Effects of Psychoactive Drugs or Stress on Learning, Memory, and Performance as Assessed Using a Novel Water Maze Task

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KANT, G. J. *Effects of psychoactive drugs or stress on learning, memory, and performance as assessed using a novel water maze task.* PHARMACOL BIOCHEM BEHAV **44**(2) 287-295, 1993. — A novel water maze was used to assess the potential performance-disrupting effects of psychoactive drugs and stressors (4 mg/kg amphetamine sulfate; 1, 2, or 4 mg/kg diazepam; 30 mg/kg caffeine; 5 or 30 mg/kg atropine sulfate; 15 min of either intermittent foot-shock, forced running, or immobilization). The task utilized a traditional type of maze with walls and doorways set inside a pool. The apparatus could easily be reconfigured to present different mazes of approximately equal difficulty by opening or closing multiple doorways. Performance was measured by number of errors and time required to swim from the "start" to "finish" (a raised platform not in the rat's line of sight). After initial maze training, rats were divided into two groups. One group ran three daily trials through the same maze each day; this group was used to assess memory. The second group was challenged to swim three consecutive trials in a new maze configuration each day as a measure of learning. On any given day, rats from both groups received the same treatment. Drug or stress treatments were interspersed with vehicle or no-treatment trial days. The new maze task was more sensitive than the well-learned maze to the performance-disrupting effects of amphetamine, caffeine, and diazepam, while atropine had no significant effect on performance on either maze. Foot-shock stress impaired performance on both mazes, while the other stressors had no significant effect.

Learning   Memory   Performance   Water Maze   Stress   Amphetamine   Diazepam
Caffeine   Atropine

OUR laboratory is interested in developing and characterizing animal models that can be used to study the potentially detrimental effects of stress, fatigue, neural injury, and illness on mental and physical performance. We wish to use such animal models as an assessment tool for the evaluation of new therapeutic compounds that might prevent performance decrements. In addition, we are testing the effects of potential therapeutics when administered without prior insult or injury to evaluate unwanted neurobehavioral side effects of these drugs. Because stress, illness, injury, or drugs may affect appetite, we have chosen to characterize a nonappetitively motivated water maze task for this purpose. Successful navigation of a water maze requires sensory, motoric, and cognitive per-formance. Because swimming is within the natural repertoire of a rat, swimming itself does not need to be trained. We found that rats learn to swim the maze to reach the exit platform much more quickly than they learn to run the identical maze not filled with water to reach a food reward (10). By testing rats on previously learned and new maze configurations, both memory or learning can be evaluated and, often, deficits in motor vs. cognitive performance can be discriminated.

The maze task we characterized is a traditional maze with alleys, doorways, and choices between paths, not an open water maze such as the Morris maze (12,13,16). The maze can be configured differently by altering which doorways are open
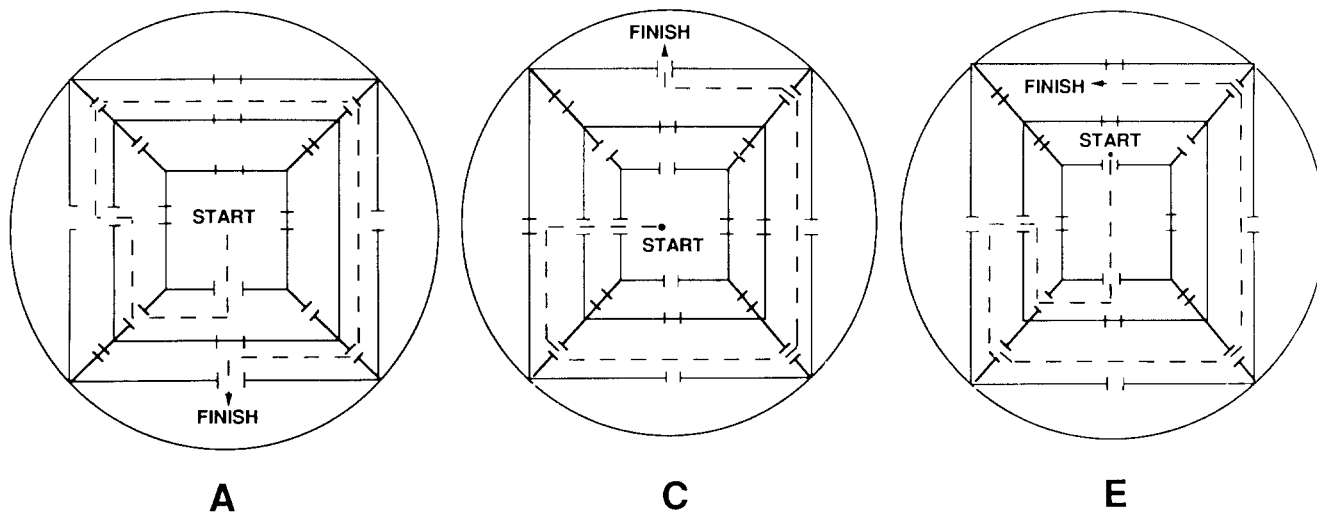
FIG. 1. Representative maze configurations. Straight unbroken lines represent the white plastic walls with removeable doorways. The dotted line represents the optimum swim path from start to finish. All rats were first trained on maze A. One group continued to be tested on maze A on each test day. A second group of rats was tested on a different maze each day of similar difficulty. Mazes C and E are representative of the other 11 mazes utilized. An out-of-the-water platform (double-stacked test tube racks) was placed at the finish. Rats were placed at the start and given a maximum of 5 min to swim to the platform. Whole-body entries through doors not on the correct path were counted as errors.

and which are closed and by altering the "start" or "finish" locations (Fig. 1). Mazes can be designed with different degrees of difficulty or different mazes of predicted similar difficulty can be constructed. Recently, we described several studies in which this water maze task was a useful paradigm. For example, we evaluated a variety of blood substitutes following hemorrhage and found the maze task to be sensitive to ischemia caused by fluid replacements that did not carry sufficient oxygen (15). We also reported that treatment of rats with 0.1 mg/kg MK-801 (an NMDA receptor antagonist) disrupted learning a new maze configuration but did not affect performance on a previously learned configuration (9). Preliminary data from our laboratory suggest that incomplete global forebrain ischemia also causes performance deficits in the maze task (8).

The present studies were conducted to characterize this task further and evaluate some drugs with known psychoactive properties for performance effects in this paradigm. In addition, experiments were conducted to examine the effects of stressors on maze performance. These experiments were performed using a different testing paradigm than we have used heretofore to minimize training trials on the maze. All rats were first trained to swim one particular maze configuration. Rats were then divided into two groups. One group was always tested on the original maze configuration, while the second group was challenged with a different maze each day selected from approximately 10 maze configurations of predicted equal difficulty, based upon number of path choices required. Both groups of rats received the same experimental treatment on each test day and each rat swam three trials for each test day.

Drug or stress testing days were alternated with vehicle or no-treatment days. This experimental design minimized training time and allowed comparison of drug effects within the same subjects at the expense of not having drug-naive animals for each drug test.

METHOD

Animals

Male Sprague-Dawley rats were purchased from Zivic-Miller and weighed approximately 340 g (314–368 g) at the beginning of the study, which lasted several months. Rats were individually housed in rack-mounted hanging cages with food and water freely available in a light-controlled animal room (lights on from 0700–1900 h). For testing, the animal rack was wheeled from the animal room to the maze room and individual animals were removed for testing.

Drugs

d-Amphetamine sulfate was a gift from Smith, Kline and French Laboratories (Philadelphia, PA). The drug was dissolved in saline prior to each test session; the dose reported is the sulfate. Diazepam was a gift from Hoffman-La Roche (Nutley, NJ). Diazepam was prepared daily in a vehicle of propylene glycol, ethanol, and saline. Caffeine and atropine sulfate were dissolved in saline (these drugs were purchased from Sigma Chemical Corp., St. Louis, MO). For drug experiments, animals were placed back into home cages between injection and maze trials. Drugs were injected 15 min prior to maze testing.

Stressors

Three stressors — intermittent foot-shock, immobilization, and forced running — that we have previously shown to increase plasma levels of the stress hormones corticosterone and prolactin were used as acute stressors (7). For each stressor, rats were removed from the home cages and exposed to one of the three stressors. For foot-shock exposure, rats were placed in a cage with parallel floorbars through which intermittent scrambled foot-shock (1.6 mA) was delivered on a variable-interval schedule with an average intershock interval

of 30 s and shock duration of 5 s, that is, approximately 30 shocks in the 15-min shock exposure period. Immobilization stress was accomplished by placing the rat in a plastic cylinder (5.7 cm diameter) constricted at one end and then placing a plastic partition behind the rat to block egress. Forced running required the rat to maintain walking speed in a motor-driven drum (38 cm diameter, 8 rpm). Rats were exposed to each stressor for 15 min immediately prior to maze testing.

Maze

As shown in Fig. 1, the maze consisted of concentric squares set inside a 5-ft. dia child's swimming pool. The maze walls (50 cm high) were white opaque plastic and the alleys between the walls were 16 cm wide. Removable doorways set in the center of each of the walls allowed for different maze configurations. The maze was located in an open laboratory with overhead lighting and numerous available spatial room cues including laboratory equipment and the position of the investigator. Water (24°C) filled the maze to a depth of 25 cm. Maze A was the first maze configured. Rats were placed into the center of the maze and given a maximum of 5 min to find the out-of-the-water exit platform located at the finish. Both the time required and number of errors (whole body entries through doorways not leading to the exit platform) were recorded for each trial. Rats not reaching the platform in 5 min were gently pushed from behind with a paddle and guided through the correct path until they reached the platform. Until rats could complete the maze within 100 s, only one trial per day was run. After all rats reached criterion and drug or stress testing was initiated, three daily trials were conducted and each rat was given a 30-s intertrial rest before

being placed back at the maze starting point. One group of 10 rats always was tested on the original maze configuration (A), while the second group of 10 rats was tested on a variety of mazes of predicted similar difficulty. Mazes B–L varied the position of the start and finish and/or which doorways were open or closed. Mazes C and E are representative and shown in Fig. 1.

Data Analysis

Swim time and errors committed for each day's three trials were recorded. Analysis of variance (ANOVA) was performed to compare selected sets of test days for group and treatment such that performance on the familiar vs. the new maze was compared and drug or stress treatment was compared to the appropriate vehicle or no-treatment trials. Where significant F-values were found, follow-up comparisons between drug or stress groups on each maze were compared to the appropriate maze control group by Student's t-test (two-tailed). Both groups of rats (familiar vs. new maze) were always tested on the same treatment on the same day, but treatment effects (control vs. drug or stress) were performed on different days. Drug days were compared to vehicle injection days performed within a few days of each other. Stress data were compared to no-treatment days.

RESULTS

Comparison of Performance on Familiar vs. New Maze Tasks

Not surprisingly, as shown in Fig. 2, rats in the group presented with the same maze each day swam faster and made
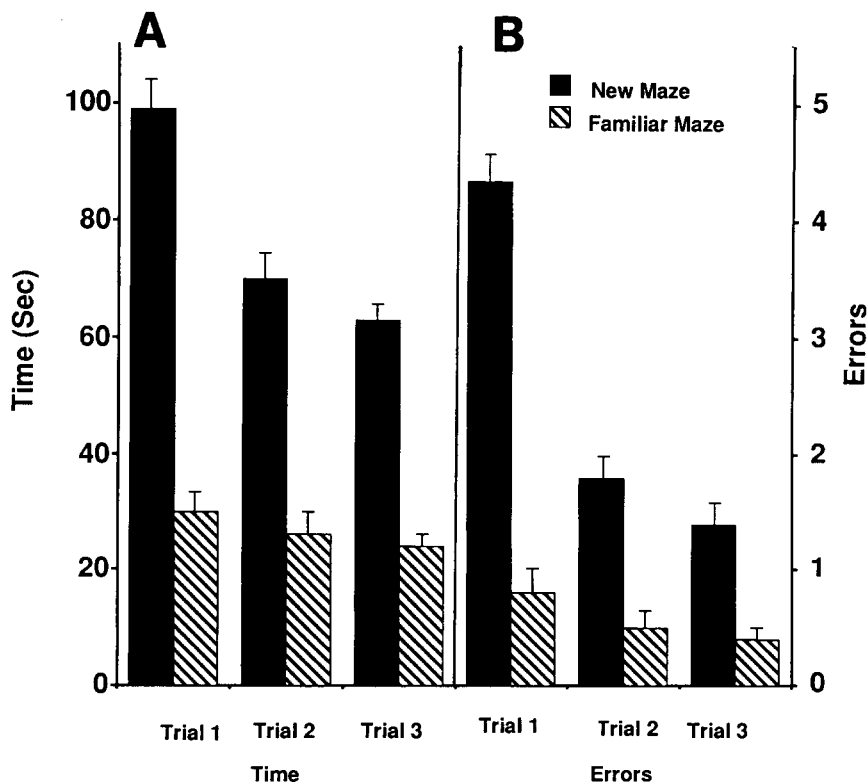

FIG. 2. Average time required (A) and errors commited (B) for three trials in the well-learned and new maze tasks. Data are pooled from 61 test days of 10 rats in each maze group.

fewer errors on the well-learned maze than did rats in the group swimming a new maze configuration each day. These data were pooled from 61 test days for each group and include drug, vehicle, stress, and no-treatment test days. Within each test day, both groups of rats improved their performance over the course of the three trials. It is worth pointing out that rats swimming a different maze each day reached the exit platform in approximately 100 s, even on the first swim. Maze-naive rats typically require several days to reach the platform in under the maximum allowable 300 s. Thus, this repeated acquisition version of the original maze task described does permit more rapid assessment of the effects of drugs on learning.

Effects of Psychoactive Drugs on Maze Performance

Amphetamine sulfate (4 mg/kg) was given on 2 separate testing days to both experimental maze groups 15 min prior to testing. These data were compared to two saline-injection test days. As shown in Table 1, amphetamine increased the swim times and errors committed for both testing groups. However, only performance on the new maze task was significantly disrupted. ANOVA for the new maze data showed for swim time, $F(1, 38) = 12.3, p < 0.001$, and for errors, $F(1, 38) = 7.6, p < 0.01$.

Caffeine (30 mg/kg) was also administered on two separate occasions 15 min prior to testing and compared to performance on 2 days when saline vehicle was administered. Caffeine did not significantly affect performance on the well-learned maze as measured by either errors or completion time (Table 1, Fig. 3). For the group of rats learning a new maze, caffeine significantly increased the average time per trial from 45 to 86 s, $F(1, 38) = 4.98, p < 0.05$, and the average errors committed from 1.8 to 3.1 but the average error increase was not statistically significant. When performance was examined for each of the three trials separately (Fig. 3), differences among trials were seen. The performance of rats on the first trial on the caffeine day was similar to the performance on the first saline treatment trial. However, for the second and third trials caffeine-treated rats required more time to reach

the finish (Fig. 3) and made more errors along the way. For the new maze, there were significant differences for time between caffeine- and saline-treated rats on the second and third trials and differences in errors for Trial 2 only.

Diazepam was administered at 1 mg/kg (on 2 test days), 2 mg/kg (on 2 test days), and 4 mg/kg (1 test day). Vehicle was administered on 6 test days. Diazepam disrupted performance in both mazes to some degree at all tested doses, as shown in Figs. 4 and 5. The 4-mg/kg dose was disruptive, as shown by the large swim time for rats in both maze groups. Two rats in the new maze and 4 of the 10 rats swimming the well-learned maze did not complete the maze in the maximum 300 s allotted on the first trial at the 4-mg/kg dose. By the third trial, only one rat in the new maze group and two rats in the well-learned maze group failed to complete the maze.

ANOVA found significant effects for maze group and drug treatment for both time and errors. Combined across maze groups and doses, the ANOVA for the effect of diazepam on swim time found, $F(1, 212) = 37, p < 0.0001$, and for errors, $F(1, 212) = 12.2, p < 0.01$. Follow-up specific comparisons are shown in Figs. 4 and 5.

Atropine sulfate was administered on 2 test days, once at 5 mg/kg and once at 30 mg/kg, each at 15 min prior to testing. No significant differences between the atropine treatment days and saline treatment days were seen (Table 1). Subsequently, atropine sulfate 30 mg/kg was tested on 2 days when given 30 min prior to maze testing. Again, no significant performance differences between atropine test days and saline test days were seen (data not shown).

Effects of Acute Stress on Maze Performance

Three stressors were assessed for their effects on performance: running, immobilization, and intermittent foot-shock. Each stressor was utilized on a different test day and compared to the data obtained from a no-treatment test day. Rats from both maze testing groups were exposed to the stressor for 15 min immediately prior to maze testing.

TABLE 1

EFFECTS OF AMPHETAMINE, CAFFEINE, AND ATROPINE ON MAZE PERFORMANCE

Drug	Well-Learned Maze				New Maze			
	Saline		Drug		Saline		Drug	
Amphetamine sulfate (4 mg/kg)								
Time (sec)	38	± 10	78	± 21	91	± 19	195	± 23*
Errors	0.9	± 0.3	2.2	± 1.3	1.8	± 0.3	5.8	± 1.4*
Caffeine (30 mg/kg)								
Time (sec)	15.6	± 0.9	18.8	± 1.8	45.4	± 7.3	86.4	± 16.8*
Errors	0.18	± 0.09	0.20	± 0.08	1.8	± 0.4	3.1	± 0.8
Atropine sulfate (30 mg/kg)								
Time (sec)	44	± 8	53	± 12	116	± 113	105	± 26
Errors	1.2	± 0.2	2.0	± 0.8	4.8	± 0.5	4.2	± 0.8

Values represent the mean ± SEM. Ten different animals were tested in each group each test day. For each day, the time (seconds) and error data from three trials was averaged for each rat. The values listed above represent the mean of those averaged scores for each group for 1 (atropine) or 2 (amphetamine and caffeine) testing days. Atropine was also tested on 1 day at 5 mg/kg (15 min after injection) and on 2 other days at 30 mg/kg (30 min postinjection). There were no significant effects under either of those conditions.

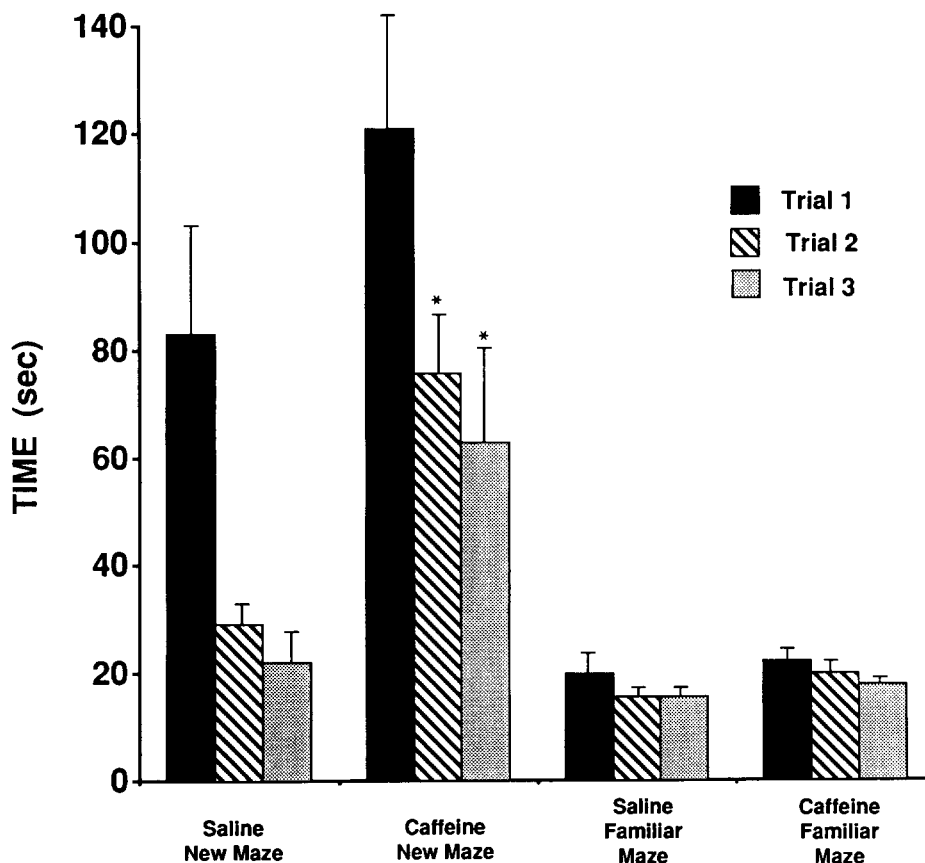*Significantly different from saline, $p < 0.05$, Student's t-test.

FIG. 3. Effects of caffeine (30 mg/kg) on maze performance. *Significantly different from same-trial saline-treated performance on new maze. Student's t-test, two-tailed, $p < 0.05$.

No differences between stressed and nonstressed rats were seen on the first trial on either maze for swim completion time (Fig. 6). However, on the second trial while nonstressed rats improved performance the foot-shocked and forced running groups' performance worsened. ANOVA for the second trial for both maze groups revealed a significant effect of stressor, $F(3, 72) = 2.99, p < 0.05$. While the trend for poor performances by the foot-shock and forced running groups continued on the third trial, the differences in performance compared to the control group did not reach statistical significance, $F(3, 72) = 2.4, p < 0.08$.

The performance of individual rats exposed to stressors prior to maze testing was variable. For example, the 10 rats in the new maze foot-shock group swam the first trial in a average of 129 s with 3 rats not finishing in the 300-s allotted time. This group finished the second trial in 191 s with four rats at the 300-s maximum and the third trial at 161 s with five rats not finishing. The other five rats swam well, finishing in under 36 s. Rats not finishing the maze in 300-s did not commit a high number of errors but, instead, tended to remain immobile in one area of the maze. The same pattern of performance disruption for the foot-shock stress group was seen in the familiar maze, with the foot-shocked group performing less well on succeeding trials and significantly more poorly on the second trial.

The forced running group did consistently worse than the control group after the first trial in both mazes, but these differences were not statistically significant. Both the non-stress and the immobilization stress groups improved performance over trials in both mazes.

Errors committed were difficult to interpret in the stress experiments because rats that failed to finish often simply stayed in one area, increasing swim times but precluding errors.

DISCUSSION

In the present study, we demonstrated that the water maze task previously described can be modified and used as a repeated acquisition paradigm. As such, the task is sensitive to the disrupting effects of known psychoactive compounds, including amphetamine, caffeine, and diazepam. In addition, performance-disrupting effects of brief exposure to foot-shock stress were seen. On the other hand, the task did not reveal performance disruption by atropine (5 or 30 mg/kg) or immobilization stress. Forced running effects were small and not consistent between the new and well-learned mazes.

Rats were run in this paradigm approximately 60 times after initial acquisition of the first maze configuration and after exposure to different drugs and treatments. Task performance appeared to remain fairly stable, although there was a gradual improvement viewed over long periods of time.
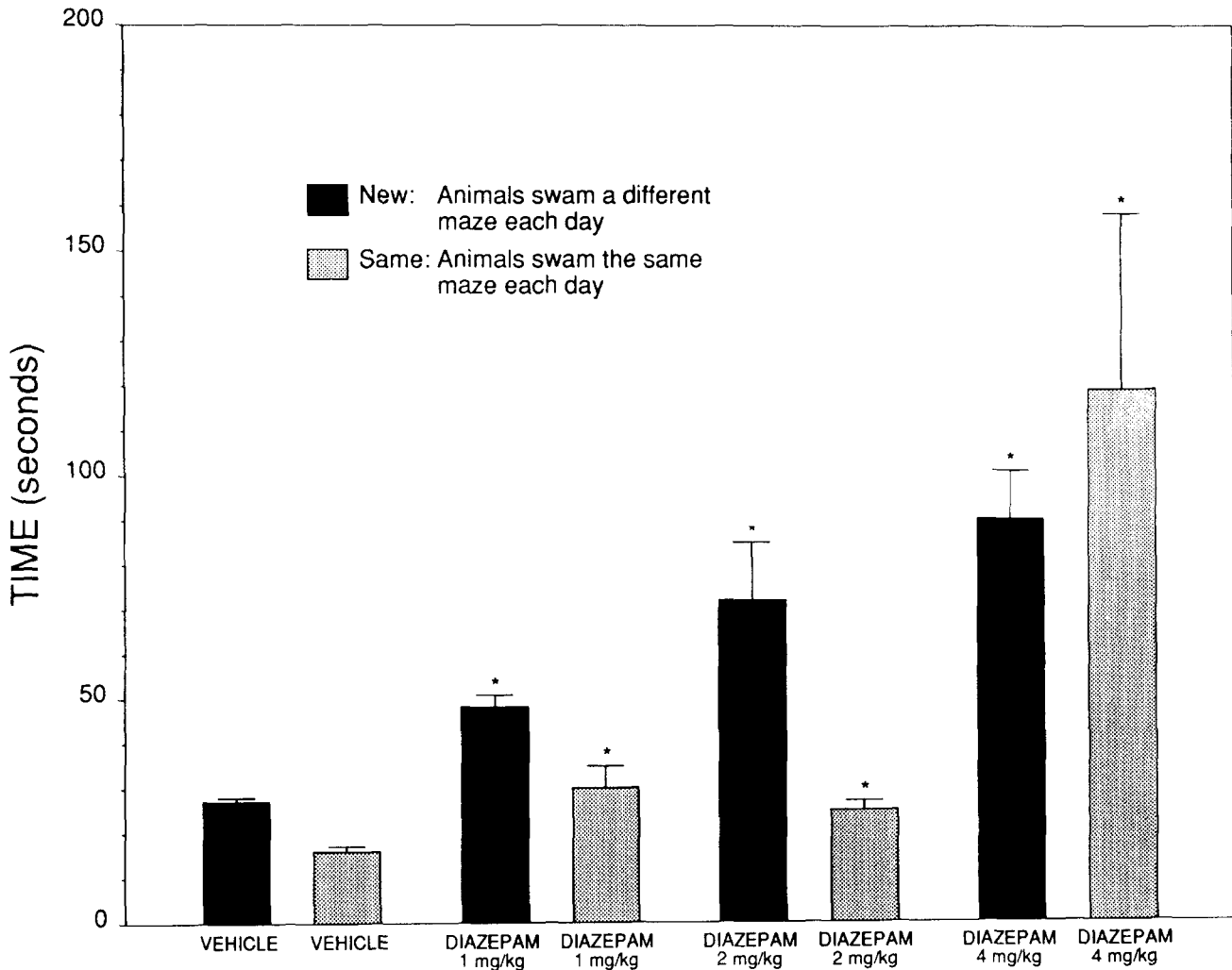
FIG. 4. Effects of diazepam on swimming time in well-learned and new mazes. *Significantly different than vehicle-treated group performance on corresponding maze. Student's t-test, two-tailed, $p < 0.05$.

Amphetamine and diazepam had robust effects, demonstrating that the task is sensitive to known performance disrupters. Repeated training did not decrease the utility of the task to detect performance disruption, as shown by the diazepam experiments, among the last to be performed.

Performance on a new maze appeared to be more difficult and sensitive to performance disruption. However, performance on the familiar maze provided important data that facilitated interpretation of the mechanisms by which particular treatments impaired performance. Fast and accurate performance on the familiar maze coupled with impaired performance of the new maze task suggested a more cognitive rather than motoric deficit. When performance on both mazes was affected, as it was at the 4-mg/kg diazepam dose, it was not possible to distinguish between motoric and cognitive impairments.

In general, speed and errors were usually similarly affected by most treatments, but on occasion both measures provided a fuller picture than either data alone. For example, rats not completing the maze in 300 s after foot-shock often had few errors; the low error rate by itself might suggest excellent performance. Instead, the time and error data together reflect a more correct picture, that is, rats were primarily behaviorally inactive and stayed in one location, neither locating the finish nor committing errors.

With respect to the effects of specific drugs, few surprises were encountered. The drugs and doses were chosen based upon reported psychoactive effects in other paradigms (2,3,6,11,14,17). Certainly, the performance-disrupting effects of amphetamine and diazepam reflect the general literature. Diazepam has been shown to affect acquisition more than recall (1,5,12), as we found in this study. Diazepam could affect performance by decreasing the motivation to complete the task via decreased anxiety, or through its effects on memory processes or through effects on motor performance or via some combination of the above factors. The diazepam experiments were run relatively late in the overall study, when rats should have been relatively habituated to the procedure
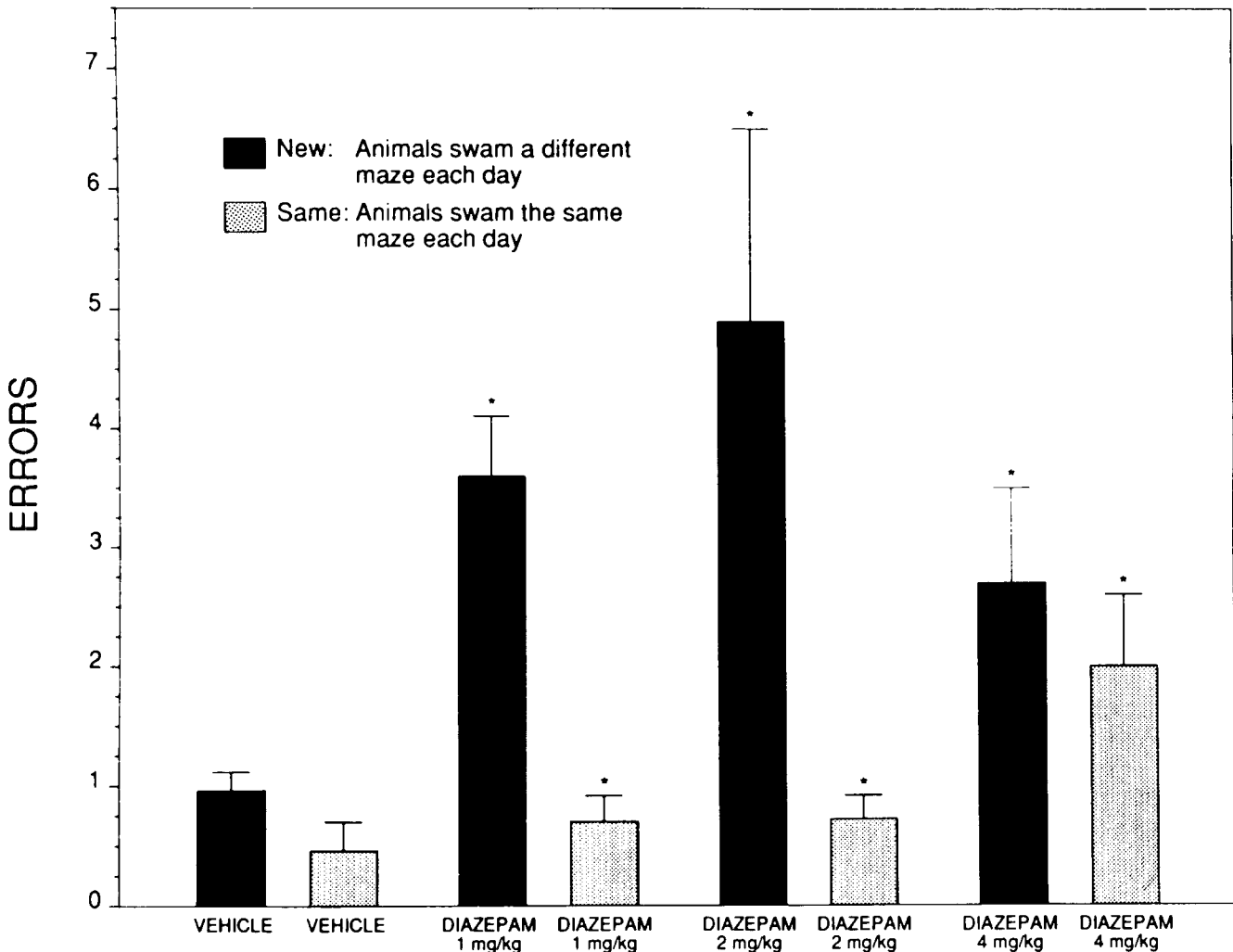
FIG. 5. Effects of dizepam on errors commited in well-learned and new mazes. *Significantly different than vehicle-treated group performance on corresponding maze. Student's t-test, two-tailed, $p < 0.05$.

and not fearful or anxious. In similar studies, we found that stress hormones markedly attenuate with experience in this task (unpublished data). Therefore, we do not believe diazepam impaired performance in the present study by decreasing fear-driven motivation to escape from the water. At the 1- and 2-mg/kg doses of diazepam, performance was significantly impaired on both the previously learned and new mazes; however, the magnitude of the deficit was much greater for the new maze. Performance deficits at the 4-mg/kg dose were similar for both mazes; in fact, rats took more time to complete the well-learned maze than the new maze. We suggest that the 4-mg/kg dose affects motor performance as well as cognitive function and that the lower doses primarily affect cognitive ability.

Because amphetamine may affect appetite, a water maze is a good assessment paradigm for this drug. As expected, amphetamine decremented performance, especially in the learning of the new maze task. Data were not collected in a way that would allow for a detailed analysis of the types of

errors committed; for example, it would be interesting to know the number of perserverative errors in door choices.

Caffeine had no effects on performance on the well-learned task; however, caffeine-treated rats took longer to complete the new maze and made more errors on this maze than saline-treated rats (Table 1). This was especially noticeable on the second and third swim trials (Fig. 3), where learning plays a larger part in task performance. On the first trial, performance will necessarily be more variable because the rat has not yet found the platform. The rat can perform better on the second and third trial if it can recall where the platform was located on the first trial.

Atropine at 5 or 30 mg/kg had no significant effects in this paradigm. While cholinergic blockade would be expected to impair learning and memory (4,16), no impairments were seen in the present study. Our failure to see an effect may indicate that this maze task is not as sensitive as the other tasks utilized.

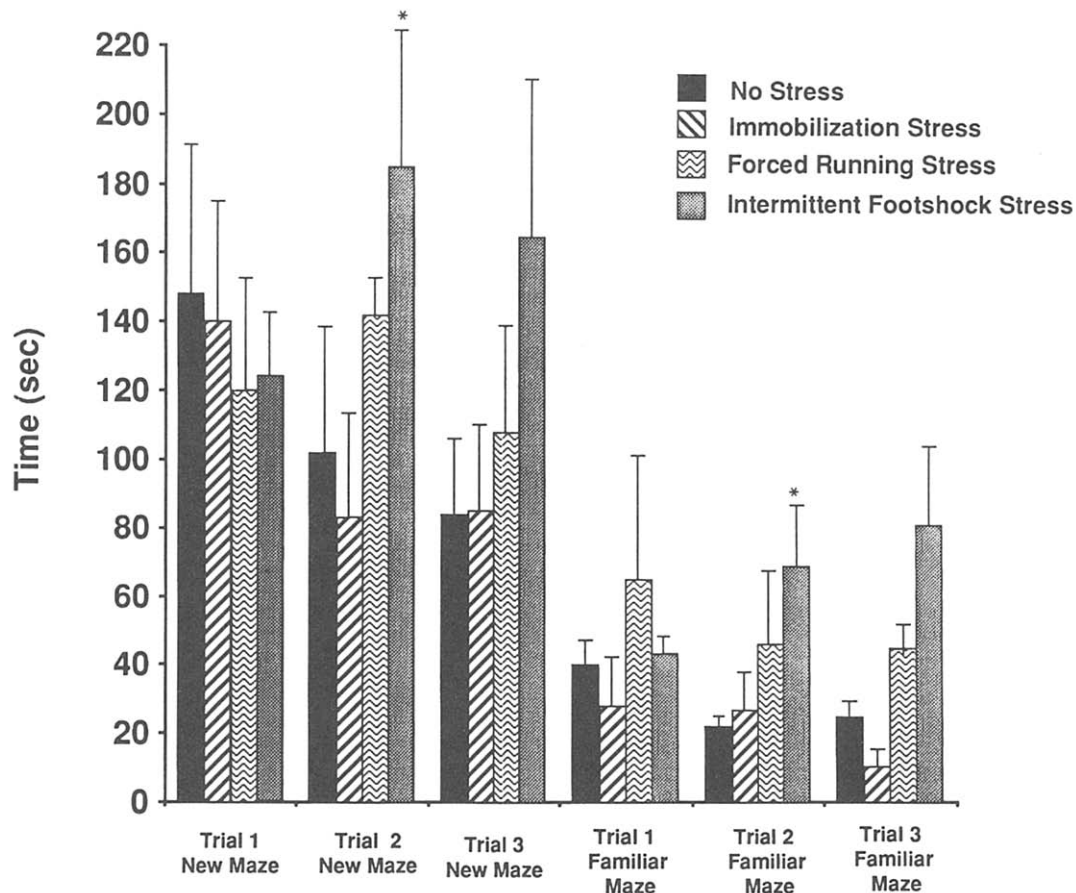The effects of foot-shock stress in impairing performance

FIG. 6. Effects of stressors on maze performance. *Significantly different from no-stress group on same trial.

were mixed. Half the rats on the new maze task performed well following foot-shock stress, while the other five rats performed poorly. It is possible that the poor performance of these five rats was either due to a "learned helplessness" effect or perhaps to shock-induced behavioral "freezing," both of which have been described following exposure to inescapable shock in rats.

Overall, the water maze task appears to be a useful addition to the collection of varied performance tasks available that can be used to assess various components of "performance." The repeated acquisition testing described herein is a

rapidly trained paradigm that reveals performance-disrupting effects of known psychoactive drugs. The ability to test rats without the need for prior food restriction and without the potential confound of drug-induced alterations in appetite makes this task appealing.

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