Pergamon

Pharmacology Biochemistry and Behavior, Vol. 47, No. 3, pp. 459-465, 1994

0091-3057/94 $6.00 + .00

# An Animal Model for Measuring Behavioral Responses to Anxiogenic and Anxiolytic Manipulations

JULIE C. STOUT[1] AND JAY M. WEISS[2]

*Department of Psychiatry, Duke University Medical Center, Durham, NC 27710*

Received 26 March 1993

STOUT, J. C. AND J. M. WEISS. *An animal model for measuring behavioral responses to anxiogenic and anxiolytic manipulations.* PHARMACOL BIOCHEM BEHAV 47(3) 459-465, 1994. – A method for measuring behavioral responses of rats to both anxiolytic and anxiogenic manipulations, the open field drink test (OFDT), is described. This method utilizes the concept that in the open field, appetitive behavior is reduced because of the ambient level of fear experienced in such an environment. For the OFDT, rats were given restricted access to water for 1 h per day for 3 days, and then their behavior was assessed in an open field that contained a water spout at its center. Use of the open field permitted a number of measures to be taken; of these, "time spent drinking" was most sensitive in detecting differences. Three experiments showed that the OFDT: a) permitted dissociation between behavioral responses to an anxiolytic (diazepam) and an anxiogenic (FG7142) drug, b) detected a dose-response relationship for an anxiolytic drug (diazepam), and c) detected behavioral responses to environmental manipulations designed to increase fear (presence of an olfactory cue from rats that had received foot shock). Advantages of this test over previously described methods are outlined, and several guidelines are provided to aid investigators in using this behavioral test.

Open field  Behavioral test  Anxiety  Rats

THIS paper details a modification of the open field that can be used for measuring both increases and decreases in anxious (i.e., anxiolytic-sensitive and mild fear-sensitive) behavior in rats. Although this method is not a major departure from some previously described methods of assessing anxious behavior in rats, it is particularly useful because it is designed to allow detection of either increases or decreases in anxious behavior using one set of experimental conditions, while simultaneously allowing observation of a wide range of behaviors from the rat's behavioral reportoire.

Animal models of anxiety have been used to investigate anxiolytic drugs as well as to study the neurophysiological basis of anxiety. While the construct of anxiety can be identified with more confidence in humans than in animals, it is widely acknowledged that anxiety as found in humans can be modeled by animal paradigms such as neophobia and conflict [e.g., (10,11)]. Historically, the open field has been used to assess emotionality, anxiety, and/or responses to mild stressin the rat (3,7-9,14). In the typical open field test, the animal is placed into a lighted arena from which it cannot escape, and for a brief period (e.g., 10-15 min) patterns of ambulation and behaviors such as rearing, grooming, and defecation are observed. Advantages of this test are that it is easy to administer and allows observation of a number of behaviors exhibited by rats. Also, in contrast to other methods used to study anxious behavior in rodents, such as the elevated plus maze (12), the Vogel test (21), and the Geller-Seifter Conflict Test (6), the open field test allows a comprehensive description of the animal's behavior, since more behaviors can be observed readily and quantified. However, this latter advantage is also a drawback in that the behaviors affected by anxiety-relevant manipulations often differ across animals, so that quantification of changes in a particular behavior may not provide a reliable indication of effects in different animals. To over-

[1] Current address: Department of Psychiatry – 0949, University of California, 9500 Gilman Drive, San Diego, CA 92161-0949.
[2] Requests for reprints should be addressed to Jay M. Weiss, Ph.D., Department of Psychiatry, Emory University, Georgia Mental Health Institute, 1256 Briarcliff Rd. N.E., Atlanta, GA 30306.

come this problem, several investigators have modified the open field test to focus anxious responses on a particular behavior that can be reliably assessed. For example, Takahashi et al. (19) tested animals in an open field containing a small enclosure (a coffee can open at one end) that was located at the edge of the field; thus, rats could enter the can to "escape" from the field. Takahashi et al. reported that the time spent in the enclosure (i.e., in defensive withdrawal) was correlated with anxiety-related environmental and pharmacological manipulations. However, a drawback to this particular method is that some rats adopt a freezing posture in a corner of the field instead of entering the enclosure, so that in some cases "time spent withdrawn into the can" is not a reliable measure of anxiety-relevant behavior.

Britton and Britton (2) described a modification of the open field in which rats were deprived of food and then placed in a small open field containing food on a pedestal at the center; latency to eat, amount of food eaten, and number of approaches to the food pedestal were recorded. Britton and Britton observed that normal rats are reluctant to eat in the open field, and that the average amount of food eaten per approach to the food pedestal increased in accord with the dose of anxiolytic drug animals were given. They reported that this method detected anxiolytic effects of diazepam, chlordiazepoxide, pentobarbital, and ethanol. An additional report has indicated that this method can detect nonbenzodiazepine anxiolytics (1). However, this test has also proven to have certain disadvantages. First, this modification of the open field is rarely suitable for detecting anxiogenic manipulations. Because little food is consumed under baseline conditions, decreases in food intake, which would indicate increased anxiety, often cannot be detected. In pilot studies conducted in our laboratory, attempts to increase baseline food intake by prolonging food deprivation or by reducing the novelty of the environment by repeated exposure were not effective. Second, because many drugs, including benzodiazepines, have marked effects on food intake per se, it is often difficult to discern whether the effects are attributable to anxiety rather than an influence of the drug on eating behavior.

In this paper, we report an alternative modification of the open field produced by depriving rats of water and then measuring their drinking behavior in an open field containing a water bottle. For the test (called the open field drink test, or OFDT), rats were restricted to 1 h of water intake per day for 3 consecutive days prior to testing, a schedule that did not appear to have ancillary adverse effects. For the actual test, the subject was placed into a novel, overhead-illuminated chamber containing a water bottle suspended above the center, and the subject was observed for 10 min. The principal measure taken was time spent drinking, but also recorded were: a) latency to begin drinking, b) number of approaches to the drinking bottle, c) number of approaches in which drinking occurred, d) number of rears, e) time spent grooming, f) time spent inactive, g) presence or absence of urination, and h) number of boli; thus, various behaviors representative of a rat's repertoire in a novel environment were measured.

The first experiment demonstrates that behavioral responses in the OFDT will detect an anxiolytic drug and an anxiogenic drug. As a test of the sensitivity of this measure to an anxiolytic drug, the prototypical benzodiazepine anxiolytic, diazepam, was administered [for a review, see (5)]; sensitivity to an anxiogenic drug was assessed using the beta-carboline, FG7142 (5). A second experiment showed that the test detected a dose-response administration of diazepam. A third experiment showed that behavioral responses to this novel open field could be enhanced by stressful procedures and olfactory cues associated with animals that had undergone foot shock.

## METHOD

### *Animals*

Subjects were male Sprague-Dawley rats weighing 250 to 450 g. Rats were housed on pine bedding, in clear plastic cages, in laminar flow cage racks that allow insulation from sound and odor of cages on other shelves. Rats were maintained on a 12 L : 12 D schedule, and all behavioral testing occurred at least 2 h, but no more than 7 h, after the onset of the light period, and was always completed by early afternoon (1400 h). Food was available ad lib except during behavioral testing. Water was available ad lib until 3 days before behavioral testing, at which time water was available for only 1 h per day (1500 to 1600). Rats were housed two per cage to avoid increased emotionality that often results from individual housing (23), and were handled for 1 min/day for at least 3 days prior to behavioral testing to reduce the effects on test behavior of the nonspecific stress of being handled.

### *Drugs*

Drugs were administered intraperitoneally (IP) 30 min before behavioral testing. Diazepam (Elkins-Sinn, Inc., Cherry Hill, NJ) was injected in manufacturer's vehicle containing propylene glycol, ethanol, water, benzyl alcohol, and sodium benzoate/benzoic acid. FG-7142 (*N*-methyl-beta-carboline-3-carboxamide; Research Biochemicals, Inc., Natick, MA) was suspended in distilled water with a drop of Tween 80 (Sigma Chemical Co., St. Louis, MO) and dispersed by ultrasound. Injected control subjects received physiological (0.9%) saline. It should be noted that since the volume of all injections given in these studies was less than 0.2 ml and thus the amount of constituents in any vehicle (i.e., propylene glycol, ethanol, etc.) was below an amount that would have an effect, additional control subjects receiving these constituents were not tested.

### *Behavioral Testing Procedure*

Testing was conducted in a square, clear Plexiglas box (36 × 36 cm) with an open top and a dark Formica floor (see Fig. 1). At the center of the test chamber, an inverted water bottle was suspended, with the end of its spout 10 cm above the floor. Illumination was provided by a 25-W incandescent light bulb 60 cm above the floor of the chamber. On all sides except one, the Plexiglas open field was surrounded by a solid dark screen to exclude extraneous visual stimuli.

Prior to testing each rat, the experimental chamber was thoroughly cleaned with a 5% acetic acid-water solution that was used to prevent the transmission of olfactory cues to the test subject. For testing, subjects were placed in the test chamber and observed for 10 min while an observer, unaware of the experimental condition, recorded the following measures: latency to begin drinking, time spent drinking, number of approaches to the drinking bottle, number of approaches in which drinking occurred, number of rears, time spent grooming, time spent inactive, and occurrence of urination and defecation. Following testing, rats were returned to the home cage.

### *Home Cage Testing Procedure*

Benzodiazepines have been found to exert appetitive effects that are independent of their anxiolytic effects (13,15,16).

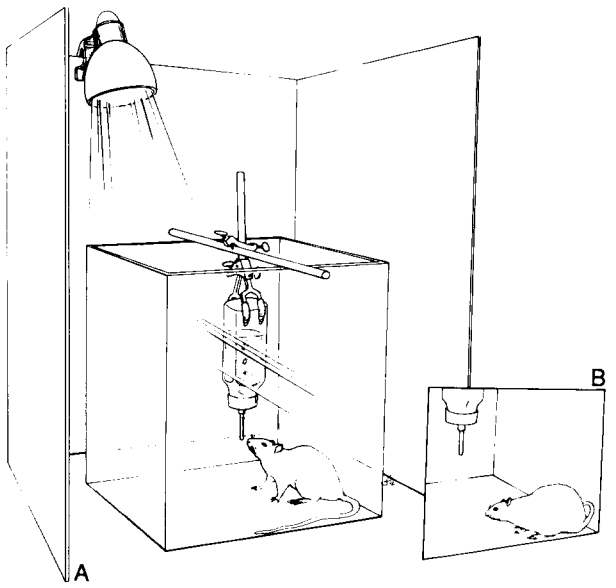


FIG. 1(A). The modified open field used in the Drink Test. Shown is the Plexiglas enclosure surrounded by a dark screen, the enclosure illuminated by an overhead 25-watt incandescent bulb, with an inverted water bottle suspended above the center of the field. An animal is shown approaching the water spout. Inset (B) shows the typical behavior of a frightened or anxious rat in this apparatus.

Therefore, it was necessary to measure the drugs' effects on drinking in the home cage (where presumably anxiety was not a factor) to determine whether effects observed in the open field might be attributable solely to appetitive, rather than anxiolytic, consequences of the drugs. Preparation for testing in the home cage was identical to preparation for testing in the open field; that is, rats were restricted to 1 h of access to water per day for the 3 days prior to testing, and on the day of testing they were injected and then replaced into home cages. For testing in the home cage, however, 30 min after injection two water bottles were placed on each home cage (one for each animal) and water consumed by each animal for a 10-min period was measured. In the first experiment, the quantity of water consumed by each animal was measured; in the second experiment, two observers who were unaware of the subjects' experimental condition recorded time spent drinking.

*Experimental Designs*

For the first two experiments, subjects received injections 30 min before testing and were then returned to their home cages until the time of the test. In experiments one and two, testing of water consumption in the home cage occurred 1 week after testing in the experimental chamber was completed.

*Comparison of an anxiolytic (diazepam) and an anxiogenic (FG7142) drug.* For the first experiment, subjects ($N$ = 22) were tested for 2 consecutive days in the experimental chamber. Data from the first day (baseline) were used to assign animals to three groups matched for mean time spent drinking. Any rat that drank for less than 15 s during the baseline session (two of 24 rats tested) was discarded from the study, since decreased drinking could not be reliably detected in such animals. On the second day, subjects in each group received IP injections of a) diazepam (1.0 mg/kg), b) FG7142 (2.5 mg/kg), or c) physiological saline, and were then tested in the experimental chamber after 30 min.

*A dose-response study of diazepam.* For the second experiment, subjects ($N$ = 30) were tested only once (i.e., no baseline data were collected). Rats were randomly assigned to one of five groups that received either diazepam (0.38 mg/kg, 0.75 mg/kg, or 1.5 mg/kg), physiological saline, or no injection, and were tested in the experimental chamber 30 min later.

*A comparison of modified environmental conditions.* For the third experiment, subjects ($N$ = 48) were tested for baseline drinking as in the first experiment and then animals with adequate baseline drinking (48 of 54 animals tested) were assigned to four groups matched for mean time spent drinking. On the second day, groups were tested as follows: one group was tested in a manner identical to the baseline day (i.e., no additional treatment). A second group (the "low anxiety" condition) was tested with the chamber floor covered with clean corncob bedding, a material that was completely novel to these animals. Although the Formica floor that is used in the control condition is more dissimilar to the bedding on which these animals are normally housed (pine bedding), we predicted that because the corncob bedding was completely novel to these animals, it would be more anxiogenic than the Formica floor condition to which they had been exposed on the previous day. A third group (the "moderate anxiety" condition) was tested on a floor covered with soiled corncob bedding that was collected from beneath the grid floor of a shock chamber immediately after several rats had received shock on the grid. Soiled corncob bedding was predicted to be moderately anxiogenic because it conveyed to the test condition olfactory stimuli produced by fearful rats, a condition that has been found to enhance anxiety-like behavior in conspecifics (24). A fourth group ("high anxiety" condition) received, beginning 2 h after the baseline session, a 30-min session of grid shock (1.0 mA shocks, each of 2 s duration, with shocks occurring on a random schedule with an average intershock interval of 2 min); these rats were then reexposed to the shock chamber immediately before testing on the second day (animals were placed in shock chamber for 5 min during which they were given a single shock of 2 s duration). These animals were tested on soiled corncob bedding as described above. This condition was predicted to be the most anxiogenic of the conditions, since for these subjects the cues of the bedding had been associated with the receipt of grid shock.

*Data Analysis*

Analysis of data from the diazepam dose-response experiment, in which no baseline data were collected, was identical to analysis of home cage data: for each measure, data were analyzed using one-way analysis of variance (ANOVA). A trend analysis was conducted to determine whether a dose-response relationship was evident. For the experiments in which baseline data were collected, data from the test day were analyzed for each measure by an analysis of covariance (ANCOVA) using the baseline data as the covariate. In each experiment, when a significant overall $F$-ratio was detected, pairwise comparisons of treatment group means to the control group were assessed for significance using Dunnett's test.

## RESULTS

*Effects of an Anxiolytic (Diazepam) and an Anxiogenic (FG7142) Drug*

Table 1 (upper section) shows the results obtained when animals were injected with either diazepam or FG7142. Of all

TABLE 1

BEHAVIOR IN THE DRINK TEST

| | Time Spent Drinking (s) | Latency to Begin Drinking (s) | Number of Approaches | Number of Approaches When Drinking Occurred | Number of Rearings | Time Spent Grooming (s) | Time Spent Inactive (s) | Amount Drunk in Home Cage |
|---|---|---|---|---|---|---|---|---|
| Experiment 1 | | | | | | | | (ml consumed) |
| Saline (0.9%) | 168.7 ± 17.1 | 52.4 ± 7.1 | 12.2 ± 0.8 | 6.9 ± 0.4 | 9.9 ± 1.4 | 29.1 ± 4.8 | 0 | 26.9 ± 1.2 |
| Diazepam (1.0 mg/kg) | 232.8 ± 14.5* | 92.0 ± 28.8 | 8.7 ± 1.1* | 4.8 ± 0.7 | 5.5 ± 1.3* | 25.2 ± 5.0 | 13.1 ± 7.7 | 26.6 ± 1.0 |
| FG7142 (2.5 mg/kg) | 98.9 ± 23.0* | 259.6 ± 64.6‡ | 7.5 ± 1.4* | 3.6 ± 1.1* | 6.9 ± 1.6 | 14.1 ± 5.5 | 16.0 ± 16.0 | 22.9 ± 6.4 |
| Experiment 2 | | | | | | | | (s) |
| Not injected | 174.0 ± 16.4 | 116.8 ± 19.4 | 11.8 ± 0.8 | 6.2 ± 0.6 | 15.0 ± 2.8 | 37.2 ± 5.4 | | 369.6 ± 20.5 |
| Saline (0.9%) | 158.2 ± 24.7 | 221.2 ± 48.9 | 8.8 ± 1.4 | 4.3 ± 1.4 | 15.3 ± 1.5 | 57.2 ± 13.4 | | 386.2 ± 32.7 |
| Diazepam (0.38 mg/kg) | 247.8 ± 14.2† | 29.7 ± 9.2‡ | 9.2 ± 0.8 | 6.5 ± 0.9 | 15.2 ± 1.0 | 36.0 ± 11.4 | NM | 464.7 ± 37.3 |
| Diazepam (0.75 mg/kg) | 294.2 ± 23.4‡ | 88.2 ± 28.3* | 7.8 ± 0.8 | 4.3 ± 0.8 | 10.7 ± 1.9 | 24.0 ± 9.2 | | 494.5 ± 21.6 |
| Diazepam (1.5 mg/kg) | 340.7 ± 16.5‡ | 155.2 ± 50.6 | 6.3 ± 1.7 | 5.3 ± 1.7 | 6.5 ± 1.6† | 21.0 ± 12.2 | | 473.2 ± 47.6 |
| Experiment 3 | | | | | | | | |
| Control condition | 149.2 ± 15.2 | 160.7 ± 60.3 | 9.5 ± 1.5 | 5.8 ± 1.1 | 15.5 ± 3.2 | 33.2 ± 4.0 | | |
| Clean bedding | 124.4 ± 27.0 | 105.2 ± 23.2 | 9.8 ± 1.8 | 5.0 ± 0.9 | 11.6 ± 2.5 | 25.8 ± 6.4 | NM | NM |
| "Soiled" bedding | 70.8 ± 18.1* | 311.6 ± 71.0 | 7.9 ± 1.6 | 3.6 ± 1.0 | 11.7 ± 2.4 | 18.4 ± 3.0 | | |
| "Conditioned fear" bedding | 23.0 ± 13.7‡ | 499.6 ± 60.9* | 2.4 ± 0.8‡ | 1.1 ± 0.6‡ | 3.7 ± 1.0* | 25.3 ± 9.6 | | |

Significant differences with respect to saline-injected controls were: *($p < .05$), †($p < .01$), ‡($p < .001$). NM = not measured.

measures taken, time spent drinking was the most reliable in differentiating treatment groups from the control group. Furthermore, this measure allowed a distinction to be made between the anxiolytic and anxiogenic conditions, since animals given diazepam drank for significantly more time than animals injected with physiological saline, while animals given FG7142 drank for significantly less time than did saline-injected animals. These differences do not seem attributable to any effects of drugs on thirst or drinking per se, in that diazepam and FG7142 did not significantly alter the amount of water consumed in the home cage.

Other measures also showed some significant differences. For example, latency to begin drinking was markedly lengthened by FG7142, but diazepam did not reduce latency to begin drinking. The overall results from the administration of diazepam, which resulted in a decreased number of approaches to the water tube, decreased rearing responses, lack of behavioral activity during the test for some animals, and reduced amount of water consumed in the home cage, suggest that the dose of diazepam used may have been mildly sedating. However, this did not appear to interfere with the time that the animals spent drinking in the open field.

### *A Dose-Response Effect of Diazepam*

Table 1 (center section) shows the effects of three doses of diazepam compared to two control groups. Again, time spent drinking showed the clearest differences of all of the measures, with increasing doses of diazepam producing increases in time spent drinking in the modified open field. As in the first experiment, these findings were not accounted for by increased thirst because all injected animals spent comparable, and not significantly prolonged, time drinking when tested in their home cage. Thus, the pattern observed in the home cage of similarly increased drinking across all doses of diazepam cannot account for a stepwise increase in drinking associated with increasing doses of diazepam observed in the OFDT. In fact, a significant positive linear trend existed across increasing doses of diazepam in the OFDT, and such a component was convincingly absent from the home cage data. As for other measures, the reduced number of rearing responses in animals receiving the highest dose of diazepam (1.5 mg/kg) again indicates that the higher doses of diazepam may well have been mildly sedating. Interestingly, the latency to begin drinking was greatly reduced by the lowest dose of diazepam (0.38 mg/kg), suggesting that anxiolytic effects were detected in this test at a very low dose of diazepam that was not associated with any sedating effects. It is noteworthy that this lowest dose of diazepam was effective as it is a lower dose than is typically used to produce anxiolytic effects in rats. However, because this drug is sometimes administered in other vehicles or may be in suspension rather than in solution, a strict comparison of this dose to doses used in some other experiments may not be possible.

### *Effects of Increasingly Anxiogenic Environmental Conditions on Behavior in the Modified Open Field*

Time spent drinking again showed the clearest relationship to the fearfulness produced by various environmental conditions (see Table 1, lower section). Whereas time spent drinking was unaffected when animals were tested over novel clean bedding, time spent drinking was reduced in animals tested over bedding where other frightened rats had previously been, and was greatly reduced when the animals themselves were shocked prior to testing in the drink apparatus and then tested over similar soiled bedding. Similar relationships were seen for several of the other measures, but again, the statistical reliability for discriminating the experimental conditions was most apparent for the time spent drinking measure.

## DISCUSSION

The data from the three experiments described above demonstrate the utility of using this modified open field procedure for measuring behavioral responses of rats to anxiolytic and anxiogenic conditions. When time spent drinking was used as an index, the procedure detected both anxiogenic and anxiolytic effects under a single set of experimental conditions; this is shown in Fig. 2. In Experiment 1, this measure was altered in opposite directions, with an anxiogenic drug (FG7142) decreasing this response and an anxiolytic drug (diazepam) increasing it. Thus, this test was useful for dissociating anxiogenic and anxiolytic behavioral responses. The second experiment showed that the test detected, in a dose-related manner, the administration of increasing doses of diazepam. Finally, the third experiment showed that the test was also sensitive to anxiogenic manipulations produced by alteration of environmental conditions.

### *Recommended General Procedure*

In our laboratory, we have used this test to measure anxious behavior in rats for more than 2 years, and have conducted the test with hundreds of rats. Behavior of different strains of rats, different genders, and even identical strains of rats received from the shipper vs. animals bred within the

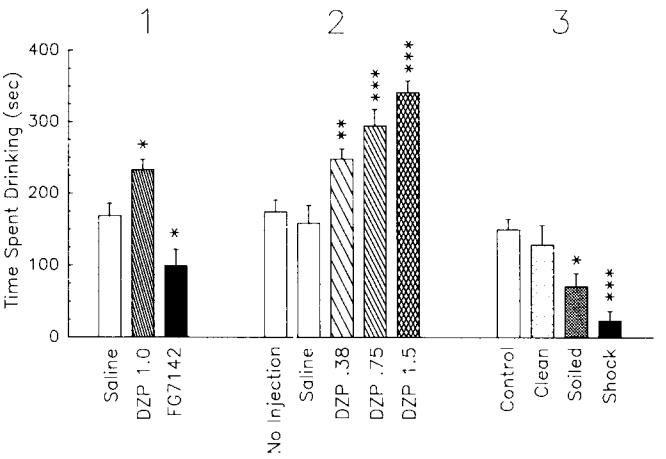


FIG. 2. The amount of time (in seconds) that thirsty animals spent drinking during a 10-minute test in the modified open field in three experiments. For each condition, the mean and standard error is shown. Experiment 1 shows the results when animals were injected with physiological saline (0.9%), diazepam (DZP, 1.0 mg/kg), or the beta-carboline FG7142 (2.5 mg/kg). Experiment 2 shows results when animals were given no injection, physiological saline, or increasing doses of diazepam (mg/kg indicated). Experiment 3 shows results when animals were exposed to test conditions designed to engender increasing levels of anxiety; Control = Formica floor (Normal test conditions), Clean = Clean corncob bedding on floor (*i.e.*, novel floor covering), Soiled = Soiled corncob bedding, and Shock = Previous exposure to shock + Soiled corncob bedding. Differences from saline-injected control group in same experiment are indicated as follows: * = $P < .05$; ** = $P < .01$; and *** = $P < .001$.

laboratory varies sufficiently (20) to require modification of the testing conditions to reliably detect increases or decreases in anxiety. As such, we have outlined the following guidelines for using this method of assessing anxious behavior in rats:

*1. Establishing a usable baseline.* A set of environmental conditions should be established that produces baseline and control levels of drinking suitable for detecting bidirectional effects. For detecting bidirectional changes, the baseline level must be in a range from which either increases or decreases are likely to occur. In the above experiments, the mean time spent drinking of the control group was 168 s, which permits a range of possible decreases in drinking before reaching its lowest level (0 s) of drinking. Additionally, for anxiolytic conditions, a baseline of 168 s of drinking allowed detection of a range of increases. We have found that on the same water restriction schedule, rats tested in the home cage drink for 300–480 s over a 10-min period, which would be the highest level of drinking for these rats under normal conditions.

To adapt this behavioral test to produce a suitable baseline for a group of rats, the environmental conditions can be altered to increase or decrease the baseline level of anxiety of the experimental conditions. Anxiety can be increased (to reduce baseline drinking) by using a larger enclosure, brighter illumination, or adding fear-relevant olfactory cues to the chamber; to decrease anxiety in the test chamber, familiarity with the chamber can be increased by previous exposures (20,22). For example, a small study in our laboratory found that repeated exposure to the testing environment produced similar behavioral effects to diazepam (18). Additionally, increasing an animal's familiarity with being handled, prior to testing in the OFDT, could reduce the stress that handling can create in the process of beginning the test, and thus a lower, more stable level of drinking at baseline may be obtainable.

*2. Use of baseline data.* Although collecting baseline as well as test-day data is time and labor consuming, we have found the benefits of doing so are significant. First, since baseline and test-day drinking are highly correlated within animals, baseline data is a useful covariate in statistical analysis. Analysis of covariance, in effect, allows between-subject variance to be removed from the error term, thus allowing a more sensitive detection of effects. Also, use of a baseline condition allows one to discard subjects that, for whatever reason, drink little or nothing (0–20 s of drinking). This is useful because rats with such low baseline drinking, we have consistently found, often do not vary from their baseline level of drinking regardless of anxiogenic or anxiolytic manipulations.

*3. Use of the enhanced fear condition.* We have found it to be useful to test animals under high anxiety as well as low anxiety (i.e., normal open field) conditions. For example, in some recent experiments, we found that manipulations of the noradrenergic system in the brain produced effects that were evident under conditions of high anxiety but not low anxiety (17). Thus, we have used an enhanced fear condition in certain experiments. For this condition, after baseline testing animals were given a 30-min session of grid shock (shocks are 1.0 mA, 2 s duration, with an average of 2 min between shocks), and then on the following day were reexposed to the shock chamber for 30 min (and are also given brief "reminder" shocks at 2, 5, and 15 min after being placed in the shock chamber) just before being given the OFDT. Reexposure to the shock chamber and then placement of the animal into the OFDT heightens the animal's fear as it begins the test (since, for the rat, handling has become associated with being introduced into a shock apparatus and is consequently a fear-producing stimulus that occurs just prior to the OFDT). This procedure has proved useful in establishing a sufficiently high background of fear/anxiety to detect the efficacy of certain manipulations.

*4. Use of additional behavioral measures.* In utilizing this test with varied experimental conditions and manipulations, we have observed a wide variety of behaviors that has included rearing, grooming, ambulation, defecation, urination, freezing, inactivity, gnawing, yawning, sleeping, and ataxia. Systematic observations of several behaviors in conjunction with notes of atypical occurrences of other behaviors allow a more detailed analysis and description of behavioral responses, and can augment the interpretability of results. For example, our observers noted ataxia and sedation in particular diazepam-injected animals. Those observations allowed us to determine that the cases in which the diazepam-injected animals spent little time drinking were associated with ataxic or sedative effects rather than an anxiogenic effect of diazepam. Modifications of this test may also be made to provide more detailed analyses of particular behaviors of interest. For example, the use of a calibrated drinking bottle to assess volume of water consumed, or a lickometer to quantify licks, would provide additional information as to the effects of experimental manipulations on drinking behavior. Additionally, ambulation could be quantified by demarcating a grid on the floor of the chamber so that the number of squares entered over the course of testing could be counted. Additional testing conditions can be also useful in clarifying results. For example, testing of water consumed in the home cage allows effects of manipulations on drinking behavior associated with thirst to be separated from those effects produced by anxiogenic or anxiolytic conditions.

In summary, the behavioral test described above is suitable for measuring increases and decreases in anxious behavior using a single set of experimental conditions, and allows the detection of pharmacological as well as environmental alterations of anxiety. Suggestions for adapting this procedure were provided to facilitate use of this test by other laboratories.

ACKNOWLEDGEMENTS

We wish to thank Mark Cierpial for his advice during the development of this measure. We gratefully acknowledge excellent technical assistance from Emily Hargrove, Christine Lorenz, Lynn Rankin, and Marc Carpenter.

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