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Pharmacology Biochemistry and Behavior, Vol. 53, No. 2, pp. 317–322, 1996

0091-3057/96 $15.00 + .00

0091-3057(95)02028-8

Effects of Triazolam and Diazepam on Learning and Memory as Assessed Using a Water Maze[1]

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Received 1 February 1995; Revised 1 May 1995; Accepted 3 May 1995

KANT, G. J., R. M. WYLIE, A. A. VASILAKIS AND S. GHOSH. *Effects of triazolam and diazepam on learning and memory as assessed using a water maze.* PHARMACOL BIOCHEM BEHAV 53(2) 317–322, 1996.—One of the reported adverse side effects of the frequently prescribed benzodiazepines diazepam (Valium®) and triazolam (Halcion®) is an impairment of anterograde memory in humans. The experiments described in this article compared the effects of triazolam and diazepam on performance in a water maze task that is sensitive to drugs that affect learning and memory. The water maze utilized is a traditional type of maze with alleyways and door choices, unlike the Morris open water maze. Time required to find an out-of-the-water platform and errors committed during the swim are used as performance measures. Rats were tested on a previously learned maze configuration and on the acquisition of new maze configurations. Neither diazepam (0.25, 1.0, or 2.0 mg/kg) nor triazolam (0.05, 0.2, or 0.3 mg/kg) injected 30 min prior to testing on the previously learned maze affected swim time or errors committed. Administration of diazepam (0.5, 1.0, or 2.0 mg/kg, IP) prior to daily training on three different new maze configurations did not affect swim time, but did increase swim errors. Triazolam administered at 0.1, 0.2, or 0.3 mg/kg markedly impaired performance as assessed by either swim time or errors. There were no differences in performance of rats previously treated with triazolam, diazepam, or vehicle in learning another new maze after drug treatment was terminated. These data demonstrate that both diazepam and triazolam affect acquisition but not recall of maze configurations and support similar conclusions reached using other types of tasks in humans and animals.

Triazolam Diazepam Halcion Valium Learning Memory Water maze Performance

HALCION® (triazolam) is frequently prescribed for insomnia in the United States. Its widespread use has been followed by reports of adverse side effects ranging from mild memory impairment to hallucinations and delusions (2). Triazolam has also been considered for use in military settings to assist soldiers to sleep during long deployments, when anxiety or noise might otherwise prevent sleep (17). However, the advantage of sleep induction must be balanced by the disadvantage of administering a drug that might impair information acquisition. The present study was performed to compare the effects of triazolam on learning and memory in a rodent water maze task with the effects of diazepam (Valium®), a drug for which much more information is available, and to provide a basis for future comparisons of new drugs with triazolam.

Diazepam has been reported to impair acquisition but not performance in the Morris open pool water maze (15). Our laboratory has utilized a different type of water maze that allows for the quantitation of errors as well as swim time for performance assessment (9,10,18). In addition, this maze is easily reconfigured so that rats can be rechallenged to learn multiple mazes. In the present study, we used mazes of equal difficulty by choosing mazes with the same number of choice points. However, easier tasks can be configured by reducing the choices.

[1] 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). Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments relating to animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NIH publication 86-23.

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We recently reported that the benzodiazepine diazepam impairs performance on this water maze task (9). The water maze task in the previous study utilized the same maze apparatus described in the present study, but rats were only given three trials on a single test day to swim either a well-learned or a novel maze configuration. Diazepam-treated rats (1, 2, or 4 mg/kg) were slightly but significantly impaired on the well-learned maze and markedly impaired on the new maze configuration. In the present study, diazepam-treated rats were given eight or more trials to learn each new maze.

METHOD

Subjects

Male Sprague-Dawley rats (purchased from Charles River) weighing 419–586 g at the end of initial maze training (beginning of drug treatment) were used as subjects. Each rat was individually housed in the animal housing area with food and water freely available. The lights were on from 0700 to 1900 h.

Drugs

8-Chloro-6-(2-chlorophenyl)-1-methyl-4*H*-1,2,4-triazolo-[4,3-*a*]-1,4-benzodiazepine (triazolam) and diazepam were purchased from Sigma Chemical Co. (St. Louis, MO). Drugs were prepared fresh daily and dissolved in dimethyl sulfoxide (DMSO), which was also purchased from Sigma Chemical Co. Drug treatments were injected IP 30 min prior to maze testing.

Water Maze Testing

The maze consisted of concentric squares set inside a 6-ft diameter child's swimming pool. The maze walls (50 cm high) were white opaque plastic and the alleys between the walls were 16 cm wide. Removeable doorways set in the center of each of the walls allowed for different maze configurations. Three of the maze configurations used in these experiments are shown in Fig. 1. Maze B (not shown) is identical to maze A, but with the start and finish reversed. Maze G (not shown) is similar in difficulty to the ones shown in Fig 1; it has an outside wall "start" and the "finish" is located in the middle square of three concentric squares. The maze apparatus was located in an open laboratory with overhead lighting and numerous available spatial room cues, including laboratory equipment. Tap water (23–27°C) filled the maze to a depth of 25 cm. Maze A was the first maze configured. Rats were placed in the center of the maze and were given a maximum of 5 min to find the out-of-the-water exit platform located at the "finish." Both the time required and the 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. Training was conducted until all rats completed

TABLE 1

EFFECTS OF TRIAZOLAM AND DIAZEPAM ON WELL-LEARNED MAZE A

Time in Seconds (Errors)	Vehicle	Diazepam (D)	Triazolam (T)
No Injection	31.4 ± 4.0	21.9 ± 1.6	24.3 ± 2.3
Four trials	(0.36 ± 0.11)	(0.36 ± 0.11)	(0.53 ± 0.13)
0.25 mg/kg D	37.0 ± 6.8	28.2 ± 3.5	31.4 ± 5.6
0.05 mg/kg T	(1.0 ± 0.6)	(0.56 ± 0.29)	(0.44 ± 0.18)
1.0 mg/kg D	30.8 ± 5.3	33.6 ± 3.7	36.8 ± 3.0
0.2 mg/kg T	(0.28 ± 0.11)	(1.22 ± 0.30)	(0.89 ± 0.21)
2.0 mg/kg D	53.2 ± 28.0	27.7 ± 4.3	61.7 ± 30.2
0.3 mg/kg T	(0.22 ± 0.15)	(1.11 ± 0.61)	(1.33 ± 0.73)

Values represent the mean ± SEM. $N = 9$ rats/group/trial. There were four trials with no drug injection interspersed among the drug trial days. Two trials were conducted at the medium drug dose and one trial each at the highest and lowest doses. There were no statistically different effects of drug treatment for time, $F(2, 203) = 2.2$, $p = 0.11$ or errors $F(2, 203) = 2.09$, $p = 0.12$. One data point in the vehicle group for 1 day was omitted because of an inexplicably high number of errors.

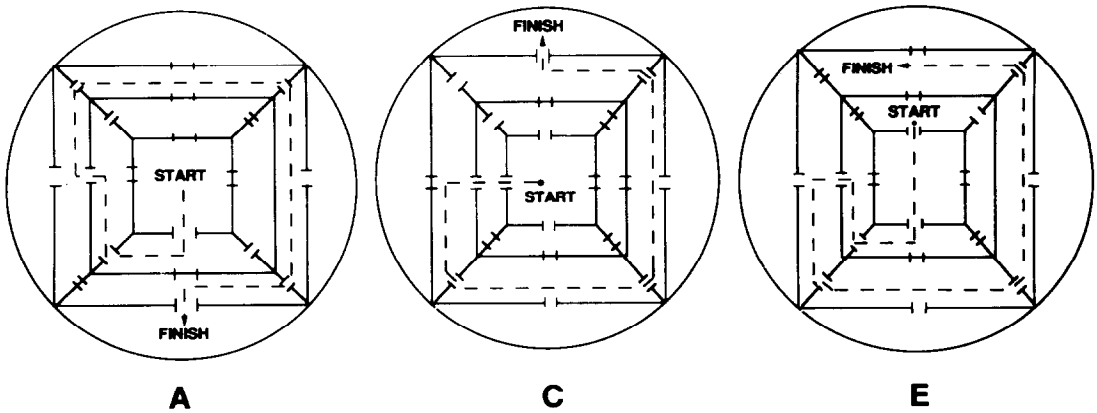

FIG. 1. Maze configurations. Straight unbroken lines represent the white plastic walls with removeable doorways. The dotted line represents the optimum swim path from start to finish. All rats were first trained on maze A. Maze B is the reverse of maze A (same path, start and finish reversed). An out-of-the-water 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.

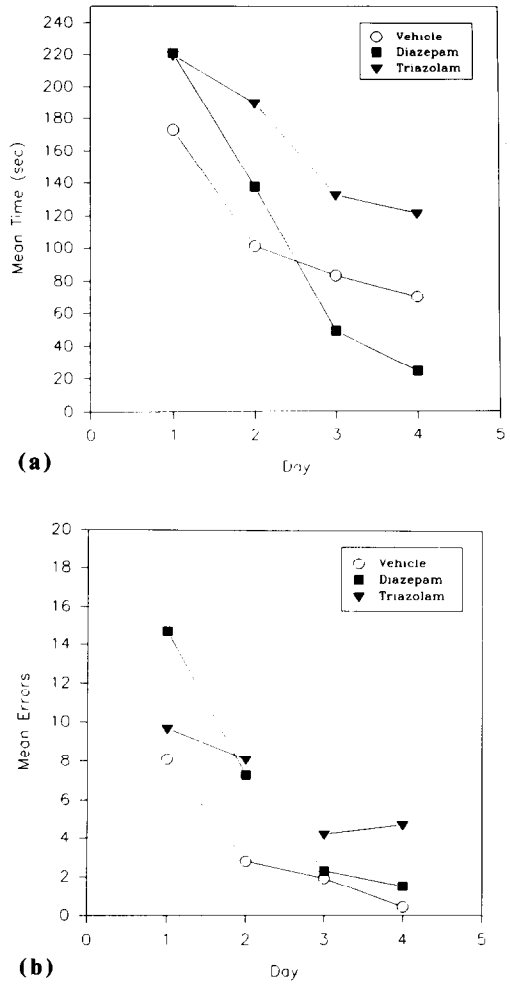

FIG. 2. Effects of diazepam and triazolam on acquisition of maze C. Two consecutive training trials per day following one injection of vehicle or diazepam (0.5 mg/kg) or triazolam (0.1 mg/kg) were performed. (a) Swim time and (b) errors.

the maze within 60 s with no more than one error. Following initial training in maze A (29 trials), the rats were divided into three groups such that maze performance was approximately equal among groups. The rats were then retested eight times over 11 days in the order: day 1, no drug injection; day 2, 0.2 mg/kg triazolam or 1.0 mg/kg diazepam or vehicle (DMSO); day 3, no drug injection; day 4, 0.05 mg/kg triazolam or 0.25 mg/kg diazepam or vehicle; days 5–7, no testing; day 8, no drug injection; day 9, 0.2 mg/kg triazolam or 1.0 mg/kg diazepam or vehicle; day 10, no drug injection; day 11, 0.3 mg/kg triazolam or 2.0 mg/kg diazepam or vehicle.

In the next phase of the experiment, the effects of triazolam and diazepam on acquisition of the maze task were assessed. Rats were challenged to learn a new maze for each drug dose tested. For each drug dose, rats were given two consecutive swim trials per day with a 30-s intertrial interval, following administration of triazolam, diazepam, or vehicle (30 min prior to first swim trial). Rats were first tested on new maze C for 4 days at 0.1 mg/kg triazolam or 0.5 mg/kg diazepam or vehicle.

After 3 no-test days, rats were challenged to learn another maze (E) over eight trials, two per day, 30 min following administration of 0.2 mg/kg triazolam, 1.0 mg/kg diazepam, or vehicle. After 3 additional no-test days, rats were challenged in another new maze (B) over 14 trials, two per day, 30 min following administration of 0.3 mg/kg triazolam, 2.0 mg/kg diazepam, or vehicle.

Finally, 5 no-test days preceded final testing on new maze G for seven trials conducted on separate days without any drug administration.

Data Analysis

Swim time required to reach the platform and errors committed for each day's trials were recorded, entered into a data base, and analyzed by the BMDP statistical software for ANOVA. For the performance testing on the previously learned maze A, each day was analyzed separately for the effects of drug treatment by one-way ANOVA. For the maze

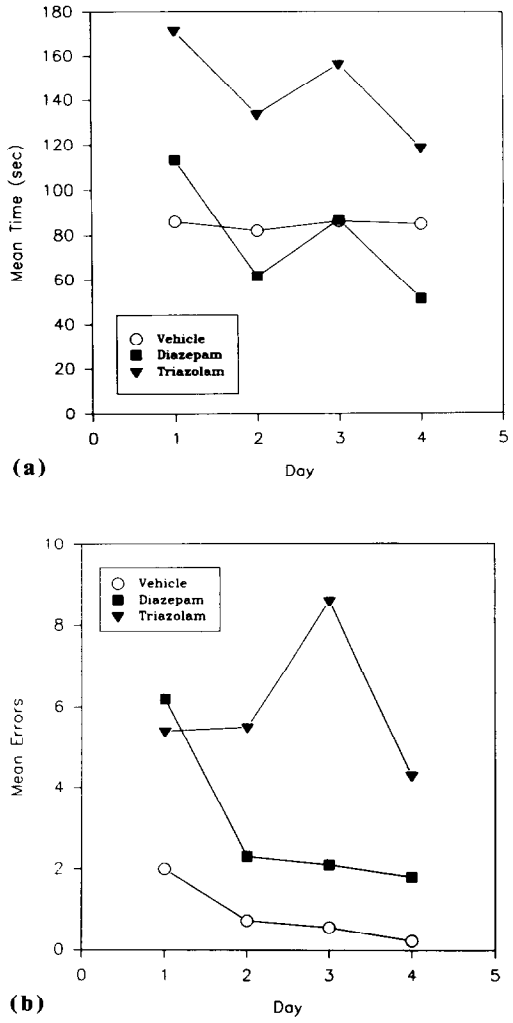

FIG. 3. Effects of diazepam and triazolam on acquisition of maze E. Two consecutive training trials per day following one injection of vehicle or diazepam (1.0 mg/kg) or triazolam (0.2 mg/kg) were performed. (a) Swim time and (b) errors.

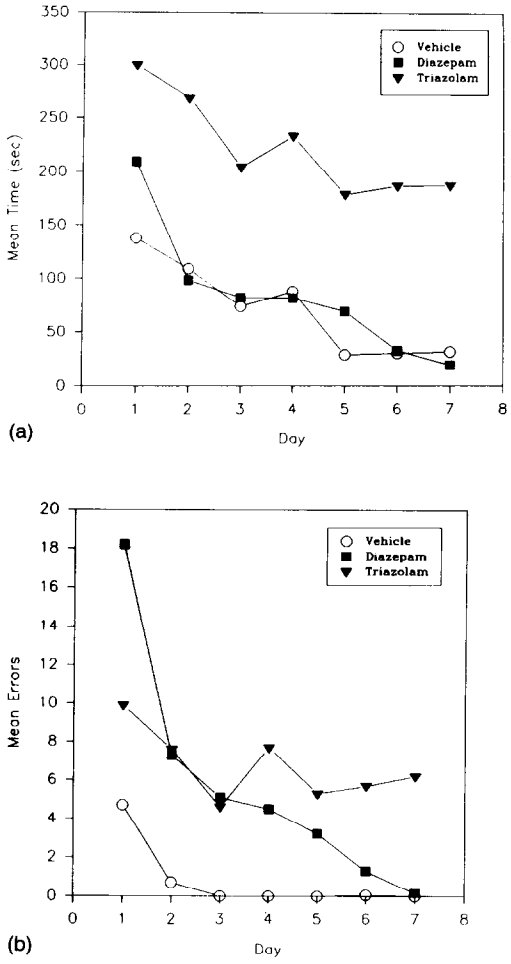

FIG. 4. Effects of diazepam and triazolam on acquisition of maze B. Two consecutive training trials per day following one injection of vehicle or diazepam (2.0 mg/kg) or triazolam (0.3 mg/kg) were performed. (a) Swim time and (b) errors.

learning experiments, data were first analyzed by two-way ANOVA for the effects of drug treatment and test day. Significant effects were followed up by vehicle vs. each drug comparisons. Group differences were considered to be significant at $p < 0.05$.

RESULTS

Effects of Triazolam and Diazepam on Performance of Well-Learned Maze A

As shown in Table 1, neither diazepam nor triazolam at any dose tested significantly impaired performance on previously learned maze A.

Effects of Triazolam and Diazepam on Learning New Mazes

Triazolam markedly impaired learning new mazes as assessed by both time and errors at all three doses tested (Figs. 2–4). Diazapam significantly affected error rate at all doses but had no effect on swim time at any dose (Figs. 2–4).

For learning maze C (Fig. 2, 0.1 mg/kg triazolam; 0.5 mg/kg diazepam), two-way ANOVA showed significant effects for drug (time, $F = 8.1, p < 0.001$; errors, $F = 8.4, p < 0.001$) and for test day (time, $F = 19.4$, $p < 0.0001$; errors, $F = 2.4$, $p < 0.05$). Follow-up comparisons between vehicle and each drug showed a significant effect of triazolam ($F = 9.9$, $p < 0.01$), but not diazepam ($F = 0.02$, $p = 0.88$), for swim time. For errors, there were significant impairments by both triazolam ($F = 13$, $p < 0.0001$) and diazepam ($F = 7.8$, $p < 0.001$).

For learning maze E (Fig. 3, 0.2 mg/kg triazolam; 1.0 mg/kg diazepam), two-way ANOVA showed significant effects for drug treatment for both time ($F = 9.07$, $p < 0.001$) and errors ($F = 18.2$, $p < 0.0001$), but not for test day (time, $F = 1.5$, $p = 0.21$; errors, $F = 2.3$, $p = 0.07$). The triazolam group differed from the other two groups by consistently requiring more time to complete the maze and making more errors on days 2–4. ANOVA found, for time, a significant effect of triazolam ($F = 11.2$, $p < 0.001$), but not diazepam ($F = 0.2$, $p = 0.69$). For errors, there were significant impairments by triazolam ($F = 32$, $p < 0.0001$) and diazepam ($F = 13.9$, $p < 0.001$).

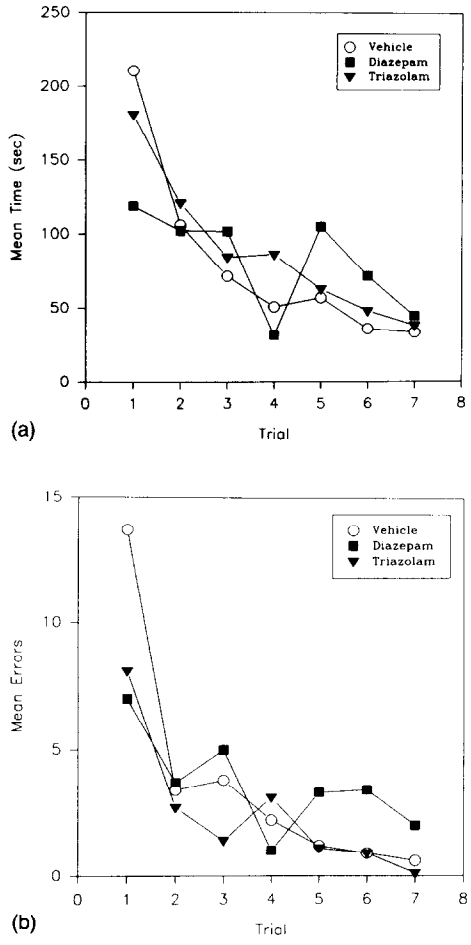

FIG. 5. Postdrug learning. One training trial per day (with the exception of two trials on the first day) with no drug administration. Rats previously treated with vehicle or with diazepam or triazolam are shown. (a) Swim time and (b) errors.

For learning maze B (Fig. 4, 0.3 mg/kg triazolam; 2.0 mg/kg diazepam) two-way ANOVA showed significant effects for drug treatment (time, $F = 107$, $p < 0.0001$; errors, $F = 27.7$, $p < 0.0001$) and test day (time, $F = 15.5$, $p < 0.0001$; errors, $F = 11.3$, $p < 0.0001$). Follow-up comparisons between vehicle and each drug found, for time, a significant effect of triazolam ($F = 144$, $p < 0.00001$), but not diazepam ($F = 1.2$, $p = 0.27$). For errors, there were significant impairments by triazolam ($F = 85$, $p < 0.00001$) and diazepam ($F = 24$, $p < 0.00001$).

Once drug administration was discontinued, there were no differences in acquisition of maze G among rats previously assigned to vehicle vs. either drug group (Fig. 5), as demonstrated by a significant effect of trial (time, $F = 10.9$, $p < 0.0001$; errors, $F = 11.1$, $p < 0.0001$), but not drug treatment (time, $F = 0.32$, $p = 0.72$; errors, $F = 1.38$, $p = 0.25$).

DISCUSSION

The major adverse side effect associated with benzodiazepine administration in humans is impairment of anterograde memory (1,2,4,7,8,12,14,17,19,20). Various neurobehavioral tasks have been used to assess this benzodiazepine-induced deficit in animal models of learning and memory, most often avoidance paradigms (3,6,11). Because of the broad spectrum of effects of benzodiazepines, it is important to differentiate memory effects from alterations in motivation, general motor activity, motor coordination, appetite, sedation, and anxiety, which may be caused by benzodiazepines (6,13). A water maze task eliminates appetite as a factor. Comparisons between performance on previously learned vs. new mazes allows estimation of the relative contributions of drug effects on general activity, coordination, and motivation to any observed performance alteration.

In the present studies, we found that triazolam was much more potent than diazepam in impairing water maze performance. The impairment was specific for learning as compared to performance of a previously learned maze, thus fitting the reported pattern for benzodiazepines affecting acquisition but not recall of previously learned information.

The present data for diazepam show that diazepam-treated rats can eventually match the performance of vehicle-treated rats on a new maze, and provide additional support for our suggestion in a previous report (9) that the performance deficit caused by diazepam is primarily cognitive rather than motoric. The absence of an impairment on the well-learned maze and the unaffected swim times on the new mazes at all diazepam doses demonstrate that the diazepam-treated rats are not ataxic nor unmotivated to reach the exit platform. Thus, error rate may be a more sensitive indicator of task impairment than swim time. At higher doses or with different testing paradigms, diazepam can affect swim speed. Swim times were increased following diazepam in our previous report, which utilized a different testing protocol, and in the Morris water maze task (9,16).

Although the doses of triazolam selected for the present study were based on previous literature, and did not affect performance on the well-learned maze, all doses greatly impaired acquisition of new maze configurations. It cannot be concluded from the data whether the triazolam-treated rats would have eventually performed as well as vehicle-injected rats. However, at the lowest dose tested the error mean dropped by approximately 50% over the eight trials. It is possible that additional improvement would have occurred with more trials.

The doses utilized of either drug should not have caused sedation or ataxia because similar doses did not affect swim times in the well-learned maze. Generally, one would expect increased errors to result in increased swim times, as was seen for triazolam, but not diazepam, in the present study. However, errors "cost" an animal variable amounts of time, depending upon how long it takes the animal to discover the mistake and correct it by swimming back through the incorrect door and forward to the correct door. We did not collect this data during each swim trial, nor did we record the amount of time that rats engaged in clinging to a doorway or swimming in place before choosing which doorway to swim through. Because the diazepam-treated rats made more errors during acquisition without significantly increasing swim times, we hypothesize that these rats spent less time than vehicle-treated rats on floating/clinging behaviors or exploration of dead ends. It is possible that floating and clinging are related to fear and that the anxiolytic properties of diazepam reduced this factor. Alternatively (or additionally), the diazepam-treated rats may have spent very little time in exploring dead ends after committing errors.

Neither drug had long-term effects on acquisition performance as assessed by testing after drug termination on maze G. However, that maze testing was conducted several days after termination of drug administration, at which time the drugs should have cleared from the animals. Because the half-life of triazolam is much shorter than diazepam, a future study in which learning trials are conducted at several time points hours after drug administration, instead of the single 30-min time point utilized in the current study, might reveal different time course effects of the two compounds.

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