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The Elevated T-Maze: A New Animal Model of Anxiety and Memory

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VIANA, M. B., C. TOMAZ AND F. G. GRAEFF. *The elevated T-maze: A new animal model of anxiety and memory.* PHARMACOL BIOCHEM BEHAV **49**(3) 549-554, 1994. – In an attempt to analyze different types of anxiety, and at the same time assess memory, a new experimental model was developed. The apparatus, named the elevated T-maze, consisted of three arms of equal dimensions (50 × 10 cm) elevated 50 cm from the ground. One arm, enclosed by 40-cm high walls, was perpendicular to two open arms. The first experimental session was conducted 25 min after IP injection of either drug or saline. To assess inhibitory (passive) avoidance, the rat was placed at the end of the enclosed arm and the time taken to withdraw from this arm was recorded three times in succession. Soon afterwards, the rat was placed at the end of one of the open arms and the time taken to withdraw from this arm was measured, thus estimating one-way escape. To assess memory, inhibitory avoidance and escape were measured again 3 days later, without drug. Dose-response curves were determined for the benzodiazepine anxiolytic and amnestic agent diazepam (DZP, 0.5-4 mg/kg), as well as for ipsapirone (IPS, 0.25-2 mg/kg), an azapirone anxiolytic that is devoid of clinically significant amnestic effects. The doses of 1, 2, and 4 mg/kg DZP and of 1 and 2 mg/kg IPS impaired inhibitory avoidance, an effect that may be viewed as anxiolytic. Inhibitory avoidance remained impaired 3 days later in the rats treated with 1-4 mg/kg DZP, indicating anterograde amnesia. This effect was not due to state-dependent learning, because rats injected both at pretraining and pretesting with 2 mg/kg DZP still showed complete amnesia. In contrast, the doses of 1 and 2 mg/kg IPS did not significantly affect memory, indicating a dissociation between the drug effects on anxiety and memory. Neither the performance of escape nor its memory was affected by DZP or IPS. Therefore, the two aversive tasks studied are likely to generate distinct types of fear/anxiety and memory, which may correlate with different classes of psychiatric disorders. The present results also warrant further exploration of the elevated T-maze as a potential model for the combined study of anxiety and memory.

Elevated T-maze Animal model Anxiety Memory Diazepam Ipsapirone

ANIMAL models of anxiety have been developed both to detect new antianxiety drugs and to study brain mechanisms underlying anxiety disorders (8,13,36). Nevertheless, until recently the main criterion for validation of an animal model of anxiety has been its sensitivity to benzodiazepine (BZD) anxiolytics. As a result, many of the existing models do not easily detect non-BZD anxiolytics (3,7,15,37). In addition, there is suggestive clinical evidence that anxiety disorders are not only heterogeneous in their manifestations, but also have different neurohumoral substrates. For instance, phobic and panic disorders are not ameliorated by anxiolytic doses of BZDs, which are effective on generalized anxiety disorder (24). Therefore, there is a clear need for new animal models that are sensitive to non-BZD anxiolytics and/or that address specific types of anxiety disorders, including those resistant to BZDs.

Animal models of anxiety necessarily involve interfering psychobiological processes, such as motor ability, motivation (deprivation of food and/or water), perception (often of painful stimuli), learning, and memory (14). Deprivation and pain can be eliminated by using ethologically based aversive situations, such as the elevated plus-maze (16,26) and the light-dark transition (4) models. Also, many tests provide independent indexes of motor activity [e.g., the number of total or enclosed arm entries in the elevated plus-maze (8)]. However, it is remarkable that cognitive factors are not usually assessed. In particular, punishment or conflict models and inhibitory (passive) avoidance tests clearly involve learning and memory.

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Indeed, inhibitory avoidance is a widely used task for studying memory mechanisms (1). In addition, a wealth of experimental evidence indicates that the brain structures involved in anxiety and in the modulation of memory, particularly emotional memory, overlap extensively (33). Therefore, there are good reasons for studying anxiety and memory together.

The present model represents an attempt to overcome several of the above shortcomings. To avoid interference of pain and deprivation, the starting point was the elevated plus-maze (26). However, because this model apparently generates different types of fear/anxiety, resulting in complex variability of drug effects (15), the apparatus was modified by closing the entrance to one of its enclosed arms, resulting in an elevated T-maze. To separate learned from unlearned fear, both inhibitory avoidance of the open arms and one-way escape from one of the open arms were measured in each animal, after drug or control injection. Inhibitory avoidance and one-way escape behaviors were measured again 3 days later for assessing memory. A preliminary report on the effect of diazepam in this model has been published (11).

In the present study, dose-response curves for two anxiolytic drugs were determined. The first drug was diazepam (DZP), a prototypical BZD compound that can induce amnesia when clinically used, particularly at high doses (19). The second was ipsapirone (IPS), a 5-HT_{1A} partial agonist of the azapirone class reported to improve generalized anxiety disorder without causing many of the side effects of the BZDs, among which is amnesia (21,28).

METHOD

Animals

Male Wistar rats, 250–300 g in weight, were housed in groups of five with food and water freely available. Lights were on from 0600 to 1800 h.

Apparatus

The elevated T-maze was made of wood and had three arms of equal dimensions (50 × 10 cm). One arm, enclosed by 40-cm high walls, was perpendicular to two opposed open arms. To avoid the rats falling down, the open arms were surrounded by a Plexiglas rim 1 cm high. The whole apparatus was elevated 50 cm above the floor. The experiments were performed with an observer inside the room.

Drugs

Diazepam (Roche, Brazil) was suspended in distilled water with 2% Tween 80. Ipsapirone hydrochloride (Bayer, Germany) was dissolved in distilled water. The drugs were prepared on the same day of the experiments and injected, IP, in a volume of 1 ml/kg body weight.

Procedure

Experiment 1. On the third and fourth days after their arrival in the laboratory, animals were gently handled for 5 min. On the fifth day, they were randomly assigned to different treatment groups, and were given DZP (0.5, 1.0, 2.0, or 4.0 mg/kg), IPS (0.25, 0.5, 1.0, or 2.0 mg/kg), or control injection (Tween vehicle or distilled water, respectively). After 25 min, each rat was placed at the end of the enclosed arm of the T-maze and the time taken to withdraw from this arm with the four paws was recorded (baseline latency). Next, the same measurement was repeated in two subsequent trials (avoidance 1 and avoidance 2) at 30-s intervals. Following avoidance training (30 s), the rat was placed at the end of right open arm and the time taken to withdraw from the arm with the four paws was recorded (escape 1). Three days later, avoidance (avoidance 3) and escape (escape 2) latencies were measured again.

Experiment 2. To assess state-dependent learning (25), animals were randomly divided into four groups: 1) injected with vehicle both before training of inhibitory avoidance and before testing, 3 days later; 2) vehicle before training and DZP (2 mg/kg) before testing; 3) DZP before training and vehicle before testing; 4) DZP before training and DZP before testing.

Data Analysis

Because a cutoff time of 300 s was established for the avoidance latency, nonparametric statistical analysis was used for this type of data. Within-group comparisons along trials were made with Friedmann's test, whereas the tests of Kruskal–Wallis and Bonferroni were used to detect significant differences among different treatment groups within the same trial. Escape latencies were analyzed using two-factor (drug treatment and trial) analysis of variance (ANOVA). Significance level was set at $p < 0,05$. Rats that fell from the elevated T-maze were excluded from the analysis. This accounts for the variable n in the groups treated with DZP, because falls were more frequent after this drug.

RESULTS

Experiment 1

As illustrated in Fig. 1, DZP affected the performance of inhibitory avoidance both during acquisition and 3 days later, when memory was tested. The test of Friedmann showed significant changes in inhibitory avoidance latency along trials in the control group, $\chi^2(3) = 32.83, p < 0.001$, as well as in rats treated with 0.5 mg/kg of DZP, $\chi^2(3) = 21.72, p < 0.001$. This indicates good avoidance acquisition and memory. In the

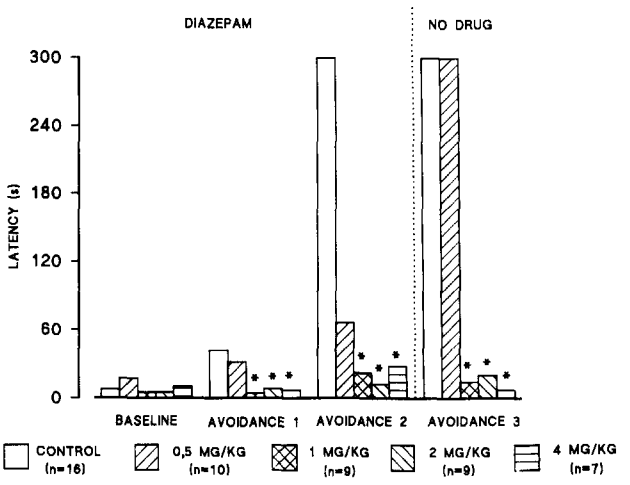

FIG. 1. Effect of diazepam (DZP) on inhibitory avoidance of open arms in the elevated T-maze. Bars represent the median. BASELINE, AVOID 1 and AVOID 2 were measured at 30-s intervals, beginning 25 min after IP injection of DZP or vehicle. AVOID 3 was measured 72 h later. The asterisk indicates significant difference from control.

remaining groups, latency to withdraw from the enclosed arm did not significantly change along trials, suggesting that no avoidance learning occurred. There were significant differences among treatment groups (Kruskal-Wallis test) at avoidance 1, $\chi^2(4) = 20.18$, $p < 0.001$, avoidance 2, $\chi^2(4) = 24.80$, $p < 0.001$, and avoidance 3, $\chi^2(4) = 30.40$, $p < 0.001$, meaning that performance was affected by the drug treatment at these trials. Multiple comparisons made with the Bonferroni test showed that the groups treated with 1, 2, and 4 mg/kg of DZP were significantly different ($p < 0.05$) from control.

In contrast to inhibitory avoidance, one-way escape was not affected by DZP (Fig. 2). Two-way ANOVA showed a significant effect of trials, $F(1, 46) = 15.60$, $p < 0.001$, on escape latency, indicating memory. However, neither the effect of drug treatment nor the drug × trial interaction was significant.

As illustrated in Fig. 3, the effect of IPS on inhibitory avoidance acquisition was qualitatively similar to that of DZP. However, in spite of a decreasing trend, no significant effect of IPS on the latencies measured 3 days later occurred (Fig. 3). The test of Friedmann showed a significant change in the latency of inhibitory avoidance along trials in the control group, $\chi^2(3) = 26.52$, $p < 0.001$, and in the groups treated with 0.25 mg/kg, $\chi^2(3) = 28.55$, $p < 0.001$, 0.5 mg/kg, $\chi^2(3) = 24.71$, $p < 0.001$, 1 mg/kg, $\chi^2(3) = 28.18$, $p < 0.001$, and 2 mg/kg, $\chi^2(3) = 21.30$, $p < 0.001$, of IPS, indicating that avoidance learning occurred in all groups. The Kruskal-Wallis test revealed significant differences among groups at avoidance 2, $\chi^2(4) = 12.18$, $p < 0.05$. In this trial, the test of Bonferroni showed significant differences ($p < 0.05$) for both 1- and 2-mg/kg groups, compared to control. Therefore, the only significant effect of IPS was to impair the performance of inhibitory avoidance at avoidance 2.

In the same way as with DZP, IPS did not affect escape from the open arm (Fig. 4). Two-way ANOVA showed only a significant effect of trials, $F(1, 60) = 27.34$, $p < 0.001$.

Experiment 2

The results of this experiment, illustrated in Fig. 5, do not support state-dependent learning. The test of Kruskal-Wallis showed overall differences among treatment groups, $\chi^2(3) = 20.29$, $p < 0.001$, whereas Bonferroni's test revealed significant differences ($p < 0.05$) between the control group (vehicle-vehicle) and the groups treated with DZP-vehicle and with DZP-DZP. This indicates that memory of the task was impaired when DZP was given during training, irrespective of the test being carried out under DZP or vehicle. On the other hand, memory was present when animals that had received vehicle injection during training were tested under DZP.

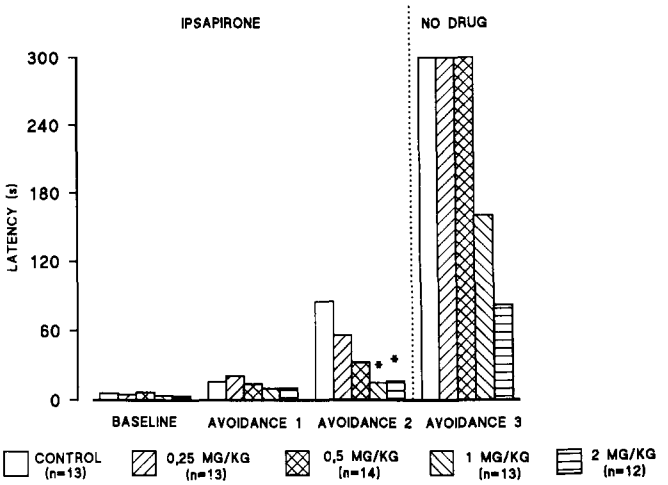

FIG. 3. Effect of ipsapirone (IPS) on inhibitory avoidance of open arms in the elevated T-maze. Bars represent medians. Measurements were taken as described in the legend of Fig. 1. Asterisks indicate significant difference from control.

DISCUSSION

The present results show that inhibitory avoidance was sensitive to acute administration of both a BZD and a non-BZD anxiolytic. Using a procedure in which only one avoidance trial was performed on the first day, it had already been shown that DZP impaired inhibitory avoidance performance (11).

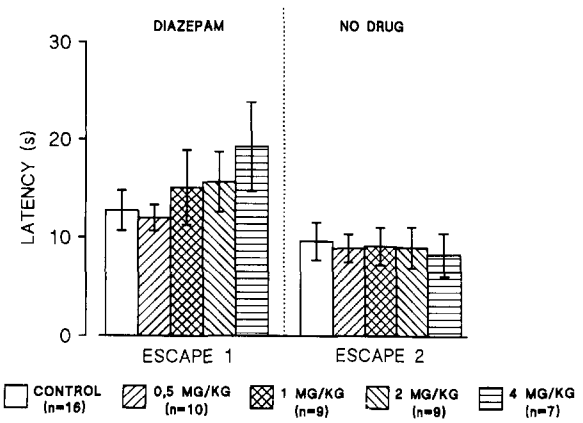

FIG. 2. Absence of DZP effect on escape from the open arm of the elevated T-maze. Bars represent the mean for groups of 7-16 rats. Vertical lines represent ± SEM. ESCAPE 1 was measured immediately after inhibitory avoidance training (see legend of Fig. 1). ESCAPE 2 was measured 72 h later.

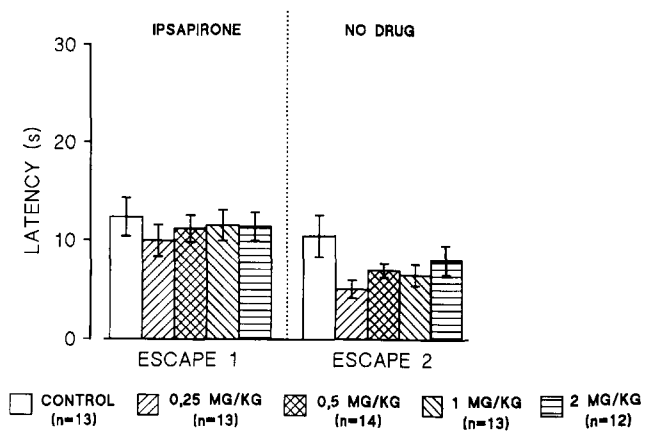

FIG. 4. No effect of IPS on escape from the open arm of the elevated T-maze. Bars represent the mean for groups of 12-14 rats. Vertical lines represent ± SEM. Measurements were taken as described in the legend of Fig. 2.

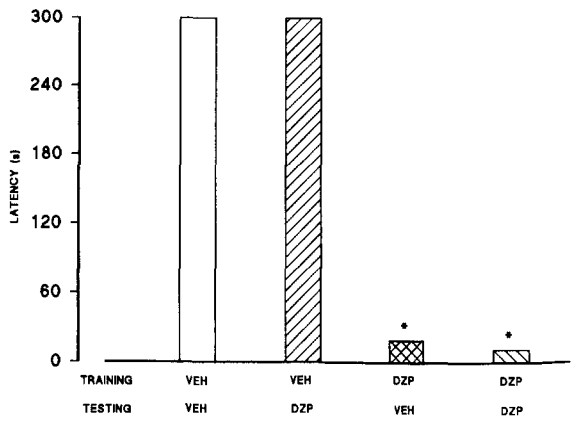

FIG. 5. Lack of state-dependent learning of inhibitory avoidance under diazepam. TRAINING refers to the first experimental session and TESTING to the session made 3 days later (see legend of Fig. 1). Only the latency measurements taken during the test session are shown. VEH indicates IP injection of vehicle and DZP of 2 mg/kg diazepam. Bars represent the median of 12 rats per group. Injections were made 25 min before either training or testing. Asterisks indicate significant difference from control (VEH + VEH).

Nevertheless, the present addition of a second avoidance trial not only made the effect of DZP more clear, but allowed the detection of the IPS effect, which became significant only at the second avoidance trial (Fig. 3). The positive result with IPS is particularly interesting because in many animal models of anxiety this and other azapirones (e.g., buspirone, gepirone) were ineffective or even had anxiogenic effects (3,7, 14,15). In agreement with the present results, however, acute IPS has been shown to decrease inhibitory avoidance latency in a conventional step-down inhibitory avoidance test (35). It may be argued that these results represent false positives, because the clinical anxiolytic effects of azapirones only appear after several days or even weeks of continuous administration (28). Nevertheless, in human experimental models of anxiety using healthy volunteers, acute anxiolytic effects of azapirones have been demonstrated. For example, buspirone accelerated extinction of skin conductance responses to a conditioned aversive sound stimulus (5), and IPS attenuated the increases in subjective anxiety as well as in arterial blood pressure induced by speaking in front of a video camera (40).

The present results also evidence an amnestic effect of DZP in rats tested without drug 3 days after inhibitory avoidance acquisition. These results agree with a wealth of published evidence indicating that BZDs induce anterograde amnesia in laboratory animals as well as in human beings (19,30). Nevertheless, because the doses 1-4 mg/kg DZP affected acquisition, the impairment of inhibitory avoidance in the test session could be due to lack of learning during training. However, results of recent studies in which rats were trained to criterion in an inhibitory avoidance task demonstrated that administration of DZP causes amnesia when acquisition is not impaired (29,31,32,34). These results also suggest that the lack of inhibitory avoidance acquisition presently observed after DZP is not due to short-term memory impairment and, thus, reflects anxiolysis. Nevertheless, it should be kept in mind that BZDs have been shown to disrupt short-term working and reference memory (17,38).

The present results showing that only the doses of DZP that were anxiolytic caused amnesia concur with previously reported data indicating that the anxiolytic and the amnestic properties of the drug are interrelated (33). In particular, different research groups have shown that the anxiolytic (anticonflict) and the amnestic effects of BZDs are localized at the same site in the amygdaloid complex, the anterolateral/basolateral amygdala (18,27,32,34). In contrast, the results presently obtained with IPS suggest that a dissociation between the anxiolytic and the amnestic effect of this drug occurred. Indeed, the two doses (1 and 2 mg/kg) of IPS that impaired inhibitory avoidance in the first session did not significantly affect memory on the test day. Nevertheless, a clear trend for a dose-dependent decrease in avoidance latency may be seen in Fig. 3. Therefore, the difference between DZP and IPS could be only quantitative. To test this hypothesis, an extra group of 15 rats was given 4 mg/kg IPS, but the avoidance latency observed on the test session (median = 300 s, interquartile interval 15-300 s) reversed the decreasing trend shown in Fig. 3. On the other hand, favoring the dissociation hypothesis, there is no compelling evidence for an anterograde amnestic effect of azapirones (20).

Although results obtained in one laboratory suggest state-dependent learning for BZDs [see, e.g., (23)], others were unable to reproduce this phenomenon (2,6,9). The present results add to the negative evidence, because they clearly show that animals given an anxiolytic dose of DZP before training of inhibitory avoidance had complete amnesia during testing, irrespective of being under saline or DZP. Conversely, if the rats had acquired the inhibitory avoidance under saline, they displayed good memory even when DZP was given before the test. The last result is also interesting because it means that, once acquired, inhibitory avoidance behavior is resistant to BZDs. This has at least two implications: 1) it indicates that this measure of memory is little influenced by anxiety (at least BZD-sensitive anxiety); 2) it also suggests that inhibitory avoidance is different from the apparently similar punishment (conflict) test, because with the latter BZDs are fully effective in overtrained animals [see, e.g., (10)].

Finally, the present results show that one-way escape from the open arm of the T-maze was not affected by the same doses of DZP and IPS that significantly impaired inhibitory avoidance. Similarly, one-way escape from foot shock is resistant to classical anxiolytics, unless very high doses that nonspecifically depress motor behavior and decrease vigilance are given. As a consequence, escape has been disregarded as an animal model of anxiety (12). However, there are reasons to reconsider this position, because one-way escape is likely to be motivated by fear. This is mostly clear in the present model, in which no pain is involved and an innately feared situation—being on the elevated open arm of the maze (22)—is used instead of electric shock. A likely possibility is that BZDs basically impair the ability of animals to withhold a highly probable response, being ineffective in anxiety models based on response emission [see (14) for a discussion]. In addition, modern classification of anxiety disorders includes conditions, such as phobias and panic disorder, that are not improved by BZDs, at least when anxiolytic doses of these drugs are given (24). Therefore, it may be worth investigating further whether escape from the open arm addresses to one of such conditions.

Still concerning one-way escape, the present results have additionally shown that memory of this task was not impaired by the doses of DZP that, in the same animals, affected inhibitory avoidance. Thus, different types of memory seem to exist, each having specific underlying brain mechanisms. Indeed,

one of us has recently demonstrated that the amnestic effects of DZP are mediated, at least in part, through influences involving the amygdaloid complex (32). It is well established that amygdala lesions attenuate the expression of emotional behavior and memory whereas its integrity does not seem to be required for other types of memory [see, e.g., (39)]. Thus, one could argue that memories for avoidance conditioning are mediated by BZD-sensitive brain areas whereas memories for escape behaviors are modulated by other brain regions, not sensitive to BZDs.

Overall, the present study suggests that a good correlation may exist between the anxiolytic and amnestic effects seen in the elevated T-maze and those observed in clinical situations. Therefore, further exploration of the elevated T-maze as a potentially useful model for the simultaneous study of anxiety and memory is justified.

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