Pharmacology Biochemistry and Behavior, Vol. 44, pp. 463–469, 1993

0091-3057/93 $6.00 + .00

Anxiogenic Stimuli in the Elevated Plus-Maze

DALLAS TREIT,[1] JANET MENARD AND CARY ROYAN

Department of Psychology, University of Alberta, Edmonton, Alberta T6G 2E9, Canada

Received 28 May 1992

TREIT, D., J. MENARD AND C. ROYAN. *Anxiogenic stimuli in the elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **44**(2) 463–469, 1993. – Untreated rats normally avoid the open arms of the "elevated plus-maze," preferring instead the closed arms, whereas rats treated with antianxiety drugs (e.g., diazepam) show far less open-arm avoidance. Although it has often been assumed that rats avoid the open arms because of novelty, height, or open space, the anxiogenic role of these stimuli in the plus-maze has not been systematically examined. In Experiment 1, rats were repeatedly exposed to the elevated plus-maze with the expectation that their "fear" of the open arms would habituate over trials. Instead, open-arm avoidance actually increased on the second trial and showed no evidence of habituating after 18 trials. In Experiment 2, three 30-min sessions of confinement to the open arms ("flooding") failed to decrease rats' open-arm avoidance. Instead, rats that had received flooding avoided the open arms significantly more than control rats during the first test. Experiment 3 showed that although diazepam-treated rats avoided the open arms less than vehicle-controls on the first test this difference dissipated across test trials. Further, diazepam had no carryover effect on rats' subsequent avoidance of the open arms in a nondrugged state. In Experiment 4, plus-maze height was varied from 50 to 6 cm, but rats did not display more open-arm activity as maze height decreased. In Experiment 5, height cues were manipulated by placing a "floor" 8 cm beneath one open arm while leaving the floor of the other open arm at 50 cm. Rats did not avoid the "low" open arm less than the "high" open arm. In Experiment 6, rats explored an open arm with a raised Plexiglas edge more than an open arm with standard flat edges despite identical height cues under these arms. Thus, it appears that open space, rather than height or novelty, is the anxiogenic stimulus in the elevated plus-maze.

Elevated plus-maze Anxiogenic stimuli Habituation Diazepam Height Thigmotaxis Animal models

RATS normally avoid the two open arms of the elevated plus-maze and instead stay in the two enclosed arms of the maze (3,16,26,27,32). When rats are forced to stay in the open arms, they show fear reactions such as freezing, defecation, and increased plasma corticosteroids (26). Standard anxiolytic drugs, such as diazepam, increase both the percentage of entries rats make into the open arms of the maze and the percentage of time they spend in the open arms (26,27). Drugs from other therapeutic classes (e.g., antidepressants) either have no specific effect on open-arm activity or suppress open-arm activity below baseline control levels. [For summaries of methods and results, see (25,35)].

Although it is clear that rats find the open arms of the plus-maze aversive, the precise source of this aversion has not been determined. One possibility is that the novelty of the plus-maze is aversive, in particular the novelty of the open arms (21). Another possibility is that rats are afraid of the height cues associated with the open arms of the maze (25). Finally, it is possible that the open spaces surrounding the open arms of the plus-maze are anxiogenic for rats (26).

Although any (or all) of these factors could motivate rats' open-arm avoidance, the relative contribution of each these factors to rat "anxiety" in the plus-maze has not been systematically studied. This is somewhat surprising given the widespread use of this test as an animal model for studying novel anxiolytic drugs and the neurobiologic mechanisms of anxiety (4,6,7,17,22,23,27,28,37). Thus, the purpose of the present studies was to systematically investigate novelty, height, and open spaces as putative anxiogenic stimuli in the elevated plus-maze.

EXPERIMENT 1

Pellow et al. (26) exposed rats to the elevated plus-maze on each of three daily 5-min test trials. Their expectation was that rats' open-arm avoidance, like other unconditioned aversions to novel stimuli, would habituate rapidly (2,5,10). Although rats' open-arm avoidance did not habituate in this experiment, Pellow et al. cautioned that three daily trials may not have been sufficient for habituation to occur. The purpose

[1] To whom requests for reprints should be addressed.

of the present experiment was to determine whether more extensive exposure to the elevated plus-maze would result in habituation of rats' open-arm avoidance.

METHOD

Subjects

Subjects were 10 naive, male Sprague-Dawley rats weighing 195–285 g. Rats were individually housed in polycarbonate cages, under a 12 L : 12 D cycle, with food and water available ad lib. Rats were tail-marked and handled for 4–6 days prior to testing. Testing occurred between 1000 and 1200 h.

Apparatus

The apparatus was a wooden, plus-shaped maze, elevated to a height of 50 cm. Two opposite arms were open (50 × 10 cm) and the other two opposite arms were enclosed with walls (50 × 10 × 40 cm). The junction of the four arms measured 10 × 10 cm.

Procedure

On each of 18 consecutive days, rats were placed individually in the center of the plus-maze facing a closed arm and allowed 5 min of free exploration. An observer, sitting quietly 1 m from the maze, measured a) total time in the open arms, b) total time in closed arms, c) number of entries into open arms, and d) number of entries into closed arms. An entry was defined as all four paws in the arm. The maze was cleaned with water after each rat was tested.

For the purpose of analysis, open-arm activity was quantified as a) time spent in the open arms relative to the total time spent in the maze (open/total × 100) and b) number of entries into open arms relative to the total number of entries into any arm (open/total × 100). Habituation would be indicated by a significant increase in the percentage of open-arm activity across trials.

RESULTS AND DISCUSSION

As can be seen in Fig. 1, there was no substantial increase in rats' open-arm activity, even after 18 test trials. Repeated-measures analysis of variance (ANOVA) showed no significant change in percentage of time spent in open arms, $F(17, 153) = 1.56, p > 0.05$. The ANOVA for percentage of open-arm entries was marginally significant, $F(17, 153) = 1.91$, $p < 0.05$, but a subsequent t-test showed that the percentage of open-arm entries actually decreased between the first and last trials, $t(9) = 2.24$, $p < 0.05$. These results suggest that rats' open-arm avoidance does not habituate after repeated exposures to the plus-maze. If anything, rats appear to become more fearful of the open arms after the first trial [see also (18)].

EXPERIMENT 2

Although habituation of open-arm avoidance was not apparent in Experiment 1 after 18 test trials, it could be argued that rats did not habituate because they were not sufficiently exposed to the open arms. This could occur either because rats were not given enough trials or because, regardless of the number of trials, rats could easily avoid exposure to the open arms and thus habituation might not occur. The latter possibility was explored in Experiment 2 by using a forced-exposure ("flooding") procedure (20).

METHOD

Methods were basically the same as those used in Experiment 1 with the following exceptions. Twenty naive, male Sprague-Dawley rats (265–345 g) were handled and randomly assigned to two groups ($n = 10$). Rats in the experimental

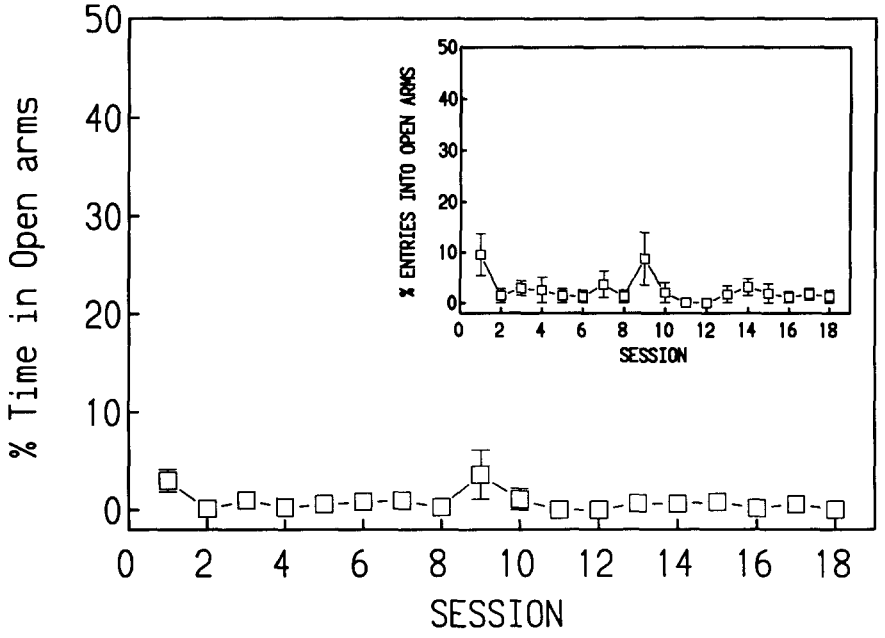

FIG. 1. Mean (±SEM) percentage of time in and entries into (inset) the open arms of the plus-maze across the 18 trials of Experiment 1.

group were individually confined to an open arm of the plus-maze for 30 min on each of 3 consecutive days (forced exposure), whereas rats in the control group were given equivalent handling but no forced exposure. On each of the 3 following days, all animals were given a 5-min, free-choice test in the standard plus-maze.

RESULTS AND DISCUSSION

Figure 2 illustrates that forced exposure to the open arms (flooding) failed to attenuate open-arm avoidance on subsequent standard test trials. In fact, rats that received the flooding treatment showed lower levels of open-arm activity than nontreated controls on the first trial [see also (16)]. Group × trials ANOVA of percentage of open-arm entries revealed a significant effect of trials, $F(2, 36) = 7.80$, $p < 0.002$, and a significant group × trials interaction, $F(2, 36) = 8.21$, $p < 0.002$. The main effect for group was not significant, $F(1, 18) = 0.06$, $p > 0.5$. A similar ANOVA of percentage of open-arm time produced the same pattern of results [trials, $F(2, 36) = 4.96$, $p < 0.02$; group × trials, $F(2, 36) = 9.61$, $p < 0.0007$; and group, $F(1, 18) = 0.15$, $p > 0.5$]. The only significant pair-wise comparisons between groups were on the first test trial, when the open-arm activity of control rats was higher than that of experimental rats [percentage time, $t(18) = 2.16$, $p < 0.05$; percentage entries, $t(18) = 2.00$, $p < 0.06$].

EXPERIMENT 3

Free or forced exposure to a anxiogenic stimulus is often an effective behavioral treatment for fears and phobias in both humans and lower animals (8,20). Thus, it is surprising that free or forced exposure in the elevated plus-maze failed to reduce rats' fear of the open arms. One trivial explanation of our negative results is that, for some reason, our test procedures are insensitive to variables that normally suppress rat anxiety in the elevated plus-maze. Thus, the main purpose of Experiment 3 was to replicate the widely demonstrated anxiolytic effect of diazepam on rats' open-arm avoidance (3,15, 22,26,32) and thereby confirm the sensitivity of our test procedures to antianxiety agents. Another purpose was to examine the longevity of anxiolytic drug effects across repeated test trials and determine whether diazepam would have any long-lasting effect on rats' subsequent avoidance of the open arms in a nondrugged state.

METHOD

Methods were similar to those used in the previous experiments with the following exceptions. Twenty naive, male Sprague-Dawley rats, weighing 270–330 g, were randomly assigned to two groups ($n = 10$). On each of three daily drug trials, experimental rats received an injection of diazepam (2 mg/kg, IP) 30 min prior to a standard 5-min plus-maze test. This dose of diazepam produces clear anxiolytic effects in the elevated plus-maze (15,22,26). Control rats were treated in the same way except they received vehicle injections. After the last drug trial, all injections stopped and both groups of rats received three more daily test trials in the plus-maze.

RESULTS AND DISCUSSION

Although diazepam-treated rats showed substantially higher percentages of open-arm activity than vehicle-treated rats in the first drug trial, this difference virtually disappeared by the third drug trial and was not apparent on any of the three subsequent nondrug trials (see Fig. 3). ANOVAs revealed significant effects of drug, trials, and drug × trial interaction for both measures of open-arm activity [percent entries: drug, $F(1, 18) = 11.69$, $p < 0.003$; trials, $F(5, 90) = 26.96$, $p < 0.001$; drug × trials, $F(5, 90) = 11.91$, $p < 0.001$; percent time: drug, $F(1, 18) = 14.71$, $p < 0.001$; trials, $F(5, 90) = 22.41$, $p < 0.001$; drug × trials, $F(5, 90) = 11.16$, $p < 0.001$]. Pair-wise comparisons confirmed that the percentage of time diazepam-treated rats spent in the open arms was significantly greater than that of vehicle-treated rats on the first drug trial, $t(18) = 4.09$, $p < 0.001$, as was the percentage of open-arm entries, $t(18) = 5.57$, $p < 0.001$. However, there was no significant difference between the two groups on either measure of open-arm activity by the third drug trial [percent time, $t(18) = 1.46$, $p > 0.1$; percent en-

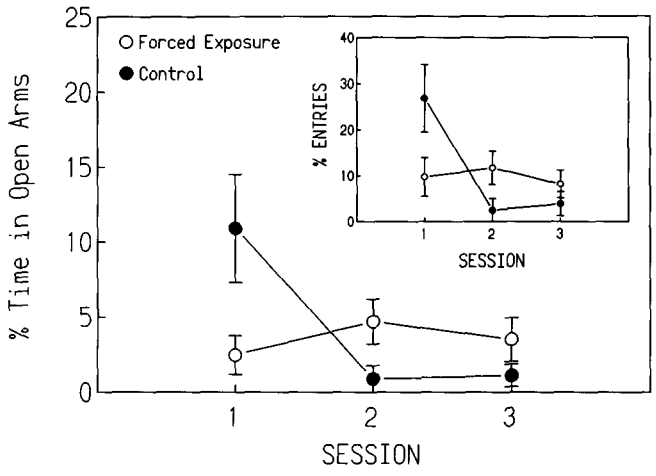

FIG. 2. Mean (±SEM) percentage of time spent in and entries into (inset) the open arms of the plus-maze by rats previously given forced exposure to the open arms (○) or no exposure (●) across the three test trials of Experiment 2.

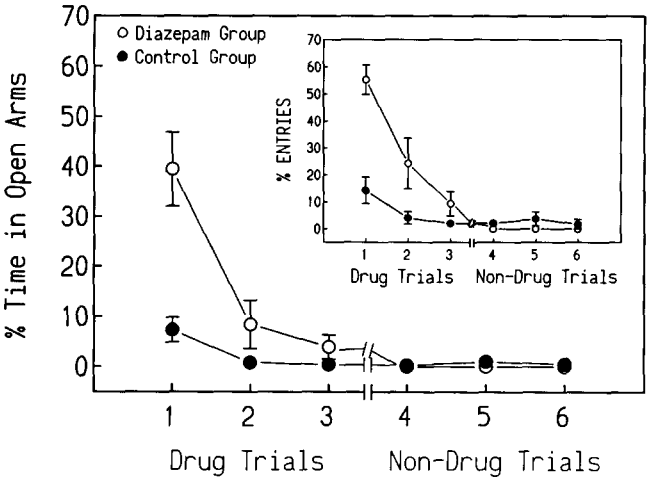

FIG. 3. Mean (±SEM) percentage of time spent in and entries into (inset) the open arms of the plus-maze by rats in the diazepam group (○) and control group (●) across the drug trials (1–3) and nondrug trials (4–6) of Experiment 3.

tries, $t(18) = 1.52, p > 0.1$. There were no significant differences between the two groups on any of the three nondrug trials. These results suggest that the anxiolytic effect of diazepam in the elevated plus-maze is transient even during drug trials and does not carry over to nondrug trials. Nevertheless, the first-trial drug data provide pharmacological confirmation of the sensitivity of our procedures for detecting anxiolytic effects in the elevated plus-maze.

EXPERIMENT 4

Although rats' open-arm avoidance is sensitive to the acute effects of anxiolytic drugs, it appears to be impervious to other treatments that ordinarily reduce novelty-related anxiety (e.g., habituation, flooding). These results suggest that novelty per se is not an important anxiogenic stimulus in the elevated plus-maze.

The next two experiments examined an alternative source of anxiety in the plus-maze, that is, height. In this regard, Barnett (1) reported that both laboratory- and wild-reared rats display an unconditioned aversion to heights. This observation was supported by the early work of Gibson and associates (12,38), who found that rats in the middle of a "visual cliff" apparatus avoided any side on which apparent depth was created. These results suggest that height is an anxiogenic stimulus for rats in general, and in particular it may be an anxiogenic stimulus for rats in the elevated plus-maze (26). However, the hypothesis that height is an anxiogenic stimulus in the elevated plus-maze has not been systematically tested. If height is an anxiogenic stimulus, one would expect that rats' open-arm avoidance would vary as a function of maze height. Experiment 4 tested this prediction.

METHOD

The methods were similar to those used in previous experiments. Subjects were 30 naive, male Sprague-Dawley rats (250–350 g). Rats were randomly assigned to one of three groups ($n = 10$) tested at maze heights of either 50 cm (the standard height), 25 cm, or 6 cm. If height is an anxiogenic stimulus in the plus-maze, there should be a significant increase in open-arm activity as maze height is decreased.

RESULTS AND DISCUSSION

Figure 4 shows that there was no systematic increase in open-arm activity as maze height decreased. There were no significant differences between groups in percentage of open-arm time, $F(2, 27) = 0.73, p > 0.5$, or in percentage of open-arm entries, $F(2, 27) = 1.35, p > 0.2$.

EXPERIMENT 5

Although the results of Experiment 4 were clearly negative, it seemed possible that a different method of manipulating height might show that it is anxiogenic in the elevated plus-maze. This method was explored in Experiment 5.

METHOD

Thirty naive, male Sprague-Dawley rats (250–360 g) served as subjects, and the plus-maze was elevated to the standard height (50 cm). However, a sheet of hardboard (120 × 120 cm) was used to adjust the depth of the floor under the two open arms, that is, the "floor" was raised to within 8 cm of one of the open arms or lowered to the standard depth of 50 cm on the opposite open arm. The hardboard floor passed completely under the center of the plus-maze and extended 50 cm beyond each of the three sides of the open arm.

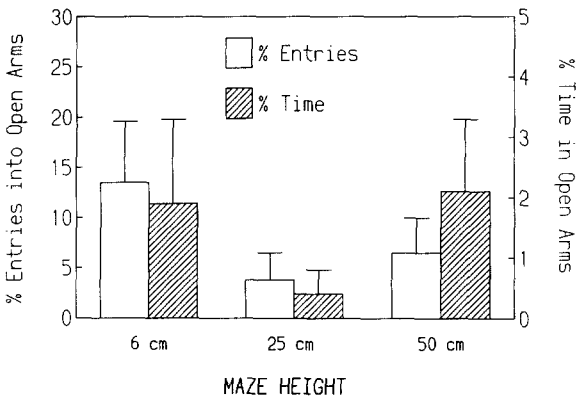

FIG. 4. Mean (±SEM) percentage of entries into the open arms (left axis; open bars) and mean (±SEM) percentage of time in the open arms (right axis; hatched bars) for rats in Experiment 4 tested at maze heights of 6, 25, and 50 cm.

All 30 animals were individually placed on the center of the plus-maze and allowed to freely explore the maze for 5 min. The depth under each of the two open arms was alternated after every fifth subject. The experimenter scored the number of entries into each of the two open arms (the open arm 50 cm from the floor and the open arm 8 cm from the floor) and the time spent on each of the two open arms. If rats are afraid of height cues associated with the open arms of the plus-maze, then they should explore the open arm 50 cm above the floor less than the open arm 8 cm above the floor.

RESULTS AND DISCUSSION

Contrary to expectations, Fig. 5 shows that rats spent more time in and made more entries into the open arm 50 cm above the floor than the open arm only 8 cm above the floor. Although the difference in entries was not significant, $F(1, 29) = 2.83, p > 0.05$, the difference in time was, $F(1, 29) = 4.50, p < 0.05$. These results are clearly inconsistent with the

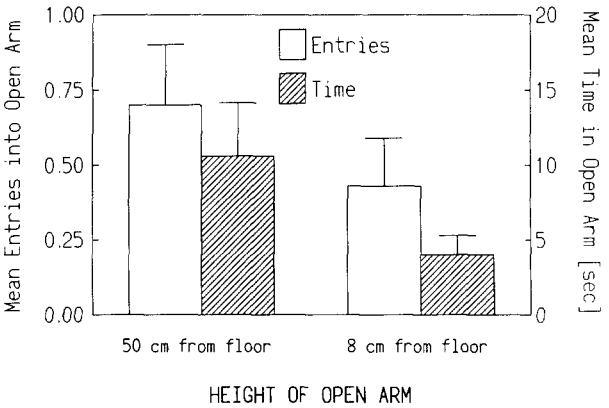

FIG. 5. Mean (±SEM) entries into (left axis; open bars) and time spent in (right axis; hatched bars) the high and low open arms in Experiment 5.

hypothesis that height is an anxiogenic stimulus for rats on the elevated plus-maze.

EXPERIMENT 6

The results of the previous experiments suggest that neither novelty nor height are strongly anxiogenic stimuli for rats in the elevated plus-maze. The remaining alternative is that rats avoid the open arms of the maze because they are "afraid" primarily of the open space around the open arms rather than the novelty or height of the open arms. Consistent with this idea, Barnett (1) reported that rats have a strong tendency to avoid open spaces, where they are more exposed to predation, and instead prefer to stay near vertical surfaces. Others have argued that this "positive thigmotaxis" is part of the rodent's natural defensive repertoire (14). More recently, it has been shown that thigmotaxis can be reduced by anxiolytic drugs (36).

In view of rats' thigmotaxic tendencies, it seems reasonable to assume that one reason rats avoid exploration of the open arms of the elevated plus-maze is because these arms lack a vertical surface that could support thigmotaxic behavior. Experiment 6 was designed to test this assumption.

METHOD

Twenty-two naive, male Sprague-Dawley rats (250–375 g) served as subjects. The apparatus was a standard plus-maze elevated to 50 cm. However, a clear sheet of Plexiglas (13 × 50 cm) was secured vertically along one edge of one of the open arms, whereas the other open arm had standard, flat edges. The raised Plexiglas edge was alternated to the opposite open arm after every fifth subject.

During test trials, each animal was allowed to freely explore the maze for 5 min. The experimenter recorded the number of entries into each of the two open arms (i.e., standard flat-edge arm and raised Plexiglas-edge arm) and the time spent on each of the two open arms. If rats avoid the open arms of the standard plus-maze because these arms do not allow thigmotaxic behavior, then they should avoid the open arm with the standard flat edges more than the open arm with the Plexiglas edge.

RESULTS AND DISCUSSION

Figure 6 shows that rats made more entries into, and spent more time on, the Plexiglas-edge open arm than the standard, flat-edge open arm [entries, $F(1, 21) = 10.65$, $p < 0.003$; time, $F(1, 21) = 7.81$, $p < 0.01$]. Because the height cues under the two open arms were identical, and each open arm was equally novel, it seems likely that rats' preference for the Plexiglas-edge open arm was driven by their thigmotaxic bias. It also suggests that rats in the standard plus-maze avoid the open arms not because of the height or novelty of these arms but because they do not permit rats to engage in thigmotaxic behavior.

GENERAL DISCUSSION

In Experiment 1, repeatedly exposing rats to the plus-maze did not diminish their avoidance of the open arms. Instead, open-arm avoidance actually increased between the 1st and 18th trials. In Experiment 2, confining rats to the open arms (flooding) for 30 min on each of 3 consecutive days also failed to diminish their open-arm avoidance on subsequent tests in the standard plus-maze. In fact, rats previously confined to the open arms (flooding) entered these arms less often and

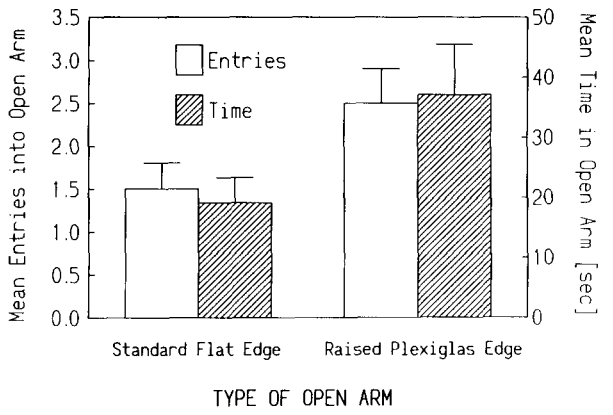

FIG. 6. Mean (±SEM) entries into (left axis; open bars) and time spent in (right axis; hatched bars) the Plexiglas-edge and the standard-edge open arms in Experiment 6.

spent significantly less time there than nontreated controls in a subsequent test. In Experiment 3, the sensitivity of our apparatus and test procedures was validated: Diazepam-treated rats showed significantly more open-arm activity than vehicle-treated rats in the first drug trial. However, this drug effect was no longer evident by the third drug trial, and no differences between the two groups were apparent in the three subsequent nondrug trials.

In Experiment 4, rats tested in a plus-maze lowered to a height of 6 cm did not display more open-arm activity than rats tested on plus-mazes elevated to heights of 25 or 50 cm, suggesting that height is not an anxiogenic stimulus in the plus-maze. In Experiment 5, the floor underneath the plus-maze was either raised to within 8 cm of one of the open arms of the maze or lowered to the standard depth of 50 cm. Rats did not selectively avoid the open arm on the "deep" side of this apparatus, suggesting again that fear of heights does not motivate rats' open-arm avoidance. Although it could be argued that rats could not discriminate between the height cues to which they were exposed in Experiments 4 and 5, this explanation seems unlikely for two reasons. First, in visual cliff experiments, Gibson et al. (12,38) reported that rats could discriminate between heights equivalent to those used in the present experiments (e.g., 8 vs. 25 cm). Second, some rats in Experiment 4 stepped off the maze when it was lowered to a height of 6 cm but none stepped off the maze when it was elevated to heights of 25 or 50 cm, suggesting that rats could discriminate between these maze heights. Although rats can apparently discriminate between these heights, height clearly did not motivate their avoidance of the open arms.

Experiment 6 assessed the possibility that the open space surrounding the open arms of the maze, rather than novelty or height, is what rats "fear" in this apparatus. When a Plexiglas edge was added to one of the open arms of the plus-maze, rats explored this arm more than the open arm with standard, flat edges. It is important to note that these two open arms were at identical heights and were equally novel to rats. Thus, the results of Experiment 6 suggest that rats' fear of the open arms is driven by thigmotaxis, a natural defensive response in which rats remain close to vertical surfaces, thereby shielding themselves from predators (1,14,36).

If fear of open spaces does account for rats' open-arm avoidance in the elevated plus-maze, however, it appears to

be resistant to antianxiety treatments. For example, repeated exposures to other "fearful" stimuli often result in a decrement or habituation of animals' fear responses to those stimuli (31,39). In contrast, rats in the first three experiments showed no signs of habituation with repeated test trials. Instead, they appeared to become more fearful of the open arms, even after forced exposure [see also (16,30)]. Further, the anxiolytic effect of diazepam did not persist beyond the first trial in Experiment 3 and was virtually abolished by the third drug trial. Other researchers (9,11,19,30) also found that even minimal previous exposure (either drugged or nondrugged) to the plus-maze abolished the anxiolytic effect of benzodiazepines in subsequent tests, a result that is consistent with Experiment 3 and cannot be easily explained in terms of simple drug tolerance (9,34). In any case, it is clear that the anxiolytic effects of benzodiazepines in the elevated plus-maze are short-lived, perhaps because open spaces are prepotent fear stimuli for rats.

It has been suggested that habituation of aversions is affected by the evolutionary significance the aversive stimulus has for an organism (29). For example, rats habituate at a slower rate and less completely to the sound of a rat's distress cry than to a pure tone (24). Further, repeated exposures to an intense or highly significant stimulus may strengthen, or sensitize, organisms' unconditioned fear reaction to that stimulus (13,33). Sensitization of fear is consistent with our data: Open-arm avoidance in general increased in the second trial of the first three experiments and showed no signs of habituating after either 18 trials or forced exposure. Further, sensitization might have accounted for the loss of diazepam's anxiolytic effect in Experiment 3. [For a similar argument, see (30)]. In any case, the results of the present experiments suggest that rats' fear of open spaces in the elevated plus-maze is surprisingly resistant to modification by variables that in general produce long-term reductions of other fear reactions in both animals and humans.

ACKNOWLEDGEMENTS

This research was supported by NSERC and AMHAC grants awarded to D.T. The authors thank Sandra Widgiz for assistance with the data collection for Experiment 6 and Marcia Spetch for helpful comments on the manuscript.

REFERENCES

1. Barnett, S. A. The rat: A study in behavior. Chicago, IL: Aldine; 1966.
2. Bodnoff, S. R.; Suranyi-Cadotte, B. E.; Quirion, R.; Meaney, M. J. Role of the central benzodiazepine receptor system in behavioral habituation to novelty. Behav. Neurosci. 103:209-212; 1989.
3. Brett, R. R; Pratt, J. A. Chronic handling modifies the anxiolytic effect of diazepam in the elevated plus-maze. Eur. J. Pharmacol. 178:135-138; 1990.
4. Briley, M.; Chopin, P.; Moret, C. Effects of serotonergic lesions on "anxious" behavior measured in the elevated plus-maze in the rat. Psychopharmacology (Berl.) 101:187-189; 1990.
5. Carlton, P. L.; Advokat, C. Attenuated habituation due to parachlorophenylalanine. Pharmacol. Biochem. Behav. 1:657-663; 1973.
6. Copland, A. M.; Balfour, D. J. K. Spontaneous activity and brain 5-hydroxindole levels measured in rats tested in two designs of elevated X-maze. Life Sci. 41:57-64; 1987.
7. Critchley, M. A. E.; Njung'e, K.; Handley, S. L. Actions and some interactions of 5-HT1a ligands in the elevated X-maze and effects of dorsal raphe lesions. Psychopharmacology (Berl.) 106: 484-490; 1992.
8. Emmelkamp, P. M. G. Behavior therapy with adults. In: Garfield, S. L.; Bergin, A. E., eds. Handbook of psychotherapy and behavior change. New York: Wiley; 1986:385-442.
9. File, S. E. One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. Psycopharmacology (Berl.) 100:281-282; 1990.
10. File, S. E.; Hyde, J. R. G. Can social interaction be used to measure anxiety? Br. J. Pharmacol. 62:19-24; 1978.
11. File, S. E.; Mabbutt, P. S.; Hitchcott, P. K. Characterization of the phenomenon of "one trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology (Berl.) 102:98-101; 1990.
12. Gibson, E. J.; Walk, R. D. The "visual cliff." Sci. Am. 210:64-71; 1960.
13. Graham, F. K. Habituation and dishabituation of responses innervated by the autonomic nervous system. In: Peeke, H. V. S.; Herz, M. J., eds. Habituation. New York: Academic Press; 1973: 163-218.
14. Grossen, N. E.; Kelley, M. J. Species-specific behavior and acquisition of avoidance behavior in rats. J. Comp. Physiol. Psychol. 81:307-310; 1973.
15. Handley, S. L.; Mithani, S. Effects of alpha2-adrenoceptor agonists and antagonists in a maze-exploration model of fear-motivated behavior. Naunyn Schmiedeberg Arch. Pharmacol. 327:1-5; 1984.
16. Itoh, J.; Nabeshima, T.; Kameyama, T. Utility of an elevated plus-maze for the evaluation of memory in mice: Effects of nootropics, scopolamine, and electroconvulsive shock. Psychopharmacology (Berl.) 101:525-532; 1990.
17. Kostowski, W.; Plaznik, A.; Setfanski, R. Intra-hippocampal buspirone in animal models of anxiety. Eur. J. Pharmacol. 168: 393-396; 1989.
18. Lee, C.; Rodgers, R. J. Antinociceptive effects of elevated plus-maze exposure: Influence of opiate receptor manipulations. Psychopharmacology (Berl.) 102:507-513; 1990.
19. Lister, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl.) 92:180-185; 1987.
20. Marks, I. M. Fears, phobias, and rituals. New York: Oxford University Press; 1987.
21. Montgomery, K. C. The relation between fear induced by novel-stimulation and exploratory behavior. J. Comp. Physiol. Psychol 48:254-260; 1958.
22. Moser, P. C. An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. Psychopharmacology (Berl.) 99: 48-53; 1989.
23. Moser, P. C.; Tricklebank, M. D.; Middlemiss, D. N.; Mir, A. K.; Hibert, M. F.; Fozard, J. R. Characterization of MDL 73005EF as a 5-HT1a selective ligand and its effects in animal models of anxiety: Comparison with buspirone, 8-OH-DPAT and diazepam. Br. J. Pharmacol. 99:343-349; 1990.
24. Peeke, H. V. S.; Zeiner, A. R. Habituation to environmental and specific auditory stimuli in the rat. Comm. Behav. Biol. 5:23-29; 1970.
25. Pellow, S. Anxiolytic and anxiogenic drug effects in a novel test of anxiety: Are exploratory models of anxiety in rodents valid? Meth. Find. Exp. Clin. Pharmacol. 8:557-565; 1986.
26. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Meth. 14:149-167; 1985.
27. Pellow, S.; File, S. E. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24:525-529; 1986.
28. Pesold, C.; Treit, D. Excitotoxic lesions of the septum produce anxiolytic effects in the elevated plus-maze and the shock-probe burying tests. Physiol. Behav. 52:37-47; 1992.

29. Petrinovich, L. A species meaningful analysis of habituation. In: Peeke, H. V. S.; Herz, M. J., eds. Habituation. New York: Academic Press; 1973:141-162.
30. Rodgers, R. J.; Lee, C.; Shepherd, J. K. Effects of diazepam on behavioural and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. Psychopharmacology (Berl.) 106:102-110; 1992.
31. Shalter, M. D. Predator-prey behavior and habituation. In: Peeke, H. V. S.; Petrinovich, L., eds. Habituation, sensitization and behavior. New York: Academic Press; 1984:349-391.
32. Taukulis, H. K.; Goggin, C. E. Diazepam-stress interactions in the rat: Effects on autoanalgesia and a plus-maze model of anxiety. Behav. Neural Biol. 53:205-216; 1990.
33. Thompson, R. F.; Groves, P. M.; Teyler, T. J.; Roemer, R. A. A dual-process theory of habituation: Theory and behavior. In: Peeke, H. V. S.; Herz, M. J., eds. Habituation. New York: Academic Press; 1973:239-271.
34. Treit, D. Evidence that tolerance develops to the anxiolytic effect of diazepam in rats. Pharmacol. Biochem. Behav. 22:383-387; 1985.
35. Treit, D. Anxiolytic effects of benzodiazepines and 5-HT1a agonists: Animal models. In: Rodgers, R. J.; Cooper, S. J., eds. 5-HT1a agonists, 5-HT3 antagonists and benzodiazepines: Their comparative behavioral pharmacology. New York: John Wiley; 1991:103-131.
36. Treit, D.; Fundytus, M. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol. Biochem. Behav. 31:958-962; 1988.
37. Treit, D.; Pesold, C. Septal lesions inhibit fear reactions in two animal models of anxiolytic drug action. Physiol. Behav. 47:365-371; 1990.
38. Walk, R. D.; Gibson, E. J.; Tighe, T. J. Behavior of light- and dark-reared rats on a visual cliff. Science 126:80-81; 1957.
39. Wyers, E. J.; Peeke, H. V. S.; Herz, M. J. Behavioral habituation in invertebrates. In: Peeke, H. V. S.; Herz, M. J., eds. Habituation. New York: Academic Press; 1973:1-57.