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BRIEF COMMUNICATION

Maze Performance: A Direct Comparison of Food vs. Water Mazes[1,2]

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KANT, G. J., M. H. YEN, P. C. D'ANGELO, A. J. BROWN AND T. EGGLESTON. *Maze performance: A direct comparison of food vs. water mazes.* PHARMACOL BIOCHEM BEHAV 31(2) 487–491, 1988.—The purpose of these experiments was to evaluate the utility of a water maze for testing performance in nonfood-restricted rats. Water maze performance was compared to performance in a food-rewarded (food) maze. Separate groups of rats were given single daily trials of 34 days in one of two mazes. The path through each maze was identical; in fact, the same maze was used with the exception that the maze was filled with water during water maze testing and left dry during the food maze testing. In the food maze, a chocolate peanut butter chip was placed at the finish. In the water maze an out-of-the-water platform was placed at the finish. The time to reach the finish was measured for each trial. Both free-feeding and food-restricted rats were tested in each maze. Free-feeding rats learned the food maze with great difficulty, requiring more than 30 trials. Food-restricted rats learned the food maze more quickly than did free-feeding rats. Free-feeding rats learned to solve the water maze more quickly than the food maze. Food-restricted rats also learned the water maze more quickly than the food maze and learned both mazes faster than free-feeding rats. Plasma levels of corticosterone, ACTH and prolactin were measured in all rats immediately following completion of the last maze trial. Plasma corticosterone levels were elevated and plasma prolactin levels were decreased in both food-restricted groups as compared to free-feeding rats, demonstrating that food restriction was chronically stressful. The maze testing procedure was also a source of acute stress, as shown by elevated plasma corticosterone levels in all groups relative to levels in untested rats. However, low levels of two other stress-responsive hormones, ACTH and prolactin, that habituate to stress more rapidly than corticosterone suggest that rats had considerably habituated to the testing by the 34th trial. We conclude that testing performance using a water maze requires fewer training trials than a food maze and can be accomplished without food restriction.

Water maze Maze Performance Corticosterone ACTH Prolactin Learning Memory Food deprivation

OUR laboratory has been studying the effects of acute, repeated and sustained stress on neuroendocrine and neurochemical parameters (plasma hormones, neurotransmitters, central neurotransmitter and neuromodulator receptors) in animal models (3, 4, 10–18, 23, 24). One of our current objectives is to correlate stress-induced changes in biochemical measures with stress-induced alterations in physiological function or performance. Since stress often causes voluntary decreased food intake (17,21) and, conversely, since food restriction is in itself a stressor, we needed to characterize performance tasks that were not reinforced by food. Escape from footshock has also been used as a performance motivator, but since some of our stress models involve intermittent footshock, use of this reinforcer in a performance task designed to test stressed animals would also be confounded. Several reports have described the use of various water mazes to test performance in rats (1, 2, 6, 32, 33). In this report we describe the results of a study that compared the use of a water maze vs. a food-rewarded maze to assess performance in both free-feeding and food-restricted rats.

METHOD

Animals

Forty male Sprague-Dawley rats were used in these experiments. Prior to the beginning of the experiment, rats

[1]Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 85-23. All procedures were reviewed and approved by the WRAIR Animal Use Review Committee.

[2]The views of the author(s) do not purport to reflect the position of the Department of the Army or the Department of Defense (para 4-3, AR 360-5).

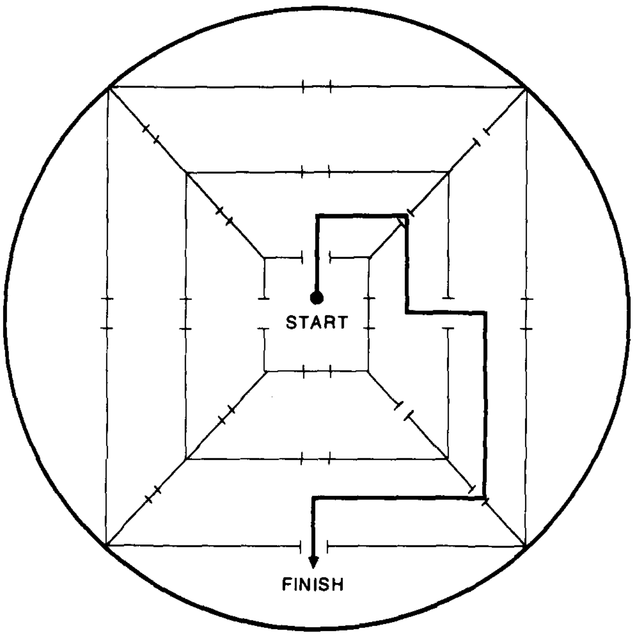

FIG. 1. Top view of maze. During water testing, the maze was filled to a height of 12" with water and an out-of-the-water platform was placed at the "finish." During food maze testing, the maze was dry and a chocolate peanut butter chip was placed in a small plastic dish at the finish.

weighed 300±20 grams. Twenty of the rats were then subjected to food restriction such that their weights dropped to 80% of their original preexperimental weight. This weight was then maintained during the duration of the experiment by adjusting daily food availability. Ten of the free-feeding rats and ten of the food-restricted rats were tested daily in the water maze. Separate groups of ten animals from the free-feeding and food-restricted groups were tested in the food maze. Rats that were to be tested in the food maze were given chocolate peanut butter chips in their home cages for several days prior to the initiation of testing to familiarize them to the novel food.

Mazes

The same maze was used as the food maze and water maze (Fig. 1). During water maze testing, the maze was filled to a height of 12" with 28°C water and the finish was an out-of-the-water-platform. During the food maze testing, the maze was dry and a peanut butter chip in a small plastic tray was placed at the finish. The maze itself consisted of sets of white opaque plastic walls arranged as 3 concentric squares set inside a 5 ft diameter swimming pool. Each of the wall sections had a removeable door that could be opened or closed. The same configuration of open doors was used for both mazes during the entire testing period. Rats had a series of choices between 2 alternative open doors. Rats were free to move forwards or backwards and no doors were closed after the rats moved through them. Rats were placed in the center of the maze and allowed a maximum of 5 min to reach the finish. Time to reach the finish was recorded and the rats were removed from the water exit platform or allowed to eat the peanut butter chip and then removed from the food maze. Rats not reaching the food reward were given the chip in their home cage after trial. Rats were tested for 1 trial per day for 34 days.

Hormone Assays

Immediately following completion of the last trial, animals were sacrificed by decapitation and trunk blood was collected in heparinized beakers. The blood was centrifuged after the addition of Trasylol, a peptidase inhibitor, and plasma was stored at −40°C until assayed for corticosterone, ACTH and prolactin by radioimmunoassay as described previously (13, 17, 20, 25).

Statistics

Data were analyzed for the effects of group (free-feeding vs. food-restricted) and maze type by analysis of variance and for the effects of trial (over days) by repeated measures ANOVA.

RESULTS

All groups of rats decreased the time required to finish the mazes over repeated trials although there were marked differences between the performances on the two mazes and between performances of food-restricted vs. free-feeding groups. As shown in Fig. 2, free-feeding rats decreased the time per trial in the food maze task very slowly although a significant effect of trial was found over the 34 trials, $F(33)=4.1$, $p<0.0001$. If the data for trials 33 and 34 were omitted, no significant learning effect over trials was seen, $F(31)=1.4$, $p>0.05$. Food-restricted rats also solved the food maze more quickly as the number of trials increased, $F(33)=3.36$, $p<0.001$. There was, however, a significant difference in the performance of the two feeding groups on the food maze, $F(1)=26$, $p<0.001$. The better performance of the food-restricted rats was especially evident following the initial 17 trials.

Both food-restricted, $F(33)=7.3$, $p<0.0001$, and free-feeding rats, $F(33)=5.0$, $p<0.001$, reached the finish of the water maze more quickly as trials progressed (Fig. 2). As in the food maze, food-restricted rats also performed better than the free-feeding rats on the water maze, $F(1)=6.3$, $p<0.05$.

In analyzing the performance of each feeding group on the different mazes, both free-feeding, $F(1)=19.8$, $p<0.001$, and food-restricted rats, $F(1)=24.9$, $p<0.0001$, learned the water maze more quickly than the food maze.

Plasma levels of ACTH and prolactin (Table 1), two stress-responsive hormones that habituate rapidly to repeated stress, were in the normal range for free-feeding rats sacrificed directly upon removal from their home cages for all groups (16,17). Plasma corticosterone, which habituates much more slowly than ACTH or prolactin and which is sensitive to any change in the environment, was elevated in all groups as compared to animals sacrificed immediately upon removal from their home cage (e.g., plasma corticosterone levels of approximately 1.0 μg/dl for animals sacrificed and assayed within the same time period in our laboratory). Food restriction markedly increased levels of plasma corticosterone in both maze groups, $F(1)=25$, $p<0.001$, and decreased levels of plasma prolactin, $F(1)=12.2$, $p<0.01$, but did not affect ACTH levels. This pattern of hormonal changes has been reported by us and others following chronic stress of various types (5, 17, 29–31). Levels of plasma corticosterone and ACTH were similar following both types of maze trials, but plasma prolactin was elevated following the water as compared to the food maze in the free-feeding groups.

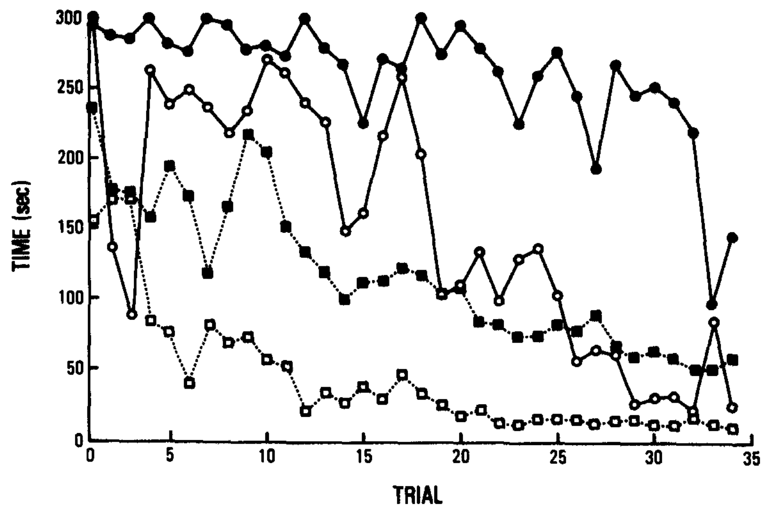

FIG. 2. Maze performance over 34 daily trials for free-feeding and food-restricted rats in the food and water mazes. ● Free-feeding, food maze; ○ food-restricted, food maze, ■ free-feeding, water maze; □food-restricted, water-maze.

TABLE 1

PLASMA HORMONES FOLLOWING 34 MAZE TRIALS

	Corticosterone (μg/dl)	ACTH (pg/ml)	Prolactin (ng/ml)
Water Maze			
Free-Feeding	24.0 ± 3.5	54 ± 10	6.7 ± 1.4†
Food-Restricted	34.0 ± 1.9*	74 ± 20	1.2 ± 0.4
Food Maze			
Free-Feeding	23.2 ± 2.4	57 ± 12	2.1 ± 0.5
Food-Restricted	38.8 ± 2.2*	96 ± 25	1.1 ± 0.3

Values represent the mean ± SEM. N=10 rats per group. Two-way analysis of variance showed a significant effect of feeding condition for corticosterone, $F(1)=25$, $p<0.001$, and prolactin, $F(1)=12.2$, $p<0.01$, but not for ACTH. A significant effect of maze type was found only for prolactin, $F(1)=5.5$, $p<0.05$. Follow-up selected comparisons of individual groups were made by Student's *t*-test. *Significantly different from free-feeding groups. †Significantly different from all other groups.

DISCUSSION

In the present study, two types of mazes were used to assess performance in free-feeding and food-restricted rats. Without food restriction, learning a maze for a food reward, even a highly desirable chocolate peanut butter chip, appears to be very slow. Free-feeding controls appeared to be easily distracted (occasional noise outside the testing room) and did not always eat the food chip within the maze even when they reached the finish. Free-feeding rats did quickly consume the chip once back in the home cage. This relative disinterest in the food reward was reflected in the very slow learning rate. In contrast, food-restricted rats learned the path to the finish where the food reward awaited much more quickly. Obviously, this is the reason why food restriction is commonly used prior to training rats to perform appetitively reinforced tasks.

However, food restriction is not a trivial factor to introduce into experimental designs. The degree of food restriction imposed in the present experiment, which is similar to many regimens in the literature (7–9, 19), has been shown by us and others to be a significant stressor in itself as shown by chronically elevated levels of corticosterone in rats (18,28). Although the maze testing procedure itself was sufficiently stressful or arousing to increase plasma corticosterone levels in both free-feeding and food-restricted rats, the underlying chronically elevated corticosterone baseline in the food-restricted groups was still evident. Corticosterone levels were only measured immediately following the maze testing procedure. However, if rats had been sacrificed 1 hour later, after rats had been returned to their animal housing area, levels of corticosterone in the free-feeding rats would have returned to low baseline levels since the effects of acute stress on plasma corticosterone are limited in duration. Food restriction, however, is a chronic stressor that results in around-the-clock elevations of plasma corticosterone. The maze testing procedure was not very stressful after the 34th presentation. Fecal boli, which were numerous during the first few trials, had greatly decreased in number after 34 trials. Although the levels of plasma corticosterone measured following testing were elevated, ACTH and prolactin levels were not. Both of these hormones are excellent indices of acute stress response. Plasma corticosterone in the rat has a very low response threshold to even very mild stressors or changes in the environment. This may be inferred from the present experiment in which plasma corticosterone levels were high even in free-feeding rats simply placed in a familiar dry maze for 5 min and given the opportunity to find and eat a favored food. Except for the higher levels of plasma prolactin in the free-feeding water maze group as compared to the food maze group, all hormonal indices of stress were similar following the 34th trials in the food vs. water mazes. Thus, at least over repeated trials, the water maze does not seem to be especially stressful.

Food restriction also increases locomotor activity in rodents (22, 26, 27). This increased activity may also affect task performance and may explain the better performance of the food restricted rats in the water maze. In untreated animals, food restriction can be used to motivate rats to perform tasks for a food reward, albeit at the cost of exposing animals to a chronic stressor. In experiments where animals have been exposed to stress or to drugs which may affect their desire for food, interpretation of performance data for appetitively motivated rewards may be confounded. For these reasons, nonappetitively motivated performance tasks may be more appropriate for some experimental situations.

The maze design and water temperature used in the present experiments were based upon the overall experimental needs of our laboratory. For future experiments, we desired a maze that could be configured with varying degrees of difficulty to discriminate among impairments in learning or memory caused by drugs or stressors. The design selected was arbitrarily chosen and then tested in pilot experiments to determine whether rats would learn the path to the escape platform within 10 to 15 trials, a period deemed long enough to be sensitive to differences in learning yet not so long as to require substantial training time. Water temperature was also arbitrarily chosen. Colder temperatures (e.g., 21–25°) which have often been used in water mazes would probably have improved performance, by increasing the aversiveness of the task. However, since 28°C was adequate to motivate the rats to swim in the maze, we utilized the milder temperature in the hope of decreasing the stressfulness of the testing.

The water maze described in the present report can be easily modified to make the task easier or more difficult by opening various combinations of doors. Whether either the present or different maze configuration will prove to be a sensitive task that will discriminate between the performance of control vs. stressed animals remains to be determined.

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