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BRIEF COMMUNICATION

Thigmotaxis as a Test for Anxiolytic Activity in Rats

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TREIT, D. AND M. FUNDYTUS. *Thigmotaxis as a test for anxiolytic activity in rats.* PHARMACOL BIOCHEM BEHAV 31(4) 959–962, 1988.—It has been suggested that "phylogenetically prepared fear reactions" may be useful behavioral assays of the effects of anxiolytic agents. In the present experiments, rats' natural proclivity to stay near the perimeters of a novel environment (i.e., thigmotaxis) was suppressed by anxiolytic agents (diazepam 1–5 mg/kg; chlordiazepoxide 1–10 mg/kg; pentobarbital 1–10 mg/kg), with a relative potency that was similar to their relative potency in the treatment of human anxiety. Furthermore, when effects on general activity were factored out using analysis of covariance, the test also showed some degree of drug-class specificity, since neither d-amphetamine, morphine, nor chlorpromazine produced this anti-thigmotaxic effect. These results support an earlier report that thigmotaxis may be a useful test for anxiolytic activity in rats.

Anxiolytics Thigmotaxis Animal tests Diazepam Chlordiazepoxide Pentobarbital d-Amphetamine Morphine Chlorpromazine

IN a recent review, Treit (12) emphasized the potential importance of animals' untrained (i.e., "prepared") fear reactions as models for the study of anxiolytic drug action. Examples of "prepared" fear reactions are rodents' untrained responses to shock-probes, brightly lit compartments, social interactions, or elevated open platforms. It is noteworthy that each of these reactions have been shown to detect anxiolytic drug effects and to be useful for studying the neuropharmacology of anxiolytic drug action [e.g., (2, 4, 5, 9, 11)].

Barnett (1) reported that free-ranging rodents in the wild show a strong tendency to stay in contact with objects or with perimeters in the environment. It has been speculated that this "positive thigmotaxis" may be part of the rodent's natural defensive repertoire, since it may be more difficult for avian predators to attack a thigmotaxic rodent than a rodent that is out in the open (8).

Grossen and Kelley (8) were able to show that thigmotaxic behavior (i.e., duration spent near the walls of an apparatus) was significantly enhanced by the administration of a "fearful" stimulus (i.e., 1 mA foot-shock). In addition, rats' proclivity for thigmotaxis was revealed in an avoidance task: When rats were allowed to avoid shock by either jumping to a platform near a wall of the apparatus, or jumping to a platform in the center of the apparatus, rats jumped to the platform near the wall. These studies, along with other control experiments, suggested that thigmotaxis may be a "prepared" fear reaction of rodents.

In spite of these suggestive behavioral results, I found in an initial pilot study that diazepam did not have an anti-thigmotaxic effect that was independent of a suppressive effect on general activity (Treit, unpublished observations). However, in a recent abstract (10), Nichols and Schreur reported that standard anxiolytic agents such as diazepam and pentobarbital increased the amount of time that rats stayed away from the walls of a novel enclosure, whereas the nonanxiolytic agent haloperidol did not produce this anti-thigmotaxic effect. On the other hand, chlordiazepoxide did not display a significant anti-thigmotaxic effect, and drug effects on general activity were not reported.

Activity is an important consideration because it has been repeatedly shown that anxiolytic agents produce reliable, complex changes in the activity of rodents placed in a novel environment (3). These results, taken together with my pilot work with diazepam, suggest that a reduction in "thigmotaxis" could be secondary to a drug-induced change in general activity.

Accordingly, the major purpose of the present investigation was to further assess the drug-class specificity of thigmotaxis as a test of anxiolytic activity, while controlling for drug effects on general activity. I now report that the results of the present experiments generally confirm and extend

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those of Nichols and Schreur, but also illustrate that drug effects on overall activity must be factored out before selective anti-thigmotaxic effects of anxiolytics can be detected.

METHOD

Subjects

Subjects were 240 male, Sprague-Dawley rats (University of Alberta, Ellerslie), that weighed 200–400 g at the beginning of the experiments. The rats were housed individually for 3 days before each experiment, in hanging wire-mesh cages, with food and water available ad lib. Animals were tested during the light period of a 12-hr on/12-hr off light/dark cycle (lights on at 06:00 hr).

Apparatus

The apparatus was a wooden chamber (60 by 60 by 35 cm), with a 3.5 cm diameter hole in the floor in each corner. This chamber was placed in a separate testing room. The overhead fluorescent lights in this room were shaded by one layer of red Mylar film. The floor of the arena was clearly visible and demarcated with a series of black lines, drawn 1 cm apart, parallel to each of the four chamber walls. The rats' behavior was viewed and videotaped from a mirror suspended above the test chamber.

Drugs

Drugs were injected intraperitoneally 30 min before the test sessions. The range of doses (1–10 mg/kg) has been repeatedly shown to produce significant behavioral effects in other animal models of anxiety [e.g., (2, 4, 9)]. Diazepam and sodium pentobarbital were dissolved in a vehicle of 40% propylene glycol, 10% ethanol, and 50% distilled water, at a concentration of 5 mg/ml, or 10 mg/ml, respectively. Chlordiazepoxide hydrochloride, morphine sulphate, chlorpromazine hydrochloride, and d-amphetamine sulphate were dissolved in physiological saline at a concentration of 10 mg/ml. Control animals (i.e., "0" mg/kg) received IP injections of 0.5 ml/kg of physiological saline. Ongoing control experiments suggest that the diazepam vehicle by itself does not have a significant effect on thigmotaxis at the highest volumes used in the present experiments (data not shown).

Behavioral Measures

The durations of the following behaviors were scored from the video-taped sessions, using a six-channel, computer-controlled (Apple II+) event recorder.

1) *Thigmotaxis*: the duration of time in which the rat was in contact with, or within 2 cm of, any of the four walls of the apparatus. However, intrusion of the rat's tail, or the rat's snout, into this 2 cm area nearest the walls was *not* scored as thigmotaxis, if the majority of the rat's body was outside of this area.

2) *Ambulation*: locomotion at least one-half body length in any direction, anywhere in the chamber.

3) *Rearing*: the rat raising both its forepaws above the floor, anywhere in the apparatus.

4) *Investigation*: the rat making repeated sniffing movements (i.e., 3 sec or greater) directed at one spot in the chamber, anywhere in the chamber.

5) *Rest*: inactivity anywhere in the chamber.

The reliability of these behavioral measures was separately confirmed by interrater reliability coefficients computed between the paired measures of two observers viewing an equal number of instances (n=50) of the 5 behaviors. For thigmotaxis, ambulation, rearing, investigation, and rest, these coefficients were .90, .91, .92, .87, and .91, respectively.

Procedure

On each of the three days before an experiment, the rats were habituated to handling by the experimenter. On the fourth day, the rats were injected with either diazepam (0, 1, 2.5, or 5 mg/kg), chlordiazepoxide, pentobarbital, chlorpromazine, morphine, or d-amphetamine (all 0, 1, 5, or 10 mg/kg). Ten rats served in each of these combinations of drug and dose. On each day, rats were run in squads that were counterbalanced with respect to drug and dose.

Thirty min following the injection, rats were brought to the experimental room and individually placed in the center of the test apparatus. During the next 5 min, the duration of thigmotaxis was recorded, as well as ambulation, rearing, investigation, and rest. The floor of the apparatus was cleaned with a damp towel between each test to eliminate olfactory cues.

These data were analyzed in three different ways, according to the three pharmacological criteria under study: For simple sensitivity, the effects of the six drugs were analyzed with a series of one-way ANOVAs. Then, in order to establish the behavioral specificity of these drug effects (i.e., whether any of the drugs suppressed thigmotaxis *independently* of an effect on general activity), the effects of the six drugs were analyzed with a series of six analyses of covariance (ANCOVAs), using thigmotaxis as the dependent measure and general activity (i.e., the sum of ambulation, rearing and investigation) as the covariate. Thus, in each case, drug effects on thigmotaxis could be assessed independently of drug effects on general activity. Finally, in order to assess relative potency, the data for the three anxiolytics were transformed for regression analyses, according to the procedures described by Goldstein (7). The three regression lines were then used to estimate ED_{50}s, by which the relative potencies of the three anxiolytics were assessed.

RESULTS

Figure 1 shows the effects of each of the six drugs on thigmotaxis and general activity. It is apparent that although most of the drugs produced some change in thigmotaxis, they also produced a concurrent change in general activity.

Sensitivity

Consistent with the results shown in Fig. 1, simple one-way ANOVAs confirmed that almost every drug tested reliably suppressed thigmotaxis [pentobarbital: $F(3,36)=5.39$, $p<0.004$; diazepam: $F(3,36)=6.57$, $p<0.001$; chlordiazepoxide: $F(3,36)=5.31$, $p<0.004$; d-amphetamine: $F(3,36)=2.92$, $p<0.05$; chlorpromazine: $F(3,36)=4.24$, $p<0.01$; morphine: $F(3,36)=1.07$, $p>0.4$]. These results were not particularly surprising, since a parallel series of one-way ANOVAs of general activity showed that morphine, chlorpromazine, chlordiazepoxide, and diazepam all produced significant changes in activity (all ps<0.001).

Specificity

Whether or not the drugs produced a significant anti-thigmotaxic effect independently of an effect on general ac-

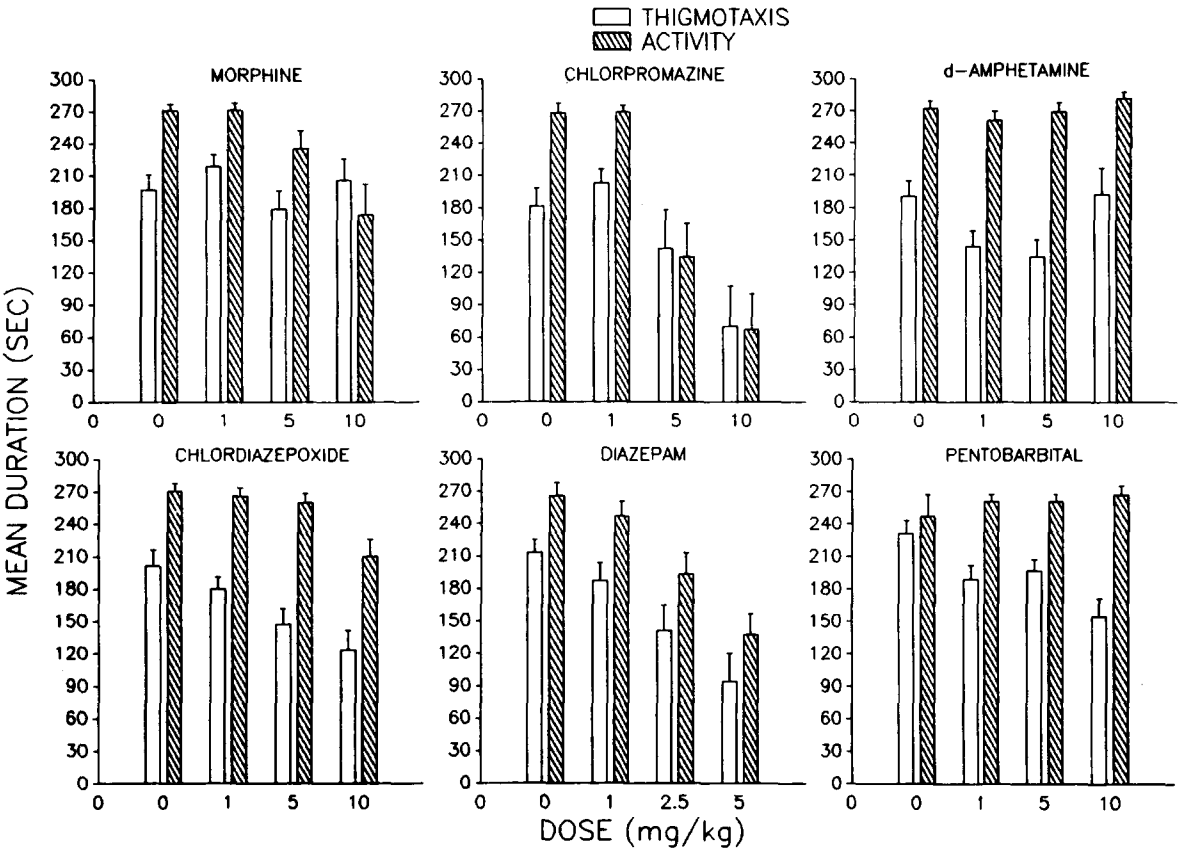

FIG. 1. Mean (S.E.M.) duration of thigmotaxis (open bars) and general activity (striped bar) during the 5 min test for rats given diazepam (0–5 mg/kg), chlordiazepoxide, pentobarbital, d-amphetamine, chlorpromazine, or morphine (all 0–10 mg/kg).

tivity was assessed with analysis of covariance. These results showed that none of the three nonanxiolytic agents produced a significant anti-thigmotaxic effect that was independent of their effects on general activity [morphine: $F(3,35)=1.79$, $p>0.1$; d-amphetamine: $F(3,35)=2.34$, $p>0.08$; chlorpromazine: $F(3,35)=0.51$, $p>0.6$]. Thus, using ANCOVA, the test showed no "false positives." Furthermore, when the same analyses were applied to the three anxiolytics, all three were detected as "true positives" [pentobarbital: $F(3,35)=5.01$, $p<0.005$; diazepam: $F(3,35)=3.41$, $p<0.03$; chlordiazepoxide: $F(3,35)=3.12$, $p<0.05$]. Although the magnitude of the anti-thigmotaxic effects of diazepam and chlordiazepoxide was reduced by factoring out effects on general activity, their anti-thigmotaxic effects were still statistically intact after ANCOVA. More importantly, ANCOVA revealed the drug-class specificity of thigmotaxis because under this analysis nonanxiolytic agents could be separated from standard anxiolytic agents.

Relative Potency

In order to compare the relative potency of the three anxiolytics, regression analyses were performed in which the duration of thigmotaxis was expressed as a percentage of the mean control durations, and then regressed against log (dose+1). These analyses yielded significant ($p<0.01$) correlation coefficients for pentobarbital ($r=-.48$), diazepam ($r=-.58$), and chlordiazepoxide ($r=-.55$). These coefficients were not significantly different from each other ($ps>0.1$), nor were they significantly different from partial correlation coefficients in which the effect of the drugs on general activity had been statistically removed from their effects on thigmotaxis (i.e., pentobarbital: $r=-.47$, $p<0.01$; diazepam: $r=-.46$, $p<0.01$; chlordiazepoxide: $r=-.46$, $p<0.01$). The latter results were consistent with the earlier analyses of covariance, and suggested that estimating ED_{50}s from the original regression lines would lead to reasonably unbiased estimates of relative potencies. And in fact, the estimates derived from these lines showed a rank-order relationship that was consistent with the relative potency of these anxiolytics in clinical settings (estimated ED_{50}s: pentobarbital=28.9 mg/kg; chlordiazepoxide=12.1 mg/kg; diazepam=4.7 mg/kg).

DISCUSSION

Taken together, the results of these experiments are consistent with the hypothesis that rodent thigmotaxis is selectively sensitive to anxiolytic agents. The issue of relative potency still needs further study, but the present results are not inconsistent with what is known about the clinical potency of diazepam, chlordiazepoxide and sodium pentobarbital. Thus, it appears that thigmotaxis might satisfy the three pharmacological criteria of dose-dependent sensitivity, relative potency, and drug-class specificity (6).

The present results also support the notion that

phylogenetically "prepared" fear reactions could serve as useful adjunctive tests for anxiolytic agents. In addition to their speed and simplicity, the neural substrates of these behaviors might be particularly amenable to studies of the mechanisms of anxiolytic drug action. This assumption is based on the notion of a strong and consistent selection pressure for rapid and reliable defense mechanisms across animal species, mechanisms which often require a relatively primitive neural substrate (11,12). This assumption, of course, may or may not be vindicated by future data, but it is hoped that at present it might serve as a useful heuristic for future research into the mechanisms of anxiolytic drug action.

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