Simultaneous Evaluation of Spatial Working Memory and Motivation by the Allocentric Place Discrimination Task in the Water Maze in Rats

Takefumi KIKUSUI*, Toshiyuki TONOHIRO and Tsugio KANEKO

Neuroscience Research Laboratories, Sankyo Co., Ltd., 2–58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140–8710, Japan

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ABSTRACT. In order to evaluate learning and memory deficits separately from and simultaneously with motivational, motor and sensory impairments in identical animals, we developed the allocentric place discrimination task test using a water maze in rats. For this assessment task, two similar, visible platforms, one was fixed and the other was floating, were simultaneously present in a pool, and the working memory of the allocentric place discrimination task was evaluated. After training, the task accuracy was high about 85% correct and animals were used repeatedly. The accuracy decreased significantly when the pool was surrounded with a black curtain. Muscarinic receptor antagonist scopolamine 0.5 mg/kg selectively impaired the accuracy. Muscle relaxant dantrolene 10 mg/kg selectively decreased swimming speed. Under low motivational condition (warm water), still time increased and swimming speed decreased, but the accuracy was not affected. Similar to warm water, opioid receptor agonist morphine 15 mg/kg increased still time and decreased swimming speed. These results suggest that the allocentric place discrimination task is useful in evaluating spatial working memory ability independently of and concurrently with also visual, motor ability and motivation in identical animals.—KEY WORDS: allocentric spatial cognition, motivation, water maze, working memory.

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The Morris water maze is the most common behavioral test of allocentric spatial learning and memory [21, 23]. The spatial memory processing in this test depends on external landmarks and is reliably sensitive to hippocampus function [24, 27], central cholinergic system [1, 7–10, 12, 15, 31, 33, 34] and aging [2–6]. Additionally, the test is useful for assessment of long-term changes in the spatial memory process with aging because the motivating stimulus (i.e. escape from water) does not require food or water deprivation necessary in other appetitive tests like the radian arm maze or T-maze. When animals perform the task poorly in the Morris water maze, it is difficult to determine if deficits lie in memory formation or in sensory, motor or motivational processes. Therefore, it is necessary to evaluate the memory formation and the other processes separately and simultaneously in the identical animals.

In this study, we modified the Morris's two-platform test [22] and established a new method, the allocentric place discrimination task test, in which the spatial learning and memory ability can be evaluated separately from as well as simultaneously with motivational, motor and sensory processes in the individual animals.

There are two distinct types of memory, working and reference memory, in psychologically mnemonic processes. Reference memory is trial independent (i.e., relevant for every trial), and the experimental animals are required to learn general rules for a task [28], for example, escape from water to a platform. In contrast to reference memory, working memory is trial dependent (i.e., relevant for one or a few trials only), and the information to be remembered, for example, the location of the platform changes in repeated sessions. This type of memory has a major temporal component and it represents the whole memory processes (encoding, retention and retrieval) in each session [28]. Generally, the acquisition of reference memory is evaluated in the Morris water maze, in which rats initially do not know that a platform is in the pool and they can escape from water on it, so they swim along the edge of the pool with high anxiety. Therefore such data included not only the spatial memory ability, but also ability of selection of strategies to solve the task and habituation to the experimental environments. Once rats have learned the task, they are familiar with the environments and perform calmly. Therefore it is considered that using animals which have already acquired the general rules for the task is a valuable way to assess the spatial memory more purely. In the present study, the working memory was evaluated in order to assess the whole memory process by using the animals that had already acquired the task.

MATERIALS AND METHODS

Subjects

Male Fisher-344 rats (n= 36) were obtained from Japan Charles River Co., Ltd. At the beginning of training, the animals were 9 to 10 weeks old. They were housed 3–5 per cage in a room with a 12:12 light/dark cycle, and the environment was kept at constant temperature (24 ± 1°C) and humidity (45 ± 5%). Food and water were provided *ad libitum*. All experiments were performed from 9 a.m. to 6 p.m.

* PRESENT ADDRESS: KIKUSUI, T., Laboratory of Veterinary Ethology, Department of Veterinary Medicine University of Tokyo, 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–8657, Japan.

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 in 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 water to a depth of 40 cm and was maintained at 23.5 ± 1.0°C. The temperature was changed to 33°C on need. 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 working memory of 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 allocentric place discrimination task tests and were located 0.5 cm above the water surface. Though these visible platforms were the same in appearance, one was fixed to the bottom with a plastic bar permitting a rat to climb onto the platform from the water, whereas the other was connected to the pool bottom with thread and would sink if a rat tried to climb on it.

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

The tank could be surrounded with a nylon black curtain, 1.5 m in height and 1.8 m in diameter, on demand.

Procedure

Handling: Rats received one daily, 10-minute handling period for three days, after which the animals were trained for two days to stand on the transparent platform. On the first day, rats were placed on the transparent platform for 60 sec, which was at the center of the tank without water, and on the second day, the rats were placed again on the platform in the same condition except that the tank was filled with water. When the rat climbed off the platform, the experimenter guided it back onto the platform.

Training procedure: Working memory of place navigation task. To increase the accuracy of response in the allocentric place discrimination task, the rats were initially trained in the working memory of place navigation task. The rats learned from extramaze landmarks (window, door, etc.) in the room visible to the animals that orientation was allocentric and that the position of the platform was fixed throughout the session (for session, see below).

The transparent platform was placed in one of the four quadrants (N, E, W or S), at one of three distances from the edge of the tank (20, 40 or 60 cm), and 1.5 cm beneath the water surface. The platform location remained the same throughout a session of 6 trials. The platform location was changed pseudorandomly for each session so that the platform was located in a different quadrant and at a different distance from the edge of the tank from the immediately preceding session.

One session consisting of 6 trials was given each day. A trial began by releasing the rat into the water facing the wall of the tank from one of the three quadrants not containing the platform. The sequence of start location was chosen in a pseudorandom manner such that the start location was different from that in the immediately preceding trial, and each location was used twice in one session; once in the first and once in last 3 trials. The trial ended when the rat found the platform or at 90 s after start of the trial, 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 re-released into the water from the next start location. After the last trial in each session, the rat was towel-wiped and placed in a drying chamber for 5 to 15 min and then returned to the home cage.

Those rats that acquired the working memory of place navigation task were used in the allocentric place discrimination task tests. The criterion for the acquisition of this task was that the rat could reach the platform within 300 cm swimming distance from the second to sixth trials for 3 consecutive sessions. All animals tested fulfilled the criterion within 12 sessions.

Test procedure: Allocentric place discrimination task. The apparatus was the same as for the working memory of place navigation task (Fig. 1). Two visible, similar platforms were used to prevent the rats from learning the difference in appearance of the platforms in order to preserve the orientation allocentricity.

One test consisted of two sessions, one session per day for two consecutive days. One session consisted of 6 trials and was given each day. The allocentric place discrimination task tests were repeatedly performed with insertion between the tests of a refresher session that was composed of one session of the working memory of place navigation task.

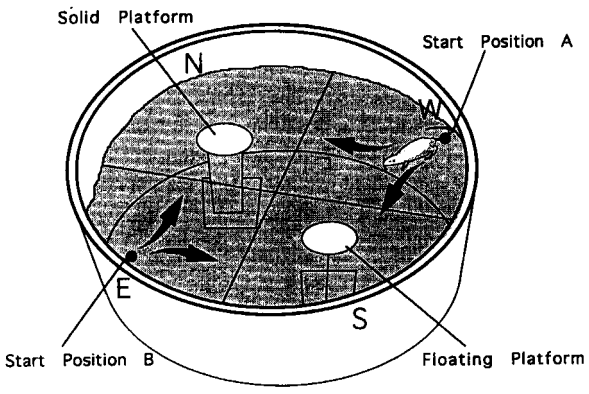

Fig. 1. Schematic diagram of the allocentric place discrimination task. The solid platform, on which a rat could climb, was placed at the center of the N or S quadrant and the float, on which a rat could not climb, was placed in the opposite quadrant. The solid platform and the float remained in the fixed positions during the first session and then were reversed in the second session. The rats were released into the water from the start position A or B. The sequence of start position was chosen in a pseudorandom manner, for example, A B A B A B or A B B A A B.

The locations of the platforms were changed in a pseudorandom manner in each session to evaluate the working memory. In the first session, the solid platform was placed at the center of the N or S quadrant and the float was placed in the opposite quadrant. The locations were kept constant during the first session and reversed in the second session. A trial began by releasing a rat into the water facing the wall of the tank from one of the remaining two quadrants (E or W). The start location was changed for each trial to prevent the tendency to swim to the left or right. The same start location was not employed 3 consecutive trials, each location was used three times during the session. The trial ended when the rat reached either the solid platform or the floating platform or at 90 s after the start, whichever came first. The rat remained on the solid platform for 45 sec and was then released into the water again for the next trial.

The accuracy was calculated as the percentage of correct responses (choose the fixed platform) in last 5 trials for each session. The first trial was excluded from the calculation because it was the information trial of the location of the solid platform in each session. Swimming distance, swimming speed, still time and swimming time were measured for each trial.

The rats used had fulfilled the criterion that the mean accuracy of the first and second session was above 80% for three consecutive tests.

All drug experiments and the warm water (33°C) experiments, except for the black curtain test, were conducted on the second sessions.

Drugs

Scopolamine hydrochloride (SCP HCl, Sigma), scopolamine methylbromide (SCP mBr, Sigma), morphine (MOR, Sankyo Co., Ltd.) and physostigmine salitilate (PHY, Sigma) were dissolved in saline, and dantrolene sodium (DAN, Sankyo Co., Ltd.) was dissolved in 0.5% tragacanth saline. SCP HCl 0.1 mg/kg (n=11) and 0.5 mg/kg (n=20), SCP mBr 0.5 mg/kg (n=11), DAN 10 mg/kg (n=13), and MOR 15 mg/kg (n=11) or vehicle (VEH) were intraperitoneally injected 30 min prior to the tests. PHY or vehicle was administrated intraperitoneally 15 min prior to the tests. There were at least 6 days between each drug administration to reduce the effects of previous drugs.

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.

A two-way ANOVA with repeated measures was performed for swimming distance, swimming speed, still time and swimming time with trials as repeated measures. When there was a significant difference among groups, post hoc analysis was performed by Tukey's WDS test. The Kruskal-Wallis test was performed for comparison of the accuracy, and post hoc analyses was performed by the Mann-Whitteny test adopted by Ryan's procedure.

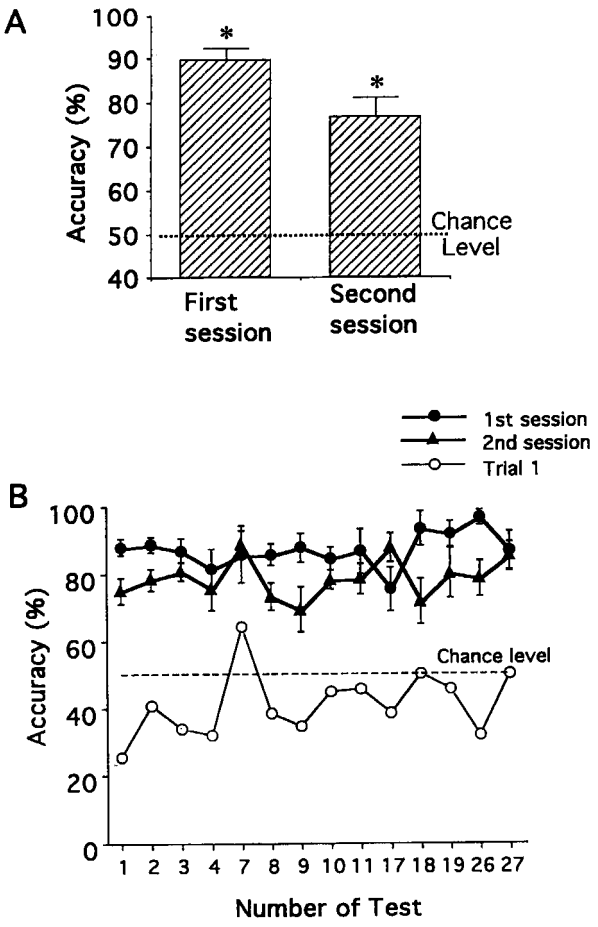

Fig. 2. Accuracy of the allocentric place discrimination task. The accuracies of the initial test after training (first and second session) were significantly higher than the chance level (A: n=36), and they were highly stable throughout the experiments (B). The ratio of the choice of the solid to floating platform in the first trial was about chance level. The data of the 5–6th, 12–16th, and 20th–25th sessions were omitted because the animals had been treated with drugs or environmental changes had occurred. Data are means ± SEM (n=10–32). *p<0.001.

RESULTS

In the initial allocentric place discrimination task test just after the training task, although the animals performed this task for the first time, they frequently chose the solid platform. The accuracy was significantly higher than the chance level (Fig. 2A: first session, 90 ± 3% (n=36), Z=8.39, p<0.001; second session, 77 ± 6% (n=36), Z=5.59, p<0.001; binomial test). Throughout the experiments, the accuracy at 80% was constantly higher than the chance level (Fig. 2B). The accuracy in the first trial, which was the information trial, was almost constant at approximately chance level (Fig. 2B: 41.1 ± 2.7%, mean ± SEM, n=14). This may be because the strategy which rats had acquired in the working memory of place navigation task was also

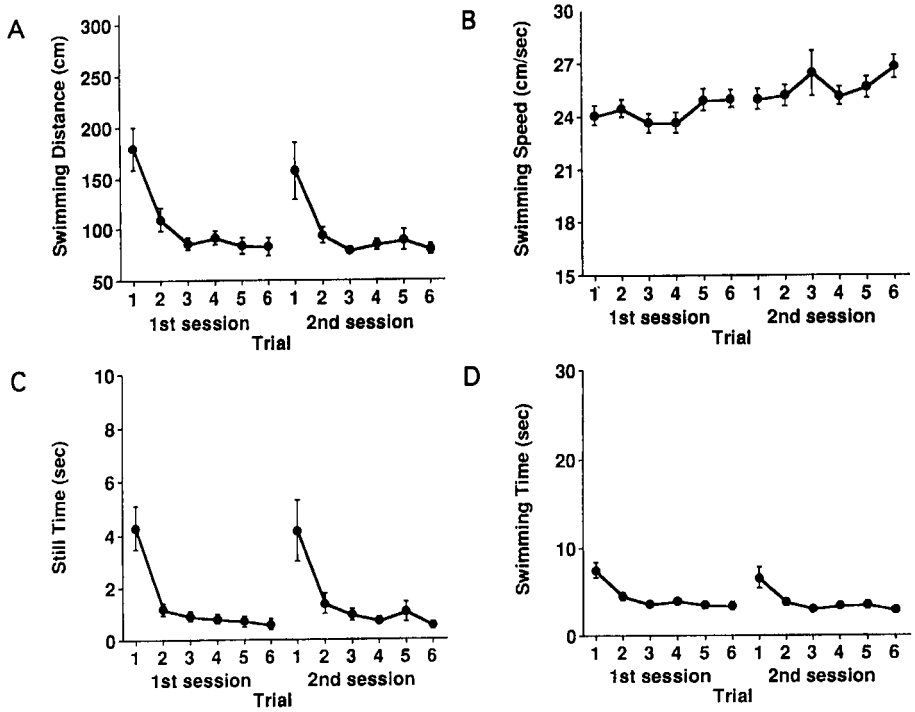

Fig. 3. Swimming distance (A), swimming speed (B), still time (C) and swimming time (D) in the initial test of the allocentric place discrimination task. Data are means ± SEM (n=36).

available in the allocentric place discrimination task; that is to say, the rats had learned that the location of the two platforms was retained throughout the session. Thirty-two out of 36 animals fulfilled the criterion and were thus used in the subsequent tests.

Other behavioral parameters, such as swimming distance, swimming speed, still time and swimming time, for the initial test are shown in Fig. 3. These parameters, except swimming speed, decreased with the repetition of trials. The swimming speed increased with the repetition of trials. Figure 4 shows the changes of these parameters with the repetition of the tests, all based on data without drugs or other manipulations of the condition. These parameters stabilized from the fourth test on (Fig. 4).

To confirm whether this task depends on allocentric spatial cognition, the effects of unavailability of the extramaze landmarks were studied by surrounding the tank with a black curtain. This significantly decreased the accuracy to about chance level (Fig. 5A: $F_{1, 20}=40.7$, $p<0.0001$). The swimming speed also decreased (Fig. 5B: $F_{1, 20}=24.2$, $p<0.0001$) and the still time increased (Fig. 5C: $F_{1, 20}=7.0$, $p<0.05$). The swimming time and the swimming distance were not different from those of the controls.

Evaluation of the learning and memory ability

The effects of scopolamine hydrochloride (SCP HCl), a muscarinic receptor antagonist, and physostigmine (PHY), an acetylcholine esterase inhibitor, on the allocentric place discrimination task were studied, as the cholinergic system is highly important for spatial learning and memory [1, 7–10, 13, 15, 31, 33, 34]. SCP HCl 0.5 mg/kg significantly decreased the accuracy (Fig. 6: $U=28.5$, $p<0.05$). But the same dose of SCP mBr, capable of only a slight penetration of the blood brain barrier, did not impair the accuracy. PHY 0.3 mg/kg ameliorated the SCP-induced impairments ($U=39$, $p<0.05$), but did not affect the accuracy by itself (Fig. 6). Neither of the still time, the swimming speed, the swimming time nor the swimming distance were affected by these cholinergic drugs.

Evaluation of motor ability

The effects of the skeletal muscle relaxant dantrolene (DAN) were studied. DAN did not decrease the accuracy (Fig. 7A). The swimming speed was decreased significantly (Fig. 7B: $F_{1, 24}=246.4$, $p<0.0001$) and the swimming time was lengthened significantly by DAN throughout the session (vehicle, 25.9 ± 0.3 cm/sec; DAN, 15.9 ± 0.5 cm/sec; mean ± SEM: $F_{1, 24}=16.8$, $p<0.0005$). The still time and the swimming distance were not different from the controls (Fig. 7C).

Evaluation of Motivational changes

To evaluate the motivational aspects separately from learning and memory, the effects of warm water (low incentive, 33°C) were studied. The accuracy was not impaired (Fig. 8A.). But the swimming speed decreased (Fig. 8B: $F_{1, 37}=15.6$, $p<0.0005$) and the still time increased, especially in the first trial (Fig. 8C: $F_{1, 37}=12.4$, $p<0.005$). The swimming time also increased (23°C, 2.75 ± 0.20 sec; 33°C, 3.01 ± 0.51 sec; mean ± SEM: $F_{1, 37}=5.3$, $p<0.05$). The swimming distance was not significantly different from

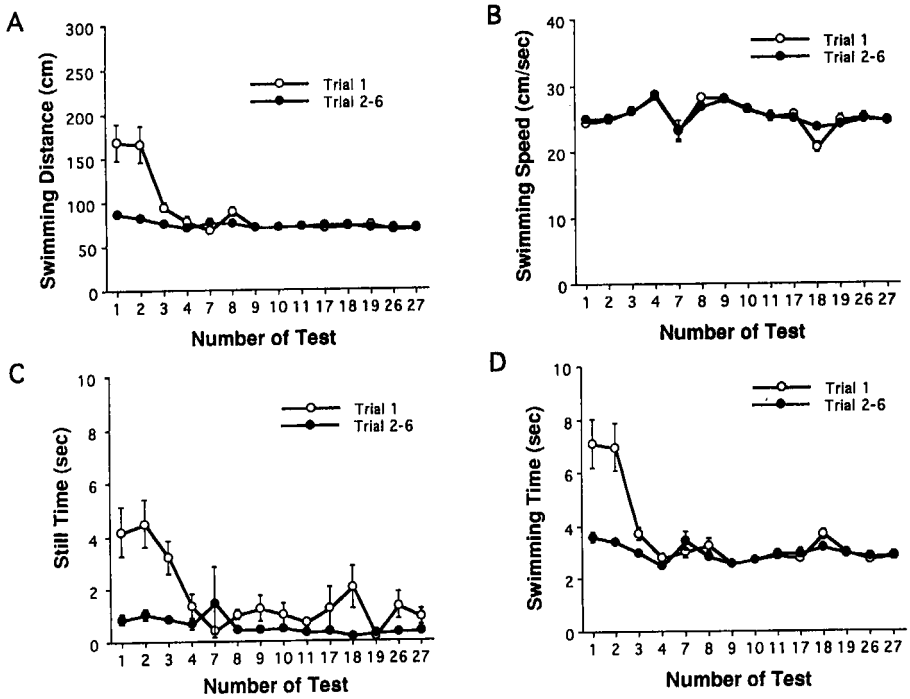

Fig. 4. Changes of the swimming distance (A), swimming speed (B), still time (C) and swimming time (D) with the repetition of the test. All parameters became stable from the fourth test on. The data of the 5–6th, 12–16th, and 20th-25th sessions were omitted because the animals had been treated with drugs or environmental changes had occurred. Data are means ± SEM (n=10–32).

the controls.

MOR, an opioid receptor agonist, is reported to impair place learning by decreasing motivation [11, 14, 19]. Therefore, the effects of MOR were also studied. The accuracy was not affected by MOR (Fig. 9A). MOR significantly decreased the swimming speed (Fig. 9B: $F_{1,24}=92.6$, $p<0.0001$) and increased the still time (Fig. 9C: $F_{1,24}=32.1$, $p<0.0001$) as well as the swimming time (vehicle, 2.74 ± 0.07 sec; MOR, 5.29 ± 1.47 sec; mean ± SEM: $F_{1,24}=16.4$, $p<0.0005$) compared to the controls. MOR did not affect the swimming distance.

DISCUSSION

In the Morris water maze, it is difficult to dissociate deficits in memory formation from other processes in impaired rats. Here, we established the allocentric place discrimination task, in which we were able to evaluate spatial working memory ability separately from and simultaneously with motivational, motor and sensory processes in individual animals. The task accuracy is a good index for allocentric spatial working memory ability. Selective decrease in swimming speed reflects motor deficits. An increase in still time accompanied by a decrease in swimming speed reflects a decrease of motivation. If rats have visual deficits, they might not be able to swim straight to the platform, so swimming distance may reflect visual sensory ability.

In the black curtain test, the accuracy decreased to the chance level (Fig. 5). This revealed that the rats chose the solid platform using the external visible cues. In addition, the accuracy in the first trial of every session remained constant at approximately chance level (Fig. 2). So, it was unlikely that the rats could discriminate between the solid platform and the float based on appearance. These results verify that this task is dependent on allocentric spatial cognition.

Muscarinic acetylcholine receptors in the central nervous system are considered to be highly important for spatial learning and memory because muscarinic acetylcholine receptor antagonists impairs spatial learning [1, 7–9, 12, 15, 31, 33, 34]. In other words, an assessment task in which SCP impairs the performance is thought to be appropriate to evaluate spatial learning and memory. In our assessment task, SCP HCl, but not SCP mBr, was found to impair the accuracy significantly (Fig. 6). The impairment was selective to the accuracy and the other parameters were not affected, suggesting that SCP impaired spatial working memory process directly, and did not affect the sensory and motor function, and motivation. These results suggest that the accuracy of the allocentric place discrimination task is a good index for the spatial working memory and that working memory ability can be evaluated separately from the other processes.

A skeletal muscle relaxant, DAN impaired the swimming speed but affected neither the accuracy nor still time. Thus,

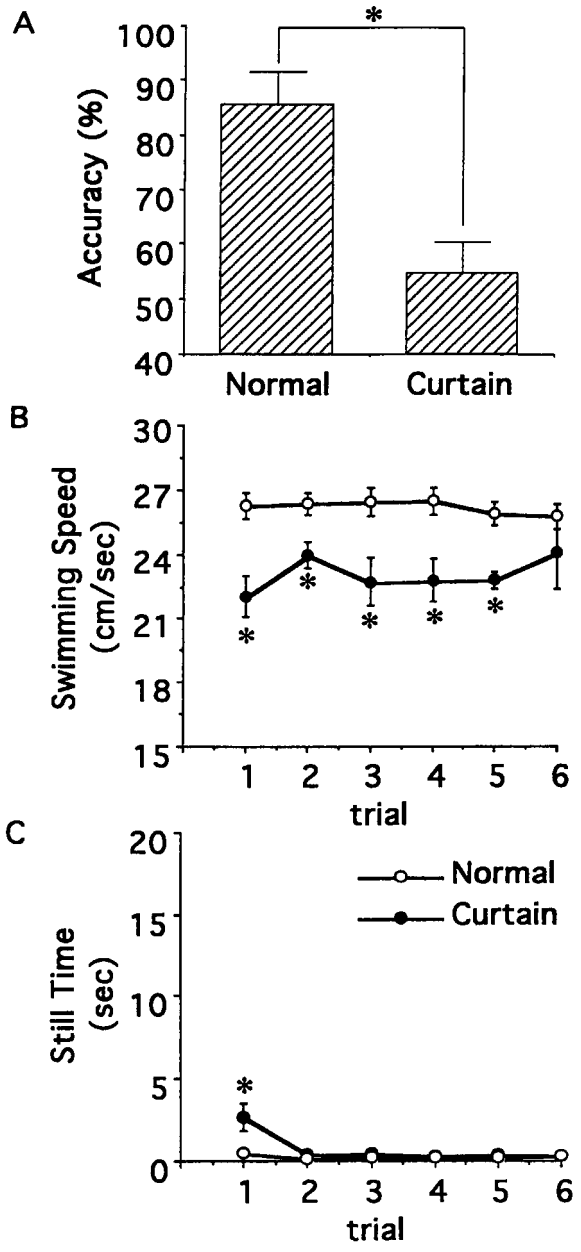

Fig. 5. The effects of surrounding the pool with a black curtain on the accuracy (A), the swimming speed (B) and the still time (C). The accuracy and the swimming speed were decreased, and the still time increased compared to the normal condition. Data are means ± SEM (n=11) *p<0.05.

motor deficits can be separately evaluated from learning, memory, and motivation; i.e., only the decrease in swimming speed represents motor deficits.

Water temperature is considered an important motive factor [11]. In the warm water test, the swimming speed decreased and the still time increased (Fig. 8.) It is, therefore, revealed that an increase in still time accompanied by a decrease in swimming speed is a good index for a decrease in motivation. In facts, upon release into the water, the rats delayed movement, then swam slowly, and sometimes did not climb onto the solid platform once they had reached it. The accuracy was not affected by the warm water. Thus, it is suggested that motivational changes can be evaluated separately from learning and memory ability in this task.

MOR did not impair the accuracy, but it increased the still time and the swimming time, and it decreased the swimming speed similar to the warm water test (Fig. 9). These results confirm that an increase in the still time accompanied by a decrease in swimming speed is a good index for a decrease in motivation and also that MOR reduces motivation rather than memory processes as previously reported [11, 14].

Two types of discrimination tasks are commonly performed in the water maze. One is the visible discrimination learning task [7–9, 34] and the other is the T-maze-type place discrimination task [16–20]. The allocentric place discrimination task, however, differs from above two tasks in some important points. First, in the visible discrimination learning tasks, only the acquisition, mainly reference memory acquisition, can be evaluated, and it is impossible to separate the learning and memory deficits from sensory, motor or motivational deficits. In contrast, in the present assessment these deficits could be separated and working memory ability can be evaluated as the platform location was changed for each session. Furthermore, the accuracy in this task reflects spatial memory ability more directly than in other assessments, because the factors which affect the acquisition of the tasks (for example, anxiety and/or ability of selection of the task solving strategies) were eliminated by using animals which had already learned the general rules of the task. Therefore this assessment is useful for evaluation of the long-term changes, such as aging, of spatial memory ability. Second, some of the commonly performed tasks are not strongly dependent on the allocentric spatial cognition even though they are performed in the water maze [9, 34]. In some of these cases the platforms used were clearly different in appearance, and rats learned the differences in appearance of the platforms and thus selected the solid one. In other cases, the working memory was evaluated in the T-maze-type place discrimination task, but rats could solve the task using odors and/or egocentric navigation (i.e., rats might select the direction, left or right by themselves) [16, 18–20]. Therefore, rats learned how to go, not where they were and where to go, in the T-maze-type place discrimination task. It is well established that knowing where to go (allocentric) is different from knowing how to go (egocentric) [25–27, 29, 30, 32]. In the present assessment task, the start position was changed for each trial and surrounding visible cues were found to be necessary for the accuracy, consequently spatial allocentric discrimination task could be evaluated.

In conclusion, the allocentric place discrimination task was developed. Not only place discrimination working memory, but also visual ability, motor disorder and

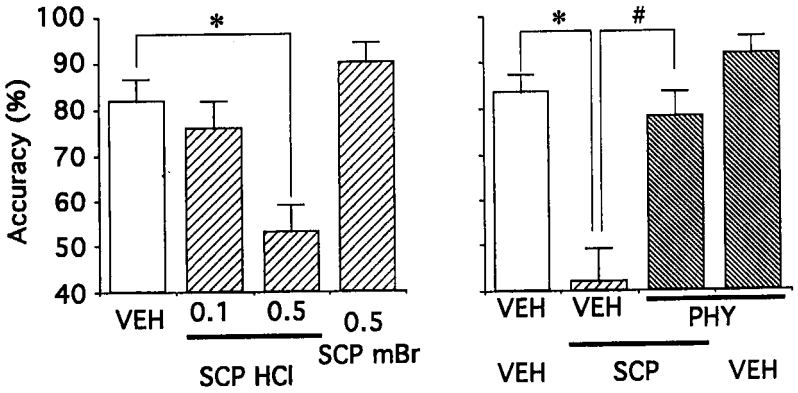

Fig. 6. The effects of cholinergic drugs on accuracy. SCP HCl (0.1–0.5 mg/kg, i.p.), but not SCP mBr (0.5 mg/kg, i.p.), impaired the accuracy dose-dependently. This impairments were ameliorated by PHY (0.3 mg/kg, i.p.), which did not affect the accuracy by itself. Data are means ± SEM (n=11–20). *p<0.05 compared to the vehicle control, #p<0.05 compared to the SCP+vehicle.

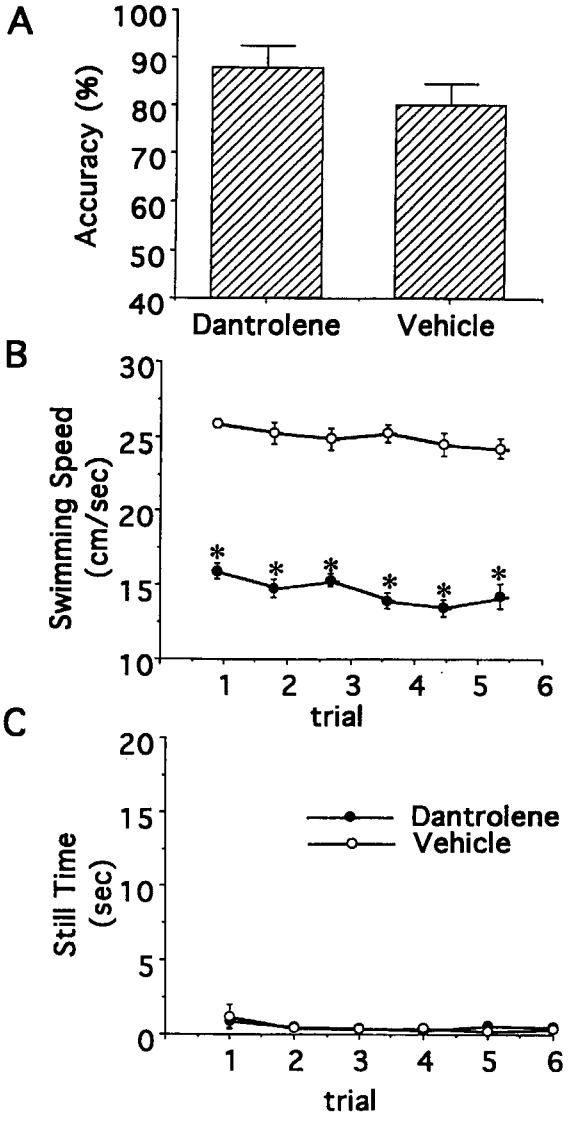

Fig. 7. The effects of DAN (10 mg/kg, i.p.) on the accuracy (A), the swimming speed (B) and the still time (C). DAN did not decrease the accuracy. The swimming speed was significantly decreased, but the still time was not affected. Data are means ± SEM (n=13). *p<0.0001.

motivational changes can be evaluated independently and simultaneously in individual animals in this assessment. These evaluated factors have been considered to affect parameters, such as escape latency, in other learning and memory assessments such as the Morris water maze.

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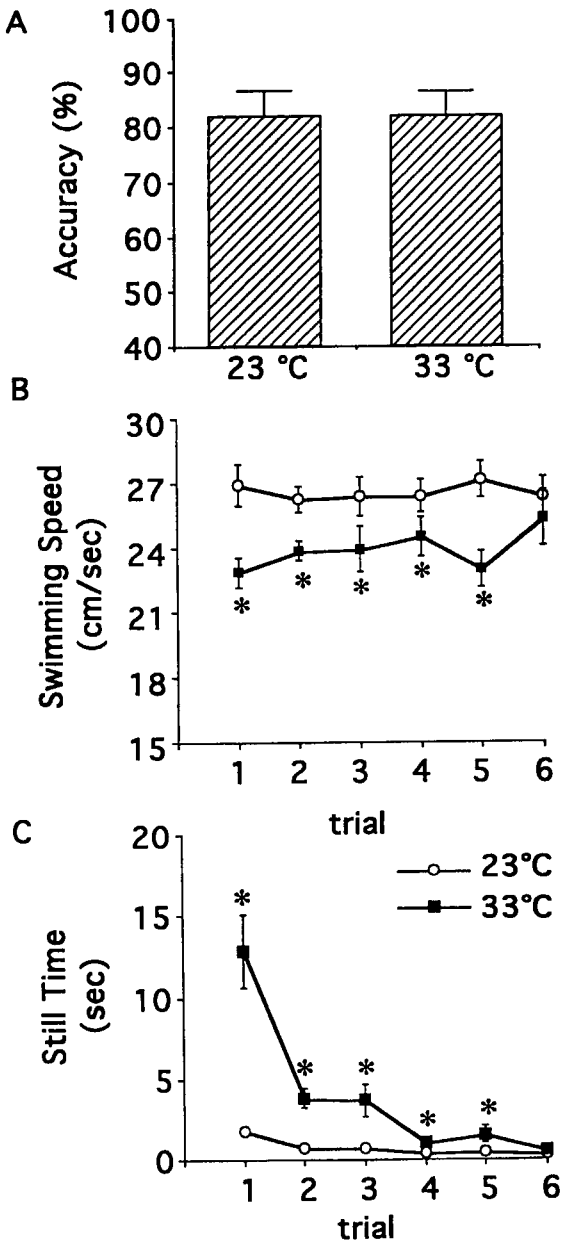

Fig. 8. The effects of the warm water (33°C) on the accuracy (A), the swimming speed (B) and the still time (C). The accuracy was not different between the warm water and normal (23°C) group. But the swimming speed was decreased and the still time was increased significantly. Data are means ± SEM (n=19, 20). *p<0.05.

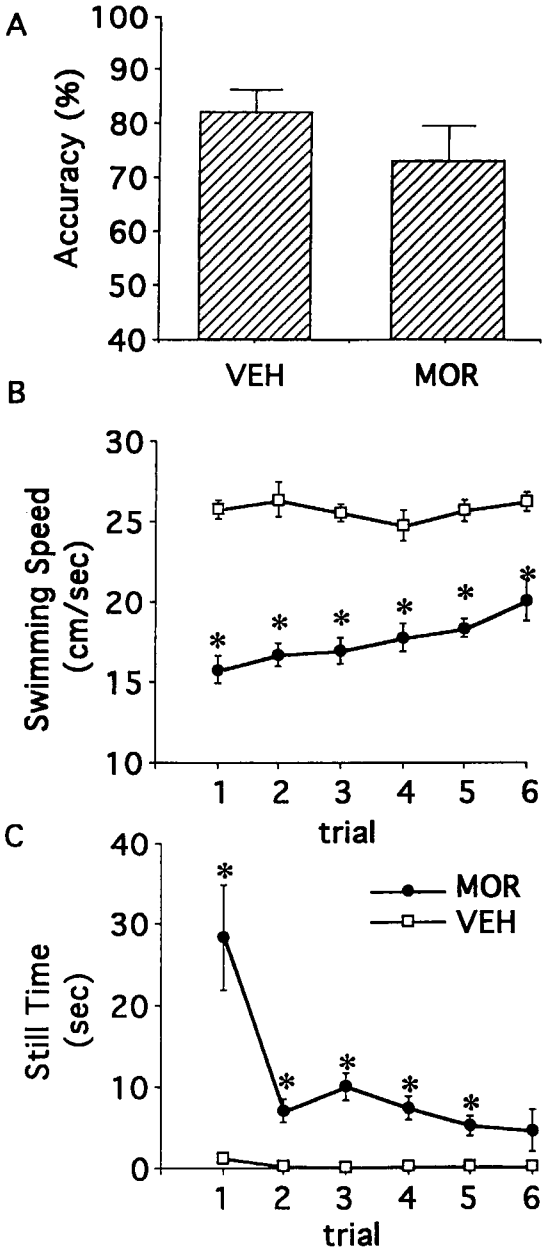

Fig. 9. The effects of MOR (15 mg/kg, i.p.) on the accuracy (A), the swimming speed (b) and the still time (C). MOR did not affects the accuracy. The swimming speed was significantly decreased and the still time was significantly increased by MOR. Data are means ± SEM (n=13). *p<0.005.

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