Pharmacology Biochemistry & Behavior, Vol. 35, pp. 121–126. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Latency to Enter a Mirrored Chamber: A Novel Behavioral Assay for Anxiolytic Agents

PAUL L. TOUBAS,* KATHLEEN A. ABLA,* WU CAO,* LANCE G. LOGAN*‡ AND THOMAS W. SEALE*†‡[1]

*Departments of *Pediatrics, †Biochemistry and ‡Psychiatry and Behavioral Sciences University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190*

Received 18 May 1989

TOUBAS, P. L., K. A. ABLA, W. CAO, L. G. LOGAN AND T. W. SEALE. *Latency to enter a mirrored chamber: A novel behavioral assay for anxiolytic agents.* PHARMACOL BIOCHEM BEHAV **35**(1) 121–126, 1990.—Many animal species exhibit approach-avoidance responses upon the novel placement of a mirror into an individual animal's environment. With a view toward identifying new behavioral measures with qualitatively or quantitatively different responses to anxiolytic agents, we developed a mirrored chamber apparatus for which adult male BALB/cByJ mice showed an extended latency to enter. Administration of diazepam significantly reduced this latency to enter a mirrored chamber in a dosage-dependent manner. The psychomotor stimulant, methylphenidate, had no effect on latency to enter the mirrored chamber at a dose which stimulated locomotor activity to the same extent as diazepam. Thus, the decreased latency to enter the mirrored chamber brought about by diazepam seems unlikely to reflect the motor effects of this benzodiazepine. The potency of diazepam was significantly lower in the mirrored chamber assay than it was on three other measures of exploratory activity—"head-dipping" performance, plus-maze performance and locomotor activity stimulation. The findings of our study indicate that the mirrored chamber method is simple to carry out, nonpunishing, rapid and quantitative and that it possesses pharmacological attributes which distinguish its response to anxiolytics from other assays of exploratory behavior.

Behavioral measures of anxiety Exploratory activity Anxiolytics Benzodiazepines Diazepam Amphetamine Cocaine Methylphenidate Responses to mirrors Inbred mice

HUMAN anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of an imagined or unreal threat, sometimes manifested by tachycardia, sweating, disturbed breathing, trembling or even paralysis (19). Fear is the emotional response accompanied by physical manifestations which results from exposure to a real, immediate danger. Definition of anxiety in animals is necessarily operational in nature. Validation of such definitions as truly representing an analog of human anxiety is difficult. The choice of animal models for anxiety studies is an important one because it may significantly affect the identification of biological and environmental factors causing both acute and chronic anxiety (1,2). Further, it could significantly affect the outcome of searches to discover new classes of anxiolytic compounds with potential therapeutic applications. Several different behavioral paradigms have been suggested to reflect pharmacologically sensitive anxiety levels in animals. These include a variety of tests related to exploratory activity [e.g., performance on the elevated plus-maze (10,13), the frequency of "head-dipping" in a hole-board test (10, 12, 17), movement transitions between a brightly lighted and a dark compartment (3)]. Other qualitatively different techniques employ punishment responses [such as the conflict test (1,18)] or social interaction behaviors (4,5). To the extent that such tests have led to the identification of clinically useful anxiolytics, they truly reflect, at least in part, some measure of relative anxiety state (2).

The chamber of mirrors procedure occurred to us as a measure of anxiety which might be qualitatively distinct from the other previously mentioned approaches. It is well established that a wide spectrum of vertebrate species show approach and withdrawal responses upon the placement of mirrors in their environment (6). Novel stimulation evokes both exploration and anxiety, and thereby generates an approach-avoidance conflict behavior. We hypothesized that distortion of the appearance of a readily traversed environment by a compartment constructed of mirrored glass might produce an aversion to entry that was quantitatively or qualitatively different from the anxiety states tapped by the plus-maze or the head-dipping assays. Further, response to an apparent animal or multiple animals reflected in the mirror might also be a source of anxiety (6). Here we report that the extended latency to enter the chamber of mirrors was dramatically affected

[1]Requests for reprints should be addressed to Dr. Thomas Seale, Department of Pediatrics, Room 2B300 CHO, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

by diazepam. This behavioral assay required significantly higher diazepam doses to elicit an effect than did the plus-maze assay of anxiety and did not exhibit the sharp biphasic dose responses of the head-dip behavioral assay.

METHOD

Animals

Experimentally naive male BALB/cByJ inbred mice (the Jackson Laboratory) weighing 27–32 g and 8 to 10 weeks of age were group housed (n = 5 per cage) in a climatically controlled environment (temperature 19–21°C) with a continuous 12-hour light-dark cycle. The litter used was hardwood chips (Sanichips, P. J. Murphy). Free access to a standard pelleted rodent food (Lab/Blox, Wayne) and water was given. Mice were allowed to recover from shipping trauma for at least one week before use.

Drug Source and Administration

Diazepam (a generous gift of Hoffmann-La Roche, Inc.) was dissolved in a 1:1 mixture by weight of dimethylsulfoxide (Fisher Scientific) and Emulphor (Emulphor El-620, GAF Corp.) and then was diluted with physiological saline solution to give a final composition of 30% dimethylsulfoxide-Emulphor to 70% saline. Diazepam solutions were prepared immediately before injection and were administered intraperitoneally in a volume of 0.1 ml per animal. This vehicle has been shown previously to be useful in administering compounds with low aqueous solubility (14–16). Diazepam was administered 10 minutes prior to the initiation of behavioral testing. Control animals received the dimethylsulfoxide-Emulphor-saline vehicle without diazepam. Methylphenidate hydrochloride was purchased from Sigma Chemical Company.

Latency to Enter the Chamber of Mirrors

The chamber of mirrors consisted of a mirrored cube open on one side which was placed into a square Plexiglas box (Fig. 1). The mirrored cube (30 by 30 by 30 cm) was constructed of 5 pieces of mirrored glass with one mirrored side and an opposite side painted dark brown. In the standard configuration, the mirrored surfaces (3 side panes, a top pane and the floor pane) faced the interior of the cube. Various combinations of mirrored and nonmirrored surfaces were also constructed. The container box, 40 by 40 by 30.5 cm, had a white floor and opaque black walls. Placement of the mirrored cube into the center of the container forms a 5 cm corridor completely surrounding the mirrored chamber. A sixth mirror was placed on the container wall positioned so that it faced the single open side of the mirrored chamber. Except for this one mirrored portion on the container wall, all portions of the container walls were black. Behavioral evaluations were carried out in a quiet room with fluorescent lighting (constant room luminance of 200 lux). Luminance in the corridor surrounding the mirrored chamber was measured to be 200 lux; within the mirrored compartment the luminance was 100 lux.

The procedure for the conduct of this behavioral evaluation was derived empirically. Group housed mice were brought into the room when the experiment was conducted and allowed to accommodate to the new environment for at least 30 minutes prior to initiation of an experiment. Mice were exposed to the chamber of mirrors and evaluated only a single time (to avoid problems with habituation). The focus of this method was to evaluate the latency to enter the chamber of mirrors from the surrounding corridor. To begin the evaluation, a single mouse previously injected with vehicle or diazepam was placed at a single, fixed starting point at

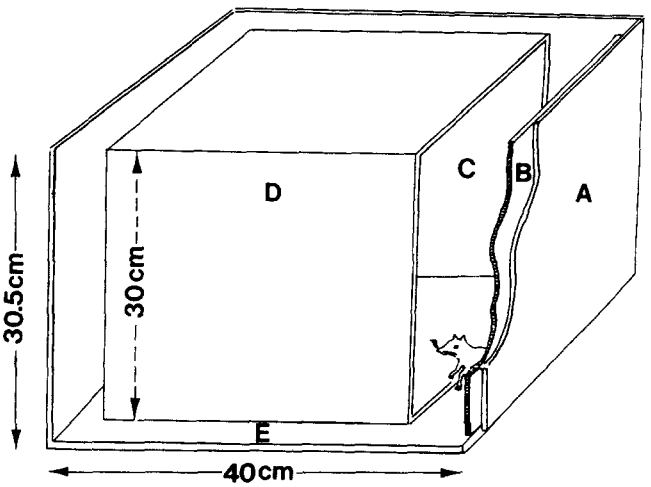

FIG. 1. The structure of the mirrored chamber apparatus. (A) The Plexiglas wall of the outer chamber was opaque and black. The chamber was 40 × 40 cm with walls 30.5 cm high. (B) Opposite the open side of the chamber of mirrors was an inward facing mirror the exact width and height of the mirrored chamber. (C) The cubical mirrored chamber (30 × 30 × 30 cm) had its 5 interior surfaces made of mirrored glass. (D) The exterior surfaces of the mirrored glass chamber were nonmirrored and painted brown. (E) An open corridor 5 cm wide surrounded the mirrored compartment on all sides.

the same corner of the corridor. The mouse was allowed free movement around the corridor and into the chamber of mirrors. A practical, empirically established evaluation period of 30 minutes (starting at the time of placement into the corridor) was chosen for use throughout these experiments. The time to enter into the chamber of mirrors was evaluated directly by an observer approximately 1 meter from the apparatus. The criterion for entry into the chamber was all four feet being placed on the floor panel of the mirrored chamber. After each animal was evaluated, the apparatus was washed thoroughly with tap water to eliminate potential cues (e.g., excreta) to entry left by the previous occupant. Typically, 10 mice were evaluated for each drug dose or condition. Latency values were expressed as the mean entry time in seconds ± SEM. Diazepam or vehicle was administered 10 minutes prior to placement of the animal in the apparatus.

To test the influence of the mirrors on the delay to enter a chamber of the same volume, the mirror chamber was replaced by a chamber of the same size made of inverted mirror tiles. The inside chamber walls were, thus, of brown color inside. The mirrors were outside. The sixth mirror was removed. The same type of experiment was described as above, but only vehicle was administered.

Plus-Maze Behavior

The plus-maze apparatus was made of Plexiglas and consisted of two open arms 30.5 cm long with a 5 cm wide runway and two enclosed arms 30.5 cm long with 5 cm wide runway enclosed by clear Plexiglas walls 15 cm high. The arms extended from a central platform, and the runways of both arms were made of black Plexiglas. The apparatus was mounted on a Plexiglas base 38.5 cm above the floor. The apparatus was similar to that described by Lister (10). Behavioral evaluations were carried out in a quiet room, with a fluorescent lighting (constant lighting of 200 lux). Animals were brought in the room half an hour prior to experi-

mentation. During the test period, the mouse was placed in the center of the plus-maze facing an open arm. During the five-minute test, the delay of entry as well as the number of entries and time spent in each of the two arms were scored by direct observation. A mouse was taken to have entered an arm when all four legs were on the arm (outside the start position). Diazepam or vehicle was administered 10 minutes prior to placement of individual mice in the test apparatus.

Head-Dipping Behavior

Head-dipping in the holeboard test has been suggested as a measure of anxiety in rodents (4,10). We used a Digiscan Optical Animal Activity Monitor equipped with infrared sensors to measure the insertion of the mouse's head and snout into a fixed array of holes in a horizontal plate. The metal plate floor was 40×40 cm wide. It contained 6 holes, 1.5 cm in diameter, spaced symmetrically in a diamond pattern. The plate was opaque and approximately 3.5 cm above the base of the apparatus. Animals ($n = 5$ per dose) were brought into the test room 30 minutes prior to the start of the experiment. They were injected with vehicle or diazepam and placed individually in the test apparatus 10 minutes after injection. The number of head-dips was recorded automatically in 1-minute increments for 5 minutes. Values are expressed as the mean ± SEM number of head-dips per 5 minutes.

Locomotor Activity Assessment

Locomotor activity was monitored in ten activity chambers. Each activity chamber consisted of a 2 foot diameter circular arena, 10 inches high, equipped with two photocell detectors. Each detector was illuminated by a 25-W light bulb (General Electric No. 25R14N) placed outside the arena with the light beam directed through a ½ inch hole in the side of the arena. The bulbs were the only source of lighting within the chamber. A Rockwell AIM 65 microprocessor system was used for data acquisition. Data recorded for each 30-minute activity session consisted of 10-minute interval counts and cumulative total counts for the 30-minute period. Activity sessions were conducted daily from 0800 to 1430 hours. The mice ($n = 8$ to 10 for each dose) were injected intraperitoneally with vehicle or diazepam solutions (0.01–5 mg/kg) immediately prior to placement in the activity chamber. Values were expressed as the mean ± SEM number of light beam breaks per time interval.

Statistics

Comparison of behavioral responses to different diazepam doses within a test regimen and between different behavioral assays was carried out by ANOVA, or, in certain cases, by the Fisher Exact Method (8). A value of $p<0.05$ was taken as statistically significant.

RESULTS

Description of the Behavioral Response to the Chamber of Mirrors

When individual mice were placed into the corridor surrounding the chamber of mirrors, they rapidly began exploration of the corridor space. While they explored all of the space of the corridor, none of 10 untreated mice entered the mirrored chamber in less than 205 seconds. The average latency to enter the mirrored chamber was 1039 ± 125 seconds. These findings were typical in that most (>90%) of the naive mice from different lots of animals behaved comparably. The mirrored surface was important to this extended latency to enter the open side of the chamber. When an identical chamber with nonmirrored (brown) walls was substituted, the latency to enter decreased dramatically. The average latency to enter was 14 ± 3 seconds under this condition. In testing of various lots of mice, >90% of the mice entered the nonmirrored chamber with similar rapidity. However, we observed that mice which had been handled extensively or which had been used previously in other behavioral assays behaved differently. They were more heterogeneous in their responses. For example, when mice had been handled for five days and subjected to various behavioral analyses, 60% of them rapidly entered the mirrored chamber. To produce consistently long latencies, animals must be naive to handling in the laboratory and to the apparatus. Once mice had spontaneously entered the mirrored chamber, they continued to do so for at least four subsequent testing days.

The behavior exhibited in initiating entry into the mirrored chamber was interesting. When a mouse approached the mirrored chamber, initially it did not touch the surface. Usually it approached, then retreated to the corridor and circled the entire corridor. Then it exhibited a series of partial entries—one foot, two feet, three feet onto the mirrored surface—in succession. This process was carried out over the entire 1039-second latency period. Once a mouse entered the mirrored chamber it left and entered freely. In contrast, mice immediately entered directly into the nonmirrored chamber. They showed no succession of partial entry and retreat.

The presence of mirrors on all sides was important. The most consistently extended latencies were obtained with a chamber constructed with floor and ceiling mirrors, 3 vertical mirrored walls and a sixth mirror on the corridor wall opposite to the chamber opening. For example, if the mirror was removed from the opposite corridor wall, the latency to enter was 269 ± 174 seconds. Only 2 of the ten mice met the criterion of exclusion for ≥205 seconds found in the completely mirrored configuration. If only a single horizontal mirror was employed, forty percent of the mice entered in <20 seconds.

The Action of Diazepam on Aversion to Enter the Chamber of Mirrors

Diazepam administration shortened the delay to enter the mirrored chamber in a dosage-dependent manner (Fig. 2A). The percentage of mice with latencies to enter the mirrored chamber of <200 seconds began to increase at a dose of 0.1 mg/kg IP (Fig. 2A). (This was the latency criterion by which ≥90% of the vehicle-treated or noninjected mice remained outside of the test chamber.) In the experiment depicted none of the ten vehicle-treated mice entered the chamber in <200 seconds. Twenty percent of the mice receiving this diazepam dose had short entry times (<25 seconds). At a dose of 0.32 mg/kg IP, 50% of the mice had latencies below the control criterion. This increased incidence of reduced latencies among the treated mice was significant ($p<0.02$). A further increase in the dose to 0.5 mg/kg IP led to an increase to 90% in the incidence of animals rapidly entering the mirrored chamber.

The number of approach-avoidance episodes decreased too as a function of diazepam dose. For example, vehicle-treated animals averaged 16 ± 3 partial entries (1, 2 or 3 feet onto the horizontal mirror), but mice treated with diazepam (1 mg/kg IP) averaged only one partial entry (range 0 to 3) before completely entering the mirrored chamber.

Another way to view the effect of diazepam on entry behavior is to determine the average latency to enter rather than the incidence of animals exhibiting latencies below the exclusionary criterion. The effect of diazepam pretreatment on the actual

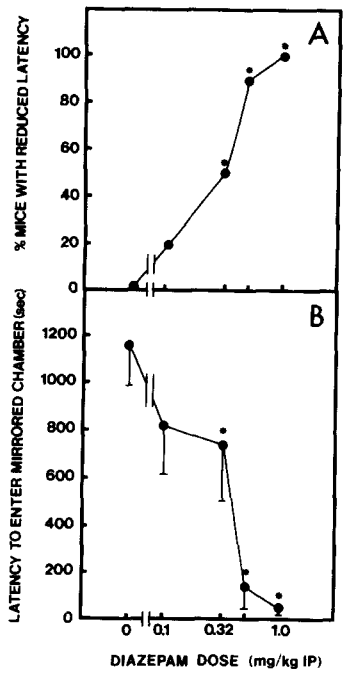

FIG. 2. The dose-dependent effect of diazepam administration on latency to enter into the chamber of mirrors. (A) Effect of diazepam on the incidence of animals exhibiting latencies below the exclusionary criterion (>205 seconds) for control mice. Values are expressed as percent of mice exhibiting significantly reduced latencies to enter compared to vehicle-treated animals. Ten different mice were used for each dose. (B) Reduction in the mean latency to enter the mirrored chamber as a function of diazepam dose. Values are the mean ± SEM of the time (seconds) taken to enter the mirrored chamber. (*) Indicates a statistically significant difference from control values.

latency to enter the mirrored chamber is shown in Fig. 2B. A marked, dosage-dependent reduction in the time mice took to enter the chamber was observed. In this experiment control mice had a mean latency to enter of 1160 ± 170 seconds. At the highest diazepam dose tested (1 mg/kg IP), the latency to enter was 48 ± 8 seconds. This value was only slightly increased above the latency to enter when mice were challenged with a nonmirrored (brown-walled) chamber. The trend toward reduction of mean latency began at the 0.1 mg/kg IP dose of diazepam. At dosages between 0.1 and 0.5 mg/kg IP, there was considerable heterogeneity in the latency to enter among individual animals. For example, at the 0.32 mg/kg IP dose, the mean latency was 740 ± 245 seconds. Fifty percent of animals receiving this dose had latencies <200 seconds, but the remainder were heterogeneous (range of these animals ≥200 seconds was from 720 to 1800 seconds). Increasing the dose to 0.5 mg/kg IP reduced the mean latency to 140 ± 88 seconds. The heterogeneity among individuals at this dose was significantly smaller compared to lower doses. Thus, diazepam markedly altered in a dose-dependent manner both the mean latency and the incidence of responding among animals. Comparison of the dosage dependency on these affects to other measures of exploratory activity is shown in Table 1 and will be described in the following sections.

The Effect of the Psychomotor Stimulant, Methylphenidate, on Aversion to Enter the Chamber of Mirrors

The possibility existed that locomotor activity stimulation rather than an anxiolytic effect might result in the altered mirrored chamber exploratory patterns seen after diazepam administration. One way to investigate this issue was to determine if a psychomotor stimulant without significant anxiolytic activity would enhance entry into the mirrored compartment. Methylphenidate at a dose of 3.2 mg/kg IP increased locomotor activity by 55 ± 15% of the basal value. The magnitude of this increase in locomotor activity level was comparable to the maximal stimulation (an increase of 62 ± 15% from the basal level) achieved after diazepam administration. Upon receiving a methylphenidate dose of 3.2 mg/kg IP, the mean latency to enter the chamber of mirrors decreased from the control value of nearly 1200 seconds to 888 ± 198 seconds. This reduction in latency induced by methylphenidate was small in comparison to the absolute latency value achieved after diazepam treatment (48 ± 8 seconds), and it was not statistically significant. Thus, motor activity stimulation per se was unlikely to be the cause of the diazepam-induced effect on entry into the mirrored chamber.

TABLE 1

COMPARISON OF THE POTENCY OF DIAZEPAM ON ENTRY INTO THE MIRRORED CHAMBER TO THREE OTHER BEHAVIORAL MEASURES OF EXPLORATION IN BALB/cByJ MICE

Behavioral Assay	Threshold (mg/kg IP)	ED_{50} (mg/kg IP)
Mirrored Chamber Entry		
Incidence change[1]	0.1	0.32
Latency change	0.1	0.4
Plus-Maze Performance		
Incidence change[1]	0.032	0.06*
Latency change	0.032	0.06*
Proportion of total time expended on open arm	0.032	0.13*
Head-Dipping Performance		
Increased frequency	0.1	0.22*
Locomotor Activity		
Stimulation	0.01	0.12*
Inhibition	3.2	>5*

[1]Refers to changes in the number of animals failing to meet criterion based on performance of vehicle-treated control mice. Values were estimated from dose-response curves composed of 7 points typically ranging from 0.001 to 1.0 mg/kg IP. At least three doses defining >0 to 100% of the maximal response were used to estimate the ED_{50} values. (*) Indicates that the potency of diazepam in the particular behavioral assay differed significantly from its potency in the mirrored chamber entry assay.

Relative Potency of Diazepam in the Mirrored Chamber Assay Compared to Three Other Assays of Exploratory Activity

The ability of diazepam to reduce latency to enter the mirrored chamber was compared to its affects on three other measures of exploratory activity—plus-maze performance, "head-dipping" behavior and modulation of locomotor activity (Table 1). BALB/cByJ mice exhibited a strong aversion to enter the open arms of the plus-maze under our conditions. Of the 300-second test period, vehicle-treated mice spent an average of 258 ± 26 seconds in the closed arms before venturing into the open arms. The latency to enter declined significantly to a value of 36 ± 17 seconds following diazepam administration and was dosage dependent. The fraction of the total time spent in the open arms of the maze also increased in a dosage-dependent way. Vehicle-treated mice spent approxi-

mately 2 ± 3% of the test period in the open arms. At a dose of 1 mg/kg IP the relative proportion of time spent in the open and in the closed arms did not differ significantly from 50%. A third way to present the plus-maze data is to identify the incidence of animals evidencing significantly reduced latencies to enter the open arm of the maze. The fraction of mice with reduced latencies increased exponentially as a function of dose. It was 70% at the 0.1 mg/kg IP dose. From these dose-response data, we estimated the ED_{50} values for each parameter shown in Table 1. Significantly lower doses of diazepam elicit changes in plus-maze performance compared to the reduction in latency to enter the mirrored chamber. Responsiveness in the other two behavioral assays also is brought about by lower doses than are effective in the mirrored chamber assay.

BALB/cByJ mice were relatively homogeneous in the explorative head-dipping behavior following vehicle or diazepam administration. Vehicle-treated mice averaged 19 ± 4 head-dips/animal during their initial 5 minutes in the apparatus. Diazepam doses >0.1 mg/kg IP significantly increased the number of head-dips compared to these control animals. The greatest increase in head-dipping frequency (to 67 ± 15 head-dips/animal) occurred at a dose of 0.32 mg/kg IP. Diazepam doses higher than 0.32 mg/kg IP did not further increase the number of head-dips. Although both the 0.5 and the 1.0 mg/kg IP doses of diazepam also significantly increased the number of head-dipping events (respectively 43 ± 6 and 29 ± 3 head-dips/animal) compared to control values, the number of head-dips clearly was reduced compared to those occurring at the 0.32 mg/kg IP dose. The head-dipping response of the 1 mg/kg IP diazepam dose was statistically different from both that of the vehicle-treated control animals and the diazepam-treated animals receiving a dose of 0.32 mg/kg IP. Diazepam doses ≥0.5 mg/kg IP were on the descending limb of this biphasic dose-response curve. The ED_{50} value for significant stimulation of head-dipping behavior was estimated to be 0.2 mg/kg IP. The biphasic nature of the dose-response curve for "head-dipping" effects and the higher potency of diazepam in this assay were significantly different from the mirrored chamber assay. As shown in Table 1, the potency and biphasic effect of diazepam on locomotor activity also differed from its mirrored chamber entry effects.

DISCUSSION

Behavioral responses to mirror images are known to occur across vertebrate species (6). Acute responses to mirrors often include aggressive threat displays and approach and withdrawal behavior (6). Even primates appear to lack the capacity for self-recognition (6,7). Although novelty seeking may be promoted with repeated exposures to mirrors, the general response to acute exposure is consistent with the initial occurrence of anxiety in the exposed animal. We expected that the distortion of normal spatial presentation and the appearance of multiple animals resulting from the placement of the mirrors would be strongly aversive to a mouse. We hypothesized that the type or degree of anxiety induced by exposure to mirrors might be quantitatively or qualitatively different from that which occurs under the circumstances of other behavioral assays used to investigate anxiolytics. In one other instance an attempt to examine the influence of mirrors on drug-induced modulation of exploratory activity in rodents has been reported (9). The methods and findings were different from those of our study. We observed that the BALB/cByJ mice had very extended latencies to enter the mirrored chamber compared to the identical chamber with partially mirrored or entirely nonmirrored surfaces. Experience in the mirrored chamber, use in other behavioral assays or extensive handling decreased the homogeneity of the aversion to the chamber among individual animals and significantly reduced the latency to enter the mirrored chamber. Diazepam administration prior to placement in the test apparatus significantly reduced the latency and increased the incidence of animals entering the mirrored chamber. This effect was markedly dosage dependent. Rapid entry into the mirrored chamber following diazepam administration did not appear to result from the activity-stimulating effects of diazepam. The dose-response curves for stimulation of locomotor activity and entry into the chamber of mirrors differ significantly. Stimulation of locomotor activity occurred at lower doses than did the increased entry into the chamber of mirrors. Also, the nonanxiolytic stimulant, methylphenidate, had no significant effect on entry into the mirrored chamber at a dose which maximally stimulated locomotor activity.

Several different observations suggest that the various behavioral assays which respond to anxiolytics are nonidentical in their pharmacological characteristics. For example, the plus-maze and the head-dipping assays have been reported to differ in their drug sensitivity. A dose of chlordiazepoxide which significantly elevated the time spent on the open arms of the plus-maze had little effect on hole-board activity of mice (10). The stimulant effects of amphetamine also differed in the two assays in the same study. Bignami has recently reviewed this issue with regard to punishment suppression systems (1). Classical anxiolytics have been shown to be ineffective in rats in some types of punishment suppression assays which were very sensitive to other agents. Effective nonbenzodiazepine anxiolytics such as buspirone and selected serotonergic agents may differ significantly in their effects on certain behavior assays compared to classical anxiolytics (1, 11, 17). From this point of view our mirrored chamber entry assay appears to have potential interest. The mirrored chamber entry assay requires significantly higher doses of diazepam to induce a change in behavior than does either the plus-maze behavioral assay or the "head-dipping" assay. The largest apparent difference in diazepam potency was between the mirrored chamber method and the plus-maze assay. Depending upon the specific criterion used, about five times more diazepam was required to bring about a half-maximal change in the mirrored chamber behavioral assay than in the plus-maze performance test. This finding implies that the aversion to enter the chamber of mirrors may be of greater intensity or qualitatively different from the aversion to enter the open arms of the plus-maze. Because of the potency difference observed for diazepam's actions on plus-maze performance and entry into the mirrored chamber, it may be that these two behavioral assays will also respond in a qualitatively or quantitatively different manner to anxiolytics acting by non-GABA-mediated mechanisms. In view of the ease with which this behavioral assay can be set up, its rapidity for quantitative evaluation and potential for computerized automation, this technique should be further characterized as a possibly valuable screening device in the identification of new anxiolytics.

ACKNOWLEDGEMENTS

These studies were supported by a grant from the Presbyterian Health Foundation. Dr. Cao Wu and Dr. Lance Logan were the recipients of Provost's Postdoctoral Research Fellowships from the University of Oklahoma Health Sciences Center. The sabbatical leave support provided to Dr. Paul Toubas by the University of Oklahoma Health Sciences Center is gratefully acknowledged. We thank Ms. Jerry Bridge for her competent assistance in preparing this manuscript.

REFERENCES

1. Bignami, G. Pharmacology and anxiety. Inadequacies of current experimental approaches and working models. Pharmacol. Biochem. Behav. 29:771–774; 1988.
2. Clody, D. E.; Lippa, A. S.; Beer, B. Preclinical procedures as predictors of antianxiety in man. In: Usdin, E.; Skolnick, P.; Tallman, J. F., Jr.; Greenblatt, D.; Paul, S. M. Pharmacology of benzodiazepines. London: Macmillan Press; 1982:341–353.
3. Crawley, J.; Davis, L. G. Baseline exploratory activity predicts anxiolytic response to diazepam in five mouse strains. Brain Res. Bull. 8:609–612; 1982.
4. File, S. E.; Curie, P. F.; Baldwin, H. A.; Neal, M. J. Anxiety in the rat is associated with decreased release of 5-HT and glycine from the hippocampus. Neurosci. Lett. 83:318–322; 1987.
5. File, S. E.; Lister, R. G. Do the reductions in social interaction produced by picrotoxin and pentylenetetrazole indicate anxiogenic actions? Neuropharmacology 23:793–796; 1984.
6. Gallup, G. G., Jr. Mirror-image stimulation. Psychol. Bull. 70: 782–793; 1968.
7. Gallup, G. G., Jr. Absence of self-recognition in a monkey *(Macaca fascicularis)* following prolonged exposure to a mirror. Dev. Psychobiol. 10:281–284; 1977.
8. Goldstein, A. Biostatistics. New York: Macmillan Co.; 1968:107–117.
9. Houri, D. Effects of central acting drugs on the mirror staircase test. Nippon Yaturigaku Zasshi 87:135–142; 1986.
10. Lister, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berlin) 92:180–185; 1987.
11. Mason, P.; Skinner, J.; Luttinger, D. Two tests in rats for antianxiety effects of clinically anxiety attenuating antidepressants. Psychopharmacology (Berlin) 92:30–34; 1987.
12. Nolan, N. A.; Parkes, M. W. The effect of benzodiazepines on the behavior of mice on a hole-board. Psychopharmacologia 29:277–288; 1973.
13. 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. Methods 14:149–167; 1985.
14. Seale, T. W.; Abla, K. A.; Roderick, T. H.; Rennert, O. M.; Carney, J. M. Different genes specify hyporesponsiveness to seizures induced by the benzodiazepine inverse agonist, DMCM. Pharmacol. Biochem. Behav. 27:451–456; 1987.
15. Seale, T. W.; Abla, K. A.; Shamim, M. T.; Carney, J. M.; Daly, J. W. 3,7-Dimethyl-1-propargylxanthine: A potent and selective in vivo antagonist of adenosine analogs. Life Sci. 43:1671–1684; 1988.
16. Seale, T. W.; Carney, J. M.; Flux, M.; Rennert, O. M.; Skolnick, P. Coincident susceptibility to seizures induced by caffeine and the benzodiazepine inverse agonist, DMCM, in SWR and CBA inbred mice. Pharmacol. Biochem. Behav. 26:381–387; 1987.
17. Soubrie, P. Reconciling the role of central serotonin neurons in human and animal behavior. Behav. Brain Sci. 9:319–364; 1986.
18. Vogel, J. R.; Beer, B.; Clody, D. E. A simple and reliable conflict procedure for testing antianxiety agents. Psychopharmacologia 21: 1–7; 1971.
19. Werkman, S. Anxiety disorders. In: Kaplan, H. I.; Freedman, A. M.; Sadock, B. J., eds. Comprehensive textbook of psychiatry III. Baltimore: Williams and Wilkins; 1984:2620–2630.