANOVA showed significant effects of MK-801 on swimming distance, $F(2, 32) = 5.35$, $p < 0.01$, swimming speed, $F(2, 32) = 56.96$, $p < 0.0001$, swimming time, $F(2, 32) = 6.21$, $p < 0.01$, and still time, $F(2, 32) = 4.35$, $p < 0.05$ (Fig. 5). Post hoc analysis revealed that MK-801 0.3 mg/kg, $F(1, 21) = 5.17$, $p < 0.05$, but not 0.1 mg/kg, $F(1, 21) = 0.44$, increase swimming distance compared to vehicle controls. There is no significant trial difference, $F(5, 32) = 1.17$, and interaction between group trial, $F(10, 32) = 1.05$, in swimming distance. Post hoc analysis revealed that MK-801 0.3 mg/kg, $F(1, 21) = 64.25$, $p < 0.05$, but not 0.1 mg/kg, $F(1, 21) = 0.03$, decrease swimming speed compared to vehicle controls. There is no significant trial difference, $F(5, 32) = 0.29$, interaction between group and trial, $F(10, 32) = 0.98$, in swimming speed. Post hoc analysis revealed that MK-801 0.3 mg/kg, $F(1, 21) = 5.74$, $p < 0.05$, but not 0.1 mg/kg, $F(1, 21) = 0.91$, increase swimming time compared to vehicle controls. There is no significant trial difference, $F(5, 32) = 1.01$, and interaction between group and trial, $F(10, 32) = 1.11$, in swimming distance. Post hoc analysis revealed that MK-801 0.3 mg/kg, $F(1, 21) = 4.98$, $p < 0.05$, but not 0.1 mg/kg, $F(1, 21) = 0.001$, increase still time compared to vehicle controls. There is a significant trial difference, $F(5, 32) = 2.40$, $p < 0.05$, but no interaction between group and trial, $F(10, 32) = 0.81$, in still time.

Discussion

DZP 3.0 mg/kg did not impair accuracy (Table 1), but decreased swimming speed and increased swimming time (Fig. 1). These effects of DZP were very similar to those of a peripheral skeletal muscle relaxant dantrolene (21). Hence, the muscle relaxant effect of DZP, but not the amnesic effect, was observed in this APDT. It has been reported that benzodiazepines had amnesic properties in humans (7,22,50), and that DZP impaired place learning ability in the Morris water maze (1,2,24,25,28,29,31,32,60). The common observation in these reports is that benzodiazepines impair acquisition of tasks while sparing short-time memory and retrieval process (1,2, 7,22,50,60). In the APDT, repeated acquisition of working

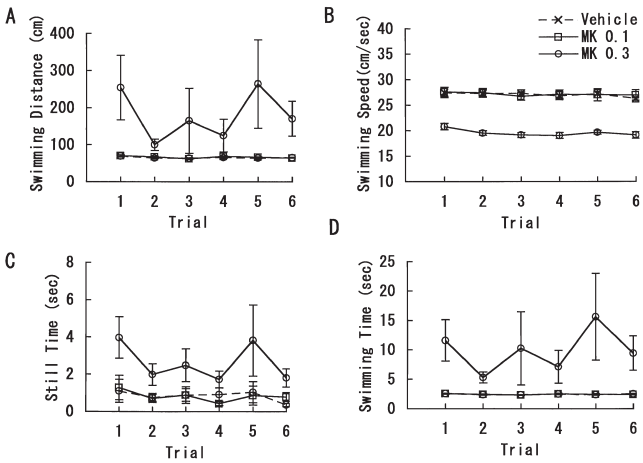

FIG. 5. The effects of MK-801 (MK) on the APDT. MK-801 0.3 mg/kg IP (n = 12) increased swimming distance (A), still time (C), and swimming time (D), and decreased swimming speed (B) compared to the vehicle control (Veh, n = 11). MK-801 0.1 mg/kg IP did not affect the parameters (n = 12). Mean ± SEM.

memory, a kind of short-term memory, was evaluated; therefore, the amnesic effect of DZP was not observed.

MCML 10 mg/kg did not affect accuracy (Table 1), but decreased swimming speed and increased still time (Fig. 2). These changes were similar to those observed in the case of a decrease in motivation in the APDT, induced by morphine or warm water (21). Therefore, MCML seems to decrease motivation. Decrease of motivation induces the spatial learning deficits in the Morris water maze as previously reported (26); therefore, MCML impaired the spatial learning in the Morris water maze, not in this APDT.

HAL increased swimming speed and decreased still time without impairing accuracy (Fig. 3, Table 1). These changes in behavioral parameters were opposite to those noted for warm water, morphine (21) and MCML. Thus, HAL seems to increase motivational process. It is uncertain that increasing motivation impairs spatial learning in the Morris water maze, but it is possible that excess of motivation upset the animal and impair the spatial learning in the Morris water maze. In this APDT, changes of motivation did not affect the accuracy; therefore, HAL did not impair the accuracy.

DPAT increased swimming distance and swimming speed, but did not affect accuracy (Fig. 4, Table 1). Actually, DPAT-treated animals did not always swim directly to the platform, and sometimes circled. Therefore, DPAT may increase locomotor activity.

MK-801 0.3 mg/kg increased swimming distance, still time, and swimming time, and decreased swimming speed (Fig. 5). MK-801 0.3 mg/kg apparently caused motor deficits; the animals failed to climb onto the platform, sometimes lost their balance, and fell down into the water again. In the MK-801 0.3 mg/kg-treated animals, there were large individual differences for the response to the drug. Seven out of 12 animals swam above 100 cm at least once in the test six trials, and the accuracy tended to decrease (60.0 ± 8.7% correct). In other animals, that swam normally, the accuracy was almost intact (76.0 ± 11.7% correct). In addition, the animals of the long swimming distance group did not touch the platform even when they swam very close to it. These observations suggest that MK-801 impaired cognitive function or caused confusion. In the Morris water maze, MK-801 impairs the spatial learning significantly, and the present results suggest that these impairments are induced by the cognitive dysfunction, rather than by impairment of the memory process directly.

EXPERIMENT 2

We previously reported that the muscarinic acetylcholine receptor antagonist scopolamine (SCP) selectively and severely impaired the accuracy in the APDT (21). The BZP-GABA receptor antagonist flumazenil (FLMZ) (27), the 5-HT_3 receptor antagonist ondansetron (OND) (10), and histamine$_3$ receptor agonist R(−)-α-metylhistamine (MHA) (53) were reported to ameliorate the SCP- or atropine-induced impairments in the place-navigation task in the Morris water maze. Therefore, these drugs were challenged to SCP-induced impairments in the APDT.

Method

Subjects, apparatus, procedure, and data analysis. The animals, apparatus, procedure, and data analysis were the same as those in Experiment 1.

Drugs. Scopolamine hydrochloride (Sigma Chemical Co., St. Louis, MO), R(−)-α-metylhistamine dihydrochloride (Research Biochemical International, Natick, MA), and ondansetron (Sankyo, Co., Ltd., Tokyo, Japan) were each dissolved in 0.9% saline. Flumazenil (Sankyo, Co., Ltd., Tokyo, Japan) were each suspended in a 0.5% tragacanth solution of saline. All injections were conducted intraperitoneally at a volume of 1 ml/kg in the home cage. MHA was administrated 60 min prior, and other drugs were administrated 30 min prior to the test. Three series of experiments were performed, each series consisted of four groups. The series contained grouping as follows: vehicle + SCP 0.5 mg/kg (n = 10), FLMZ 10 mg/kg + SCP 0.5 mg/kg (n = 11), FLMZ 10 mg/kg + vehicle (n = 12), and vehicle + vehicle (n = 11); vehicle + SCP 0.5 mg/kg (n = 10), OND 0.3 mg/kg + SCP 0.5 mg/kg (n = 11), OND 0.3 mg/kg + vehicle (n = 12), and vehicle + vehicle (n = 11); vehicle + SCP 0.5 mg/kg (n = 11), MHA 10 mg/kg + SCP 0.5 mg/kg (n = 11), MHA 10 mg/kg + vehicle (n = 10), and vehicle + vehicle (n = 11). Animals were divided into three groups, vehicle + SCP, drug + SCP, and drug + vehicle, and vehicle + vehicle-treated animals were chosen from the three groups randomly. Maximal number of tests performed on a single animal was five times. There were at least 6 days between each drug administration to ensure the withdrawal of the effects of the previous drug.

Results

SCP 0.5 mg/kg alone decreased accuracy to the chance level by itself (Table 2: FLMZ, $Z = -2.40$, $p = 0.005$; OND, $Z = -2.78$, $p < 0.01$; MHA $Z = -3.60$, $p < 0.0005$). Neither FLMZ, 10 mg/kg, OND 0.3 mg/kg, nor 10 mg/kg ameliorated SCP-induced impairments (Table 2: FLMZ, $Z = -0.62$, $p = 0.53$; OND, $Z = -0.18$, $p = 0.85$; MHA, $Z = -0.0036$, $p = 0.97$). Also, these drugs had no effect on the accuracy when administrated alone (Table 2: FLMZ, $Z = -0.107$, $p = 0.91$; OND, $Z = -0.95$, $p = 0.34$; MHA, $Z = -0.558$, $p = 0.58$).

An overall ANOVA showed significant effects of FLMZ on swimming speed, $F(3, 40) = 9.41$, $p < 0.0001$, and swimming time, $F(3, 40) = 3.91$, $p < 0.05$, but not on swimming distance and still time (Fig. 6). Post hoc analysis revealed signifi-

TABLE 2

EFFECTS OF FLMZ, OND, AND MHA ON THE SCOPOLAMINE-INDUCED DEFICITS IN THE ALLOCENTRIC PLACE DISCRIMINATION TASK

Drugs	Dose (mg/kg)	+ SCP Dose (mg/kg)	Accuracy
FLMZ	0	0	74.5 ± 5.0
	0	0.5	46.0 ± 9.0*
	10	0.5	49.1 ± 5.2*
	10	0	76.4 ± 6.2
OND	0	0	78.3 ± 5.0
	0	0.5	46.7 ± 7.1*
	0.3	0.5	47.3 ± 8.1*
MHA	0.3	0.0	78.3 ± 5.0
	0	0	83.6 ± 6.2
	0	0.5	50.9 ± 7.7*
	10	0.5	38.2 ± 7.2*
	10	0	80.0 ± 6.0

Mean ± SEM (n = 10–13).

*$p < 0.05$ compared to vehicle + vehicle group (Mann–Whitney test).

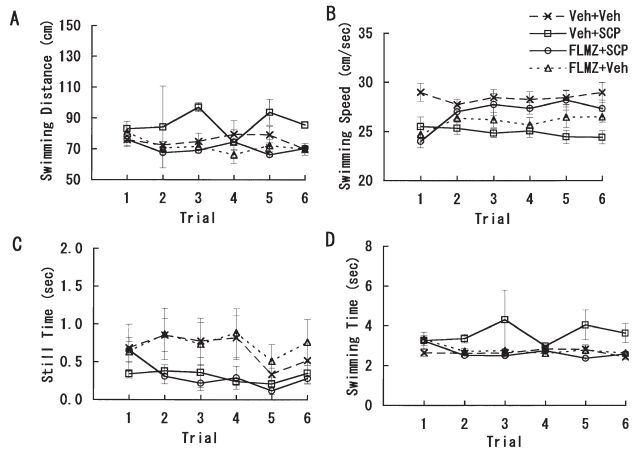

FIG. 6. The effects of SCP and FLMZ in the APDT. FLMZ 10 mg/kg IP (n = 12) and SCP 0.5 mg/kg IP (n = 10) decreased swimming speed by themselves (B). Swimming distance (A), still time (C), and swimming time (D) were not affected by any treatments. Mean ± SEM.

cant differences in swimming speed between vehicle + vehicle and SCP + vehicle, $F(1, 19) = 24.58$, $p < 0.05$, vehicle + vehicle and vehicle + FLMZ, $F(1, 21) = 16.46$, $p < 0.05$, but not between groups' comparisons. There is no significant trial difference, $F(5, 40) = 1.50$, and interaction between group and trial, $F(15, 40) = 1.70$, in swimming speed. Post hoc analysis did not reveal significant difference in swimming time for comparison of each group. There is no significant trial difference, $F(5, 40) = 0.83$, and interaction between group and trial, $F(10, 40) = 1.16$, in swimming time.

An overall ANOVA showed significant effects of OND on swimming distance, $F(3, 40) = 5.21$, $p < 0.005$, swimming speed, $F(3, 40) = 4.04$, $p < 0.05$, and swimming time, $F(3, 40) = 6.44$, $p < 0.005$, but not on still time (Fig. 7). Post hoc analysis revealed significant differences in swimming distance between SCP + vehicle and SCP + OND, $F(1, 20) = 12.44$, $p < 0.05$, and SCP + vehicle and vehicle + OND, $F(1, 19) = 8.55$, $p < 0.05$, but not between other groups' comparison. There is a significant trial difference, $F(5, 40) = 2.81$, $p < 0.05$, but no interaction between group and trial, $F(15, 40) = 1.70$, in swimming distance. Post hoc analysis revealed significant differences in swimming speed between vehicle + vehicle and SCP + OND, $F(1, 20) = 7.21$, $p < 0.05$, and SCP + OND and vehicle + OND, $F(1, 21) = 20.68$, $p < 0.05$, but not between other groups' comparison. There is no significant trial differences, $F(5, 40) = 1.78$, and no interaction between group and trial, $F(15, 40) = 0.17$, in swimming speed. Post hoc analysis revealed significant differences in swimming time between SCP + vehicle and vehicle + OND, $F(1, 20) = 12.03$, $p < 0.05$, and SCP + OND and vehicle + OND, $F(1, 21) = 14.05$, $p < 0.05$, but not between other groups' comparison. There is a significant trial difference, $F(5, 40) = 4.7$, $p < 0.05$, and the interaction between group and trial, $F(15, 40) = 2.70$, in swimming time.

An overall ANOVA showed significant effects of MHA on swimming speed, $F(3, 41) = 25.02$, $p < 0.0001$, and swimming time, $F(3, 41) = 8.77$, $p < 0.0001$, but not on swimming distance and still time (Fig. 8). Post hoc analysis revealed significant differences in swimming speed between vehicle + vehicle and SCP + MHA, $F(1, 20) = 83.08$, $p < 0.05$, vehicle + vehicle, and vehicle + MHA, $F(1, 21) = 45.37$, $p < 0.05$, SCP + vehicle and SCP + MHA, $F(1, 20) = 29.12$, $p < 0.05$, and SCP + vehicle and vehicle + MHA, $F(1, 21) = 17.55$, $p < 0.05$, but not between other groups' comparison. There is a significant trial difference, $F(5, 41) = 3.12$, $p < 0.05$, but no interaction between group and trial, $F(15, 41) = 1.21$, in swimming speed. Post hoc analysis revealed significant differences in swimming time between vehicle + vehicle and SCP + MHA, $F(1, 20) = 27.99$, $p < 0.05$, vehicle + vehicle and vehicle + MHA, $F(1, 21) = 10.31$, $p < 0.05$, SCP + vehicle and SCP + MHA, $F(1, 20) = 17.5$, $p < 0.05$, and SCP + vehicle and vehicle + MHA, $F(1, 21) = 6.67$, $p < 0.05$, but not between other groups' comparison. There is no significant trial difference, $F(5, 41) = 0.98$, and no interaction between group and trial, $F(15, 41) = 1.02$, in swimming speed.

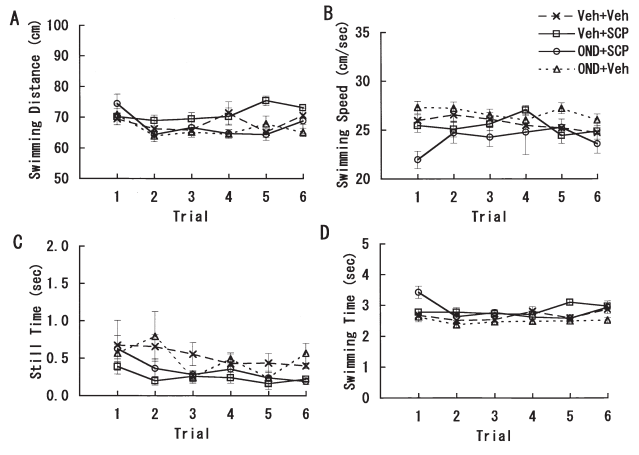

FIG. 7. The effects of SCP and OND on the APDT. OND 0.3 mg/kg + SCP 0.5 mg/kg IP (n = 11) decreased swimming distance compared to the vehicle + SCP group (n = 10) (A). Swimming speed (B), still time (C), and swimming time (D) were not affected by any treatments. Mean ± SEM.

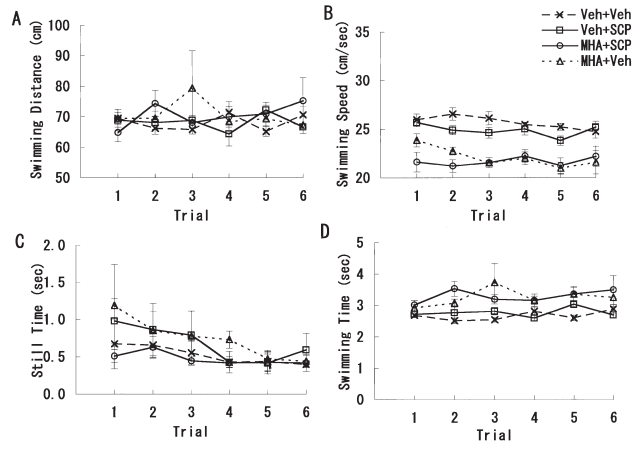

FIG. 8. The effects of SCP and MHA on the APDT. MHA 10 mg/kg IP + Vehicle (Veh, n = 10) and MHA 10 mg/kg + SCP 0.5 mg/kg (n = 11) decreased swimming speed (B) and increased swimming time (D). Swimming distance (A) and still time (C) were not affected by any treatments. Mean ± SEM.

Discussion

Neither FLMZ, OND, nor MHA ameliorated SCP-induced impairments in this task (Table 2). SCP decreased accuracy to the chance level, while other parameters were not affected. This suggests that SCP selectively impairs working memory of place discrimination, and that rats could not learn the location of the solid platform at all under a muscarinic blockade. MHA decreased swimming speed independently of SCP, so MHA may have weak muscle-relaxant effects.

GENERAL DISCUSSION

SCP delays the acquisition of place navigation task in the Morris water maze (3,15,27,35,46,53), i.e., animals can learn the task, but only slowly. So, it is possible that even under a central muscarinic blockade rats can acquire the place-navigation task using other neurotransmitter systems such as the nicotinic or the 5-HTergic system. In fact, coadministration of SCP with MCML (49), or with DPAT (46) induced severe deficits in the place-navigation task. And it is also reported that SCP-induced impairments were ameliorated by noncholinergic manipulations with FLMZ (27) or OND (10). Thus, results of the place-navigation task show that a muscarinic dysfunction can be compensated by changing other neurotransmitter systems. Summarizing these results, other neurotransmitter systems as well as the muscarinic system are important for the acquisition of the place-navigation task.

In the APDT, neither DZP, MCML, HAL, DPAT, nor MK-801 impaired the accuracy (Table 1); only SCP decreased the accuracy to chance level. Furthermore, neither FLMZ, OND, nor MHA ameliorated SCP-induced impairments (Table 2). These observations indicate that rats could not learn the platform location at all under a muscarinic blockade, and that modifications of other neurotransmitter systems were futile for compensating SCP-induced deficits. These results suggest that the central muscarinic system is highly indispensable for the accuracy in this task.

Both the place navigation task and the APDT evaluate allocentric spatial learning and memory (21,37). What are the differences between these tasks? The following comparison of these differences may contribute the elucidating the roles of the muscarinic system in spatial learning and memory. First, the period of maintaining information is different. In the place-navigation task, the acquisition of reference memory is evaluated, and information is valid for a long time (usually several days) once animals acquire it. In contrast, in the APDT, working memory is evaluated and information of the platform is valid only for several minutes, and consequently, animals are required to renew the memory for each session.

Second, the difference lies in whether or not experimental animals are naive to the environment. In the place navigation task, naive rats are used, and numerous other demands are made of them, such as habituating themselves to the experimental environment, selecting strategies to solve the task, remembering the strategies, and learning the place of the platform. Thus, drugs that do not affect the learning and memory processes directly may impair the performance in the place-navigation task. For example, it is quite possible that there is an optimal level of anxiety required for the place-navigation task, and that DZP (1,2,24,25,28,29,31,32,60), DPAT (5,46), and buspirone (34,51) impair the place-navigation task due to their anxiolytic effects. In comparison, because well-trained rats are used in the APDT, its accuracy reflects the memorys' ability directly by eliminating other factors such as the ability of habituating themselves to the experimental environment. This may be why the anxiolytic drugs DZP and DPAT did not impair the accuracy in the APDT.

Third, the characteristics of each task are different. In the navigation task, rats swim to a submerged platform using the allocentric orientation system and the path integration system (41–43,52,54,57). On the other side in the APDT, rats chose the solid platform mainly according to allocentric information (21). There are many articles that indicated that rats use two systems, "how to go there" and "where to go" in the common Morris water maze (41–43,52,54,57). Some of these articles showed that using the path integration system, animals did not swim directly from the start position to the platform, and sometimes swam with consistent distance from the wall of the pool, or swam to the center of the pool at first and made their way to the platform according to the background of it; i.e., they chose the "path" to the platform. The APDT highly depends on the allocentric orientation system rather than on the path integration system for following three points. 1) The accuracy was significantly decreased to the chance level when the pool was surrounded by a black curtain (21). This indicates that rats could not learn the location of the fixed platform completely without extramaze visible cues. 2) The fact that the accuracy was high (80–90% correct) when even the start location was varied randomly indicates that the animals could choose the fixed platform by the allocentric orientation system. If the rats had moved according to their egocentric orientation system (always turning right or left), or to the fixed left and the right sequence (start location was changed regularly), the accuracy would be decreased near the chance level by changing the start location randomly. 3) The path integration system was not necessary for the accuracy, because the platforms were visible, and the destination was apparently defined. That means the rats did not need to make the path to the platform. In fact, when the pool was surrounded by a black curtain, the accuracy was significantly decrease to the chance level, but the swimming distance was not affected (21). These results indicate that the path was apparently defined, and the rat swam straight to the platforms whether they knew which was the solid one or not, i.e., animals knew how to go there but did not know where to go. For these reasons, the accuracy of this APDT may depend on the allocentric "knowing where" system.

In humans, it is reported that the allocentric orientation system, i.e., "where to go," is functionally different from path integration system, i.e., "how to go" (23), but there is no appropriate animal model to assess the allocentric orientation system independently of the path integration system. In the present article, we showed that the results of drug manipulations were different from the common water maze, and suggest a possibility that this APDT evaluate the allocentric orientation system independently of the path-integration system. The hypothesis that the APDT is independent from the path integration system is not precisely proven at the present time, but this task can be a good tool to evaluate the spatial memory with independence of the path-integration system in a water maze.

In conclusion, the muscarinic receptor was highly and selectively important for the APDT, and based on these results, we were able to show three possibilities: 1) in spatial learning the muscarinic receptor was important for the working or short-term memory; 2) the receptor was more important for the allocentric spatial discrimination system than for the path-integration system; and 3) the receptor may not be involved in the ability of this rat model to habituate itself to the environment or to select task-solving strategies.

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