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Mice Housed in a Cage With a Maze Learn the Maze Without Explicit Training

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MASUDA, Y., S. MURAI, H. MURAKAMI AND T. ITOH. *Mice housed in a cage with a maze learn the maze without explicit training.* PHARMACOL BIOCHEM BEHAV 42(1) 101-105, 1992.—Mice were housed in a cage with a maze. A water tap was placed at the entrance of the maze. The exit of the maze connected with another cage (home cage). Food was placed in the home cage. Three different multiple mazes (types 1-3) were placed. 1) Mice were housed for 10 h a day in the apparatus and then removed to a normal cage for fasting. One trial per day was carried out after fasting for 13 h. In each trial, a mouse was put at the entrance of the maze and then the number of errors and the time till it reached the home cage was counted. Mice reached a learning criterion at Trial 2. 2) Administering scopolamine (0.125-0.5 mg/kg) 30 min before Trial four disturbed the maze work dose dependently in a type 3 maze, the most complex maze among the three, but did not in type 1 and 2 mazes. 3) Administering scopolamine (0.25-1.0 mg/kg) 30 min before Trial 11 to the mouse of the type 3 maze did not disturb the maze work. These results show that a mouse housed in a cage with a maze learns the maze without explicit training and scopolamine can differentially effect performance based upon the degree of training.

Mice Multiple maze Reference memory Scopolamine Spontaneous learning

A maze test has been widely applied for preclinical evaluation of psychotropic drugs and numerous mazes have been devised (11). A major limitation of most of these tests is the cumbersome nature of the test itself. They may require 1) usually an apparatus that occupies a wide space, 2) preliminary training of the animal, and 3) control of feed to maintain appropriate body weight. This study was conducted to see if mice housed in a cage in which a maze was incorporated and food and water were placed separately through the maze would learn the maze without explicit training. Based on these hypotheses, we developed a new method of a maze test and demonstrated its validity.

METHOD

Animals

Male ddY mice were used. They were obtained from SLC (Hamamatsu, Japan) at 5 weeks of age and housed 10-12 per normal cage with a 12 L : 12 D rhythm until body weight became 28-30 g.

Apparatus

The apparatus is illustrated in Fig. 1. Three different mazes were used. The components of the apparatus are a maze cage (26 cm in width, 40 cm in depth, and 19 cm in height), a home cage (18 cm in width, 28 cm in depth, and 12 cm in height), and a starting box. The home and maze cages are clear animal cages that are available on the market. A clear acrylic tunnel maze (4 cm in width and 6 cm in height) is placed in the maze cage. A maze with four units, each of which has two alleys (Fig. 1, upper left, type 1 maze), a maze with two units, each of which has six alleys (Fig. 1, upper middle, type 2 maze), and a maze with four units, each of which has three alleys (Fig. 1, upper right, type 3 maze) were placed in the respective maze cage. The exit of the maze and the home cage are interconnected with a tunnel. The starting box is attached an area corresponding to the entrance of the maze. A water tap is placed at the entrance of the maze and food is placed in the home cage. Mice must go and return between the water tap and the food to live in the apparatus. The home cage is painted black and covered with a black lid. Wood chips were placed in the maze and home cages. The lower panel of Fig. 1 shows the apparatus for the control, where the maze without a blind alley is placed. The apparatus is placed in the same place on a stone table in a small room (12 m^2) furnished with an extra maze during experiments.

Trials

One trial per day was carried out unless otherwise stated. A mouse was placed at the starting box. Then, the time (run-

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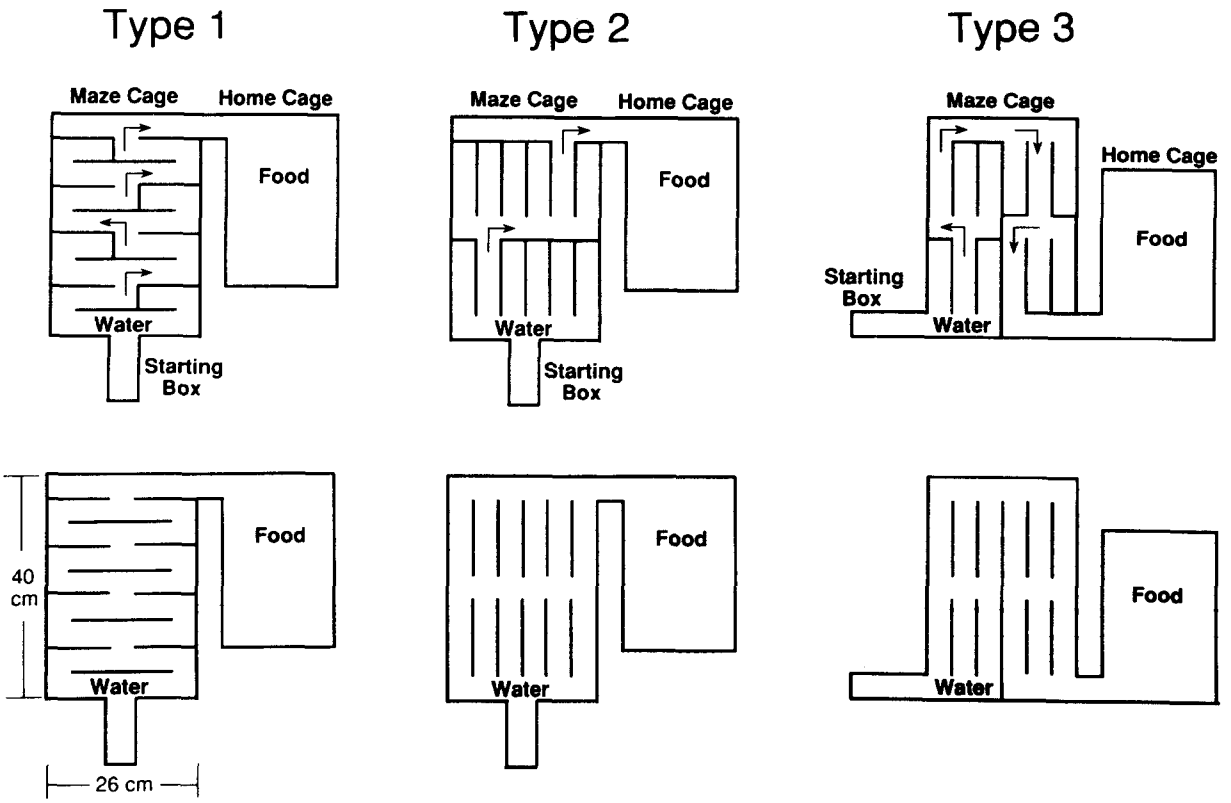

FIG. 1. Schematic drawing of the apparatus. Left, middle, and right show type 1, 2, and 3 mazes, respectively. A multiple maze (correct decision is indicated by arrows) is placed in the maze cage. Food and water are placed separately through the maze. Lower panels show the apparatus without the blind alley used in Experiment 1. Numbers in the figure indicate length in cm.

ning time) and number of errors (entering a blind alley with all four feet) until the mouse entered the home cage were counted. In the event that a mouse did not complete the maze in 5 min, the number of errors for 5 min and a running time of 300 s were recorded. At the end of each trial, the maze was cleaned and wood chips on the floor were mixed to prevent the mouse from being able to select the correct alley by odor cues.

Experiment 1

Eighteen mice were divided into two groups. One group (maze cage group) was housed in the apparatus with a type 1 maze for 10 h (10:00 a.m.-8:00 p.m.) per day. The rest group (control group) was housed in the control apparatus (Fig. 1, lower left). After housing in the apparatus, they were removed to the normal cages with raised mesh bottoms to prevent coprophagy and were deprived of food until the trial of the next morning. In the trial of the maze cage group, the mouse was placed at the starting box of the apparatus. In the trial of the control group, the mouse was placed at the starting box of the apparatus but the maze was replaced by the type 1 maze. After the finish of trials of all mice, they were housed in the respective apparatus till 8:00 p.m. Six trials were carried out. In Trial 4, the apparatus was turned 180°. In Trial 6, the correct decision of the maze was changed to a mirror image version. We considered the group to have reached a learning criterion when the number of mean errors of the group was less than one.

The experiments using type 2 and 3 mazes were carried out with the same procedure as mentioned above. In Trial 6 of the type 3 maze, the correct decision of the maze was changed from middle-left-middle-right to left-middle-right-middle.

Experiment 2

Twenty-eight mice were housed in the apparatus with the type 1 maze for 10 h per day. Two sets of the same apparatus were used. Three trials were carried out in the same manner as mentioned in Experiment 1. Thirty minutes before Trial 4, mice were divided into four groups: Scopolamine hydrochloride (Sigma Chemical, St. Louis, MO, 0.125, 0.25, or 0.5 mg/kg) or 0.05 ml/10 g saline was administered intraperitoneally, respectively. After Trial 4, mice were removed to the normal cage until Trial 5, which was carried out 6 h after the administration of scopolamine.

The same experimental procedure as mentioned above was carried out using type 2 and 3 mazes.

Experiment 3

Twenty-eight mice were housed in the type 3 maze for 10 h per day. Ten trials were carried out in the same manner as mentioned in Experiment 1. Thirty minutes before trial 11,

mice were divided into four groups and scopolamine (0.25, 0.5, or 1.0 mg/kg) or 0.05 ml/10 g saline was administered.

Statistics

The data in the figures are expressed as the mean ± SE. The statistical significance of the difference was evaluated by the Mann-Whitney *U*-test or Williams' nonparametric comparison of several groups to a control using statistical software (MUSCOT, Yukms). Differences were considered significant if $p < 0.05$.

RESULTS

Experiment 1

Figure 2 shows results of Experiment 1. The number of errors of the maze cage groups reached a learning criterion at Trial 2 in all types of mazes. When the trial was carried out with the apparatus turned 180° (Trial 4), mice could not enter the home cage within a learning criterion. When the apparatus was reset as indicated (Trial 5), mice entered the home cage within the learning criterion. When the correct decision was changed to a mirror image version in type 1 and 2 mazes, and to left-middle-right-middle in the type 3 maze (Trial 6), the number of errors increased. The curves of the running times of the groups housed in the maze cage appear almost parallel with the error curves.

The number of errors of the control groups (housed in the maze cage without a blind alley) never reached a learning criterion at any trial.

Experiments 2 and 3

Scopolamine (0.125–0.5 mg/kg) was administered 30 min before Trial 4 to mice that had mastered the maze (Fig. 3). Scopolamine increased the number of errors and running time dose dependently in the type 3 maze. In type 1 and 2 mazes, only 0.5 mg/kg scopolamine increased the number of errors and running time significantly. Six hours after administration, the effect of scopolamine disappeared.

Scopolamine (0.25–1.0 mg/kg) was administered 30 min before Trial 11 to mice that had mastered the type 3 maze (Fig. 4). Regardless of the significant increase in running time ($W_{cal} = 17.4$, $df = 3$), there were no significant differences in the number of errors among groups.

DISCUSSION

The maze test is one of the most important means for evaluating memory and learning in small animals. Usually, rats are used in a complex maze test such as a multiple maze test (7–9,12). There are few reports about multiple maze tests using mice. Our method demonstrated that mice acquired the maze work without explicit training.

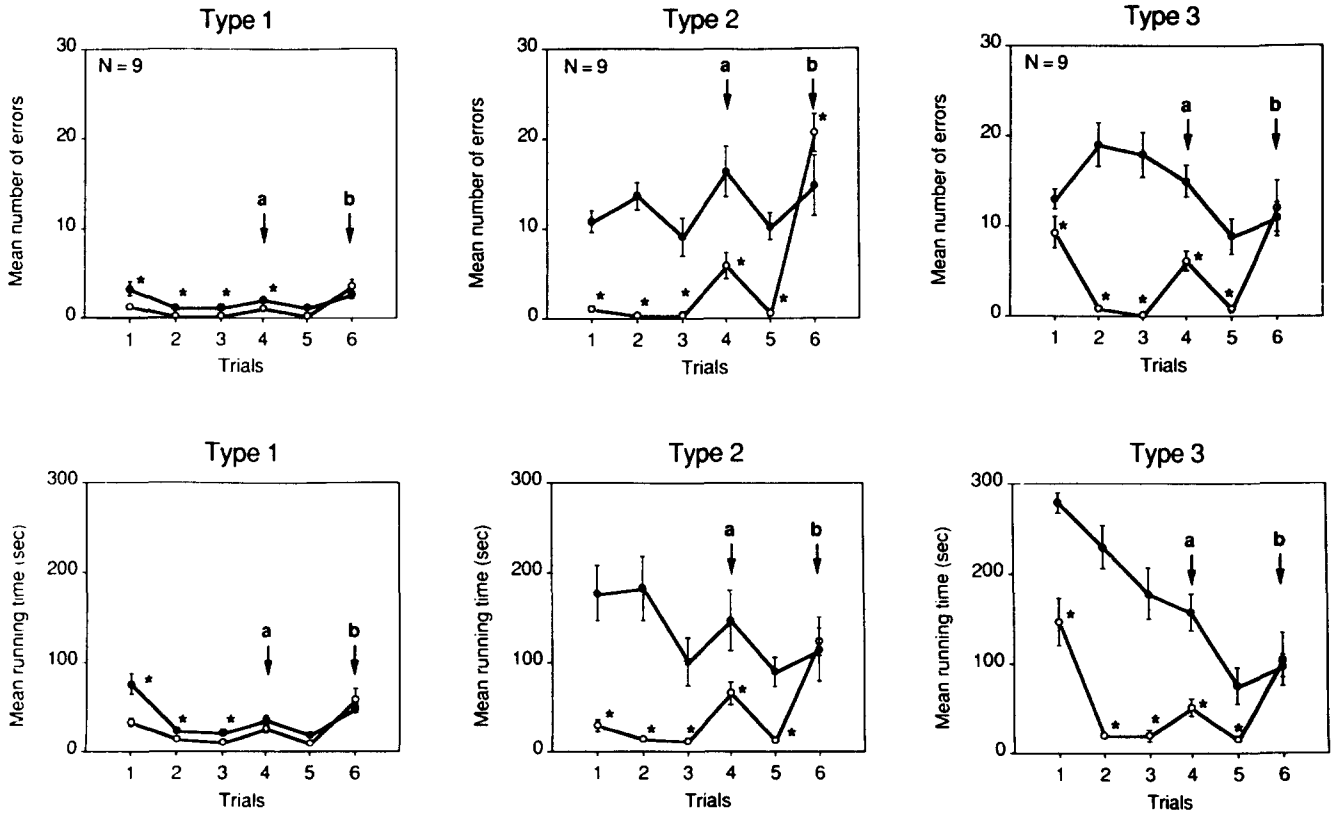

FIG. 2. Effect of the housing in the apparatus on maze work. Mice were housed for 10 h per day in the cage with type 1, 2, or 3 mazes (○). The control group (●) was housed in the apparatus without a blind alley. One trial per day was conducted. In each trial, a mouse was put at the starting box and the number of errors (upper) and running time (lower) were counted. Results are shown as mean ± SE. In Trial 4 (arrow a), the apparatus was turned 180°. In Trial 6 (arrow b), the correct decision was altered. $^*p < 0.05$ as compared with the respective control.

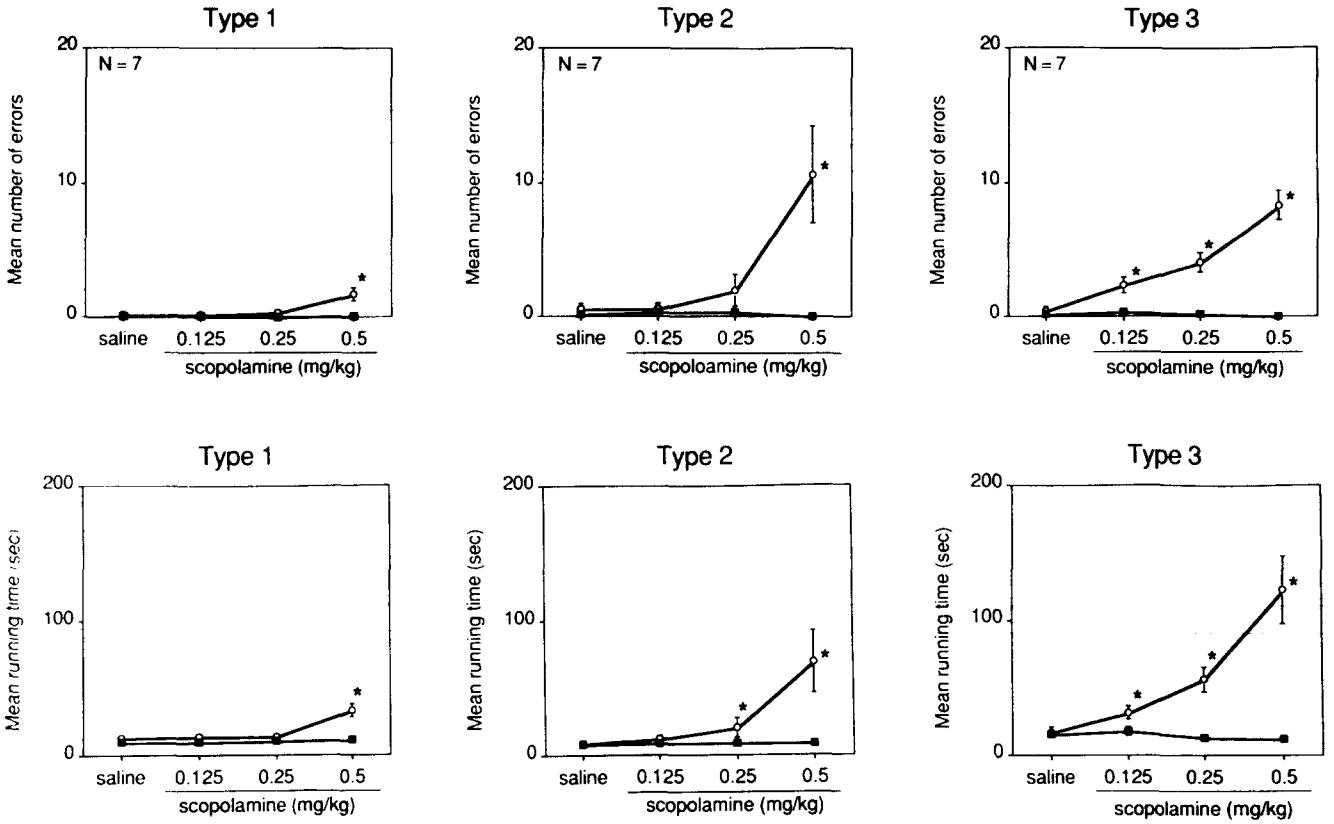

FIG. 3. Effect of scopolamine on reproduction of memory. Twenty-eight mice were housed for 10 h per day in each type of maze. Mice were divided into four groups before Trial 4. Then, each group was given either scopolamine (0.125, 0.25, or 0.5 mg/kg) or saline. Thirty minutes (○, Trial 4) and 6 h (●, Trial 5) after administration, trials were carried out. *$p < 0.05$ as compared with saline group. Upper and lower panels show mean number of errors ± SE and mean running time ± SE, respectively.

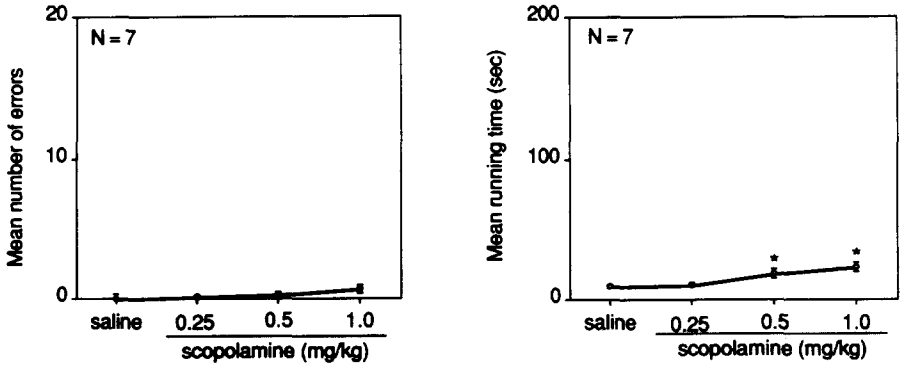

FIG. 4. Effect of scopolamine on reproduction of memory. Twenty-eight mice were housed for 10 h per day in the type 3 maze. Mice were divided into four groups before Trial 11. Then, each group was given either scopolamine (0.25, 0.5, or 1.0 mg/kg) or saline. Thirty minutes after administration, Trial 11 was conducted. *$p < 0.05$ as compared with saline group. Left and right show mean number of errors ± SE and mean learning time ± SE, respectively.

In Experiment 1, mice housed in the maze cage could enter the home cage with a smaller number of errors and less running time compared to control groups. This means mice learned the complex maze spontaneously by being housed in the cage with a maze. It is perhaps not necessarily surprising because earlier research in the field of psychology demonstrated that in spatial tasks rats and other animals have a marked ability to remember information and use this information appropriately in a flexible, adaptive manner (11). It is clear that mice did not depend on odor cues or distinguish the correct alley from a blind alley through to complete the maze work because a) at the end of each trial the maze was cleaned to prevent the mouse from being guided to the correct alley by odor cues and b) when the correct decision was changed in Trial 6 the number of errors markedly increased. The number of errors and running time increased in Trial 4 (apparatus was turned 180°). This means mice used to some degree a cue from the extra apparatus to complete the maze work. Our maze may become a useful method to estimate reference memory. The component of reference memory involved fixed stimulus–responses that are not altered over consecutive trials in the maze work (10).

It is known that perturbation of the cholinergic system interferes with learning and memory (1,4–6). To test the validity of our method, scopolamine was administered to mice that had mastered the maze. Scopolamine interfered with maze work transiently (Fig. 3). The effect of scopolamine varied with the type of maze. The clear effect of scopolamine was observed only in the type 3 maze. We think the difference was caused by the higher level of complexity of the maze. When scopolamine (0.25–1.0 mg/kg) was administered to well-trained mice (Fig. 4), a significant increase in the number of errors was not recognized, even at 1 mg/kg scopolamine. A similar result was reported in the Morris water maze, that is, when the response is overtrained higher dosages of scopolamine are needed to disrupt retention (3). From Experiment 1, reference memory plays an important role in completing the maze work. Beatty and Bierley reported that a low dose of scopolamine (0.25 and 0.5 mg/kg) had no effect on reference errors in a 12-arm radial maze and reference memory is relatively immune to disruption (2,3,13). Our results suggest that a) reference memory is disrupted by a low dose of scopolamine if the task is complex and b) when the animal is well trained reference memory is immune to scopolamine even if the task is complex.

The results show that mice housed in the cage with a maze acquired the maze work without explicit training, and the method possesses several advantages as an experimental method to estimate reference memory: a) the procedure is simple; b) it requires minimal equipment; c) it does not involve control of feed to maintain appropriate body weight; and d) it does not require the use of noxious stimuli.

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