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Conditioned Defensive Burying: A New Paradigm for the Study of Anxiolytic Agents

DALLAS TREIT, J. P. J. PINEL[1] AND H. C. FIBIGER

University of British Columbia, Vancouver, B.C., Canada V6T 1W5

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TREIT, D., J. P. J. PINEL AND H. C. FIBIGER. *Conditioned defensive burying: A new paradigm for the study of anxiolytic agents.* PHARMAC. BIOCHEM. BEHAV. 15(4) 619–626, 1981.—Behavioral paradigms that have been designed to mimic forms of learning that are important for the survival of animals in the wild, rather than to minimize the contributions of adaptive predispositions, may prove to be particularly useful for studying the behavioral effects of drugs. In the present experiments, the propensity of rats to bury sources of aversive stimulation was disrupted in a dose-dependent fashion by a single injection of the anxiolytic drug, diazepam. This suggested that the conditioned defensive burying paradigm could prove to be a valuable addition to the paradigms available for studying anxiolytic effects. Supporting this view were two additional observations. First, the relative potencies of diazepam, chlordiazepoxide, and pentobarbital in the burying paradigm compared favorably with their relative potencies in clinical settings. Second, the effects of anxiolytics on conditioned burying appeared to be dissociable from the effects of other drugs that disrupt this behavior.

Anxiolytics Conditioned defensive burying Anxiety Animal models Diazepam Chlorpromazine Pentobarbital Chlordiazepoxide

There have been a number of attempts to develop aversive learning paradigms for animals that can be used to identify potential anxiolytic compounds and the neurochemical basis of their anxiolytic activity (for reviews see [17, 22, 28]). Such endeavors are based on the implicit assumption that aversive learning paradigms are good animal models of human anxiety [13]; aversive stimulation, experienced or anticipated, is a pivotal construct in almost all theoretical accounts of anxiety (e.g., [30, 32, 33, 47, 55]).

Be that as it may, there have been two major problems associated with the use of aversive learning paradigms in the study of anxiolytic agents. The first has been variability. The effects of known anxiolytic compounds on most traditional forms of aversive conditioning have been too inconsistent for these paradigms to be of much value in identifying new anxiolytic compounds. In conditioned suppression and conditioned avoidance experiments, for example, clinically effective anxiolytics have been reported to either facilitate [40,48], inhibit [27, 49, 50], or have no effect on the conditioned behavior [18, 39, 43].

The second problem is complexity. Although anxiolytics consistently facilitate punished operant behavior (e.g., [1, 6, 15, 16, 23, 24, 28, 31, 45, 56]), this "anticonflict" effect appears to be too complex to be used as a basis for studying the mechanisms of action of anxiolytic agents. In the anticonflict test, the test response (i.e., bar pressing) is both reinforced with food and punished with electric shock, thus making it difficult, if not impossible, to determine whether the facilitative action of a particular anxiolytic is due to its selective inhibition of shock-motivated behavior (anxiety), its facilitation of food-motivated behavior (appetite), or both [54]. Unless this ambiguity can be resolved, it is difficult to see how the anticonflict test can be used as a model to study the mechanisms of anxiolytic drug effects. In addition, the procedural complexity of the anticonflict test may prohibit its routine use for screening potential anxiolytic compounds, which now number in the thousands [21].

Some of the problems associated with the use of aversive learning paradigms in the study of anxiolytic agents have been attributed to procedural factors [8]. However, consideration of the assumptions on which psychologists based the original development of these conditioning paradigms suggests an alternative explanation, and provides the rationale for the present investigation.

One of the major reasons why psychologists developed aversive learning paradigms was to discover the general principles or laws that govern the learning of associations between stimuli (respondent conditioning) or between responses and their consequences (operant conditioning). Because their purpose was to discover the *general* laws of learning, psychologists tended to study arbitrary combinations of stimuli (e.g., lights, tones) and responses (e.g., bar presses, key pecks) in arbitrary subjects (e.g., rats, pigeons). The very arbitrariness of these learning paradigms was intended to minimize the influences of stimulus-specific, response-specific, and species-specific factors and thereby

[1]Address reprint requests to John Pinel, Department of Psychology, University of British Columbia, Vancouver, B.C. Canada V6t 1W5.

insure the generality of the results that were obtained from these paradigms (cf. [42,44]). However, the study of animals in arbitrary learning situations imposes serious constraints on the animal's ability to perform adaptive responses (cf. [3, 14, 42, 44, 46]). An animal may be constrained by having to learn associations between stimuli that have little relevance to its natural environment or by having to make responses that are only indirectly related to those that help it survive in its natural habitat [3]. Such constraints on the animal's species-typical adaptations may have contributed to a large number of the inconsistencies found in the animal learning literature [3, 4, 7, 29, 36].

Accordingly, pharmacologists who study the effects of anxiolytic agents exclusively in traditional aversive learning paradigms might expect to encounter the aforementioned problems of variability and complexity. The variability of the effects of anxiolytics in these paradigms may reflect an instability of the arbitrary conditioning paradigms as much as variability associated with the pharmacology of anxiolytic agents. In addition, arbitrary forms of aversive conditioning may have a particularly complex neural basis. Forms of aversive learning that are the direct result of hundreds of thousands of years of evolutionary pressure are not only likely to be more robust and reliable, but the neural basis of these types of learning may be less complex and more directly related to the neural substrates of anxiety. Thus, the neuropharmacologist who is attempting to discover the neurochemical correlates of anxiety by studying arbitrary forms of aversive conditioning may be using paradigms that are not particularly appropriate for this purpose [36].

In a recent review paper, Pinel and Treit [36] described a new aversive learning paradigm, the conditioned defensive burying paradigm, whose remarkable robustness appears to be derived from the fact that it was designed to mimic a form of learning that is important for the survival of rodents in the wild rather than to minimize the contributions of such adaptive predispositions. They found that rats shocked once through a stationary, wire-wrapped prod mounted on the wall of the test chamber returned to the prod and buried it with bedding material from the floor of the chamber [34]. Almost every rat sprayed bedding at the shock source with forward thrusting movements of the forepaws after only a single conditioning trial, even when an identical control prod was mounted on the opposite wall of the chamber or when the conditioning-test interval was as long as 20 days (cf. [38]). Thus, a conditioned association between the shock and the prod seems to control defensive burying in such situations (cf. [51]). The remarkable speed, reliability and simplicity of this particular aversive learning paradigm suggested that it might serve as a useful assay of anxiolytic agents.

Accordingly, the purpose of the present investigation was to assess the extent to which the conditioned defensive burying paradigm fulfils three major criteria associated with a predictive animal test of anxiolytic agents: i.e., dose-dependent sensitivity, relative potency, and selectivity [5,17].

GENERAL METHODS

Subjects

In each experiment, adult, 250 to 450 g, naive, male, hooded rats, purchased from Canadian Breeding Farm and Laboratories, La Prairie, Quebec served as subjects. The rats were housed in groups of six in 84×18×18 cm wire mesh cages under a 12-hr light/dark cycle, with continuous access to Purina laboratory chow and water. Rats were tested during the light part of the 12-hr light/dark cycle.

Apparatus

All testing was done in a 44×30×44 cm Plexiglass test chamber, the floor of which was evenly covered with 5 cm of San-i-cel, a commercial bedding material made of ground corn cob (Paxton Processing Co., Paxton, IL). In the center of each of the four walls, 2 cm above the level of the San-i-cel, was a small hole through which a 6.5×0.5×0.5 cm wire-wrapped wooden dowel (i.e., the shock prod) could be inserted. Electric current was administered through the two uninsulated wires wrapped around the prod. The behavior of each rat in each experiment was monitored for 15 min from a separate room via closed circuit television.

Procedures

Habituation. Before each of the experiments, the rats were placed in the Plexiglas test chamber in groups of five or six for 30-min periods on each of 4 consecutive days.

Drug administration. On the fifth day of each experiment, shortly before they were tested, the rats were randomly assigned to drug or vehicle groups. The rats in the drug groups received an intraperitoneal injection of the drug in solution, whereas those in the vehicle control groups received an intraperitoneal injection of an equivalent volume of the appropriate vehicle. The vehicle for chlordiazepoxide, chlorpromazine, d-amphetamine, pentylenetetrazol, and morphine sulfate was 0.9% saline; for diazepam and sodium pentobarbital it was 40% propylene glycol and 10% ethanol; and for picrotoxin, 5% acacia gum.

Shock administration. Shortly before testing on the fifth day, the shock prod was inserted 6 cm into the experimental chamber through the hole in one end wall and fixed there. Each animal was then placed individually into the center of the chamber so that it faced away from the prod. When the rat first touched the prod with its forepaw, it received a brief electric shock from an 800-V power source. In some cases, the animals received a mild shock (approximately 1 mA), which typically elicited a slight flinch away from the prod followed by a slow withdrawal toward the back of the test chamber; whereas, in other cases they received an intense shock (approximately 9 mA), which typically elicited a sudden fullbody flinch, immediately followed by a rapid withdrawal toward the back of the chamber. In both cases, the shock was initiated by the experimenter and terminated by the withdrawal of the subject.

The intensity of the current administered from the 800-V power source was fixed by wiring either an 80 k or 400 k Ω resistor in series with the subjects to produce the high- or the low-shock levels, respectively. The actual intensity and duration of shocks received by rats from each of these circuits was monitored by a storage oscilloscope in a separate pilot study (n=20). The mean high-level current was actually 9.4 mA in intensity (SD=2.2) and 35.2 msec in duration (SD=10.4), whereas the mean low-level shock was 0.9 mA in intensity (SD=0.14) and 29.6 msec (SD=11.6) in duration (cf. [34, 51, 53]).

Behavioral observation and quantification. Immediately after shock administration, the behavior of each rat was viewed over the television monitor for 15 min, and the dura-

tion of each burying sequence was recorded on a chart recorder.

These burying sequences are remarkably stereotyped when rats are shocked by a prod in the presence of bedding material [35]. The rat typically moves directly toward the prod, pushing and spraying a pile of bedding material ahead with rapid alternating movements of its forepaws. It is the forward motion of the rats' forelimbs, which directs the bedding toward the prod, and thus defines burying behavior [36]. The reliability of the duration-of-burying measure has been established in several previous studies. Pinel, Treit, and Wilkie [38], Pinel, Hoyer, and Terlecki [37], Davis and Rossheim [9], and Davis, Whiteside, Dickson, Thomas, and Heck [11] have found correlations of .988, .990, .93, and .91, respectively, between the scores compiled by independent observers.

In addition to this behavioral measure of burying, once the animal was removed from the chamber after the 15-min test, the height of the bedding material was measured at the junction between the prod and the wall. Because the analyses of this height measure invariably corroborated the analyses of the duration measure [52], the results of its statistical analysis are not reported in the present paper.

Statistical Analyses

The design of the majority of experiments was centered around one set of a priori comparisons; i.e., the comparisons between the mean burying scores of drug-injected and vehicle-injected rats. The effect of different drug doses and different shock levels on burying behavior were assessed with analysis of variance, followed by a posteriori pair-wise comparisons.

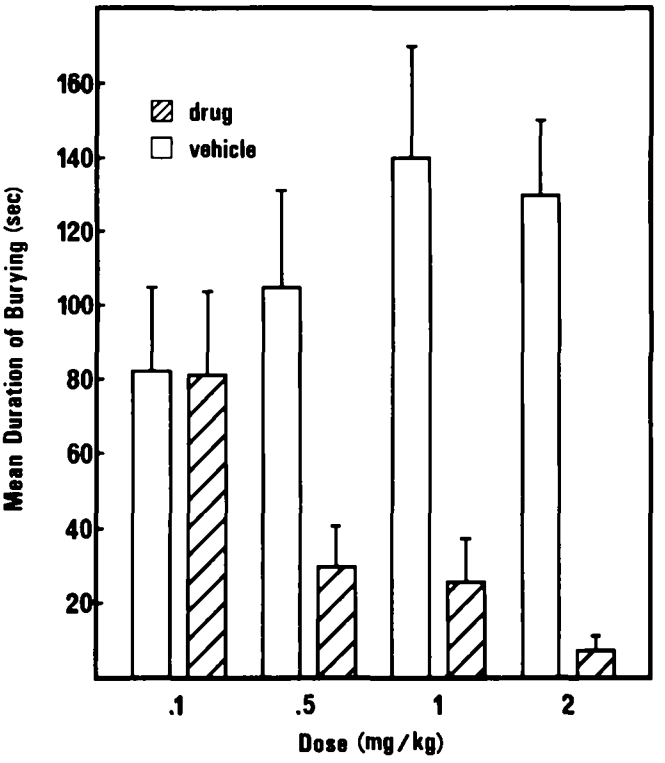

FIG. 1. Mean duration (±S.E.M.) of burying at each of four doses for diazepam-injected (diagonally-striped bars) and vehicle-injected rats (open bars) in Experiment 1.

EXPERIMENT 1

The purpose of Experiment 1 was to show that the conditioned defensive burying paradigm satisfies an important criterion of a useful screening test of anxiolytic agents; i.e., that it is sensitive in a dose-dependent manner to the effects of a known anxiolytic. Thus, in Experiment 1, the amount of conditioned defensive burying displayed by rats injected with different doses of diazepam was compared to that of vehicle-injected controls.

METHOD

On day 5, after the 4 consecutive days of habituation, the 80 rats serving as subjects were randomly assigned to one of two conditions. The rats in the experimental groups (drug-injected subjects, n=40) were injected intraperitoneally with either 0.1 mg/kg (n=10), 0.5 mg/kg (n=10), 1 mg/kg (n=10), or 2 mg/kg (n=10) of diazepam, 30 min before they were placed in the Plexiglas test chamber. When the rats first contacted the stationary wire-wrapped prod with a forepaw, they received a 1 mA shock (see General Method). The rats (n=40) in the control groups (vehicle-injected subjects) were treated in exactly the same manner, except that the rats in each group (n=10) received a volume of the vehicle that was equivalent to that received by rats of equal weight in each of the respective experimental groups. The burying behavior of each rat was recorded during the ensuing 15-min test period.

RESULTS AND DISCUSSION

It is apparent from Fig. 1 that the amount of conditioned defensive burying displayed by the diazepam-treated rats was well below that displayed by control rats. Planned orthogonal comparisons between the duration of burying in experimental and control rats confirmed that diazepam significantly suppressed burying behavior at every dose except 0.1 mg/kg (0.1 mg/kg, $t(18)=0.11$, $p>0.5$; 0.5 mg/kg, $t(18)=2.18$, $p<0.05$; 1.0 mg/kg, $t(18)=3.78$, $p<0.001$; 2.0 mg/kg, $t(18)=6.22$, $p<0.001$).

In order to assess whether or not the suppressive effect of diazepam on conditioned defensive burying was dose-dependent, the data were subjected to a 2-way analysis of variance. This 2 by 4 analysis confirmed the suppressive effect of diazepam on burying behavior $F(1,72)=25.84$, $p<0.0001$ and revealed a significant dose-by-drug interaction, $F(3,72)=3.64$, $p<0.02$, whereas the main effect of dose was not significant, $F(3,72)=0.29$, $p<0.50$. The dose-by-drug interaction was broken down into its components by subsequent a posteriori pair-wise comparisons (Duncan's multiple range test; $p=0.05$). The results of these pair-wise comparisons showed that the suppression of conditioned defensive burying by 2 mg/kg of diazepam was significantly greater than that produced by 0.1 mg/kg.

It should be emphasized that the 0.5 mg/kg dose at which diazepam was able to significantly suppress conditioned defensive burying is less than the doses that have been typically required to reveal an effect of diazepam in other behavioral paradigms (e.g., the conflict test). Thus, the conditioned burying response appears to be particularly sensitive to the effects of diazepam. Furthermore, the range of

doses at which diazepam produced a significant suppression of conditioned burying was well outside of the range that is known to produce obvious behavioral toxicity [28]. Other than the mild ataxia shown by three of the animals in the highest dose condition, the general appearance of diazepam-treated rats was not obviously different from that of vehicle-treated controls. Most of the drug-injected animals (30 of 40) sprayed at least some bedding material toward the prod, which was not substantially different from the incidence of this behavior in controls (38 of 40). Thus, the mean differences in conditioned defensive burying observed between the experimental and the control animals did not seem to occur because drug-injected animals could not make the burying response.

EXPERIMENT 2

The first purpose of Experiment 2 was to systematically replicate the findings of Experiment 1 using different anxiolytic agents. The second purpose was to assess the degree to which the conditioned defensive burying paradigm fulfills a second criterion of a valid animal test of anxiolytic agents, i.e., the criterion of relative potency. Accordingly, the effects on conditioned defensive burying of three anxiolytic agents known to differ in their relative ability to suppress anxiety in humans (i.e., diazepam, chlordiazepoxide, and pentobarbital were assessed at four different doses: 0, 1, 3, and 6 mg/kg. In clinical settings, the potency of diazepam is substantially greater than the potency of either chlordiazepoxide or pentobarbital, and the potency of chlordiazepoxide is marginally greater to that of pentobarbital (cf. [6]).

METHOD

After the 4 days of habituation, each of the 120 rats was randomly assigned to one of 12 groups (nine drug groups and three vehicle groups) of 10 subjects each. On day 5, 30 min before being placed in the Plexiglas chamber, rats in each of the groups received a 1 mg/kg, 3 mg/kg, or 6 mg/kg intraperitoneal injection of either diazepam, chlordiazepoxide, or pentobarbital, or .5 ml/kg of one of the respective vehicles (i.e., 0 dose groups). After each rat received the 1-mA shock from the prod, its behavior was observed for 15 min.

RESULTS AND DISCUSSION

It can be seen from Fig. 2 that conditioned defensive burying was suppressed by all three anxiolytics, especially at the higher dose levels. Whereas, all 30 of the vehicle-injected (0 dose) control rats displayed some burying behavior, burying was observed in only 9, 24, and 20 of the subjects treated with diazepam, chlordiazepoxide, and pentobarbital, respectively. Three sets of a priori comparisons (Dunnetts; $p=0.05$) between the duration of burying in animals in each of the three control groups and animals in each of the respective experimental groups confirmed that drug-injected animals spent significantly less time burying. Thus, the results of Experiment 2 confirmed the suppressive effect of diazepam found in Experiment 1 and extended the generality of these findings to two additional anxiolytic agents.

In order to assess the relative potency of diazepam, chlordiazepoxide, and pentobarbital, the suppressive effect of each agent was compared at the 1, 3, and 6 mg/kg dose levels using a 3 by 4 analysis of variance followed by a

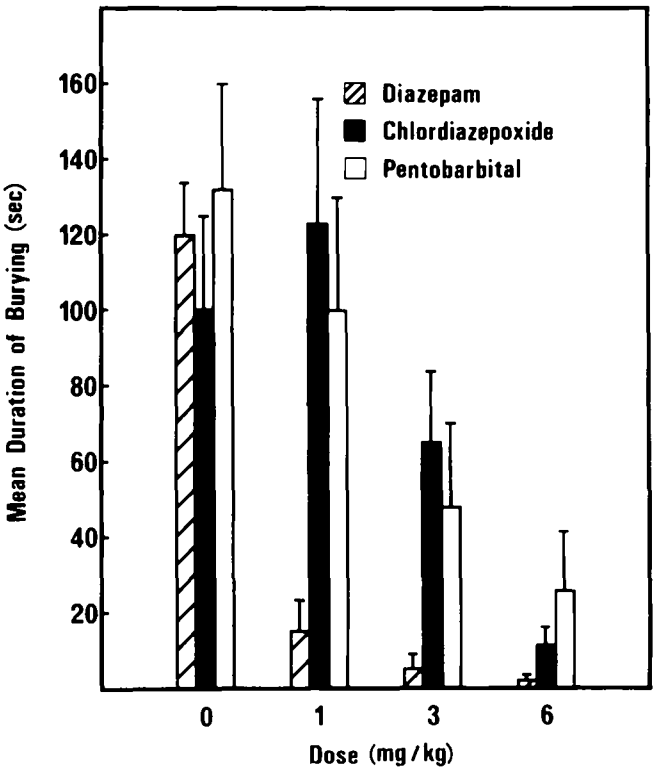

FIG. 2. Mean duration (±S.E.M.) of burying at each of four doses for diazepam-injected (diagonally-striped bars), chlordiazepoxide-injected (black bars), or pentobarbital-injected rats (open bars) in Experiment 2.

posteriori comparisons. A drug is considered more potent than another if it produces a significantly greater effect at a given dose [26].

The 3 by 4 analysis of the duration of burying resulted in a significant main effect of the type of drug, $F(2,108)=6.15$, $p<0.003$, a significant main effect of dose, $F(3,108)=16.95$, $p<0.0001$, and a significant dose-by-drug interaction, $F(6,108)=2.22$, $p<0.05$. Subsequent Duncan's multiple range tests ($p=0.05$) revealed that diazepam produced a significantly greater suppression of conditioned defensive burying than either chlordiazepoxide or pentobarbital at 1 mg/kg, and a significantly greater suppression than chlordiazepoxide at 3 mg/kg. Chlordiazepoxide and pentobarbital did not differ from each other at either 1 mg/kg or the 3 mg/kg dose levels. At 6 mg/kg, there was no significant difference between the effects of the three anxiolytics.

In clinical settings, diazepam has been shown to be 5 to 10 times more potent than either chlordiazepoxide or pentobarbital [6]. Although the clinical potencies of chlordiazepoxide and pentobarbital do not differ greatly, the potency of chlordiazepoxide is generally thought to be greater than that of pentobarbital (cf. [19, 20, 25]. The lack of a significant difference between the effects of chlordiazepoxide and pentobarbital in the present study is difficult to interpret. It is possible that the slight difference between the clinical potency of pentobarbital and chlordiazepoxide is only detectable when the sedative-hypnotic properties of these drugs are more prominant; i.e., beyond the range of

doses used in the present study. However, the significant differences between the effects of diazepam and chlordiazepoxide observed in the present study at 1 mg/kg and 3 mg/kg, and between the effects of diazepam and pentobarbital at 1 mg/kg are consistent with the relative clinical efficacy of these drugs.

EXPERIMENT 3

The purpose of Experiment 3 was to provide preliminary evidence that the conditioned defensive burying test is differentially sensitive to anxiolytic agents; i.e., that conditioned defensive burying is not affected in the same way by other psychotropic drugs. If drugs not known for their anxiolytic activity also produced a significant suppression of conditioned burying, it would be difficult to argue that the conditioned defensive burying paradigm would be a useful test of anxiolytic effects.

METHOD

On day 5, 72 rats were randomly assigned to one of six drug or six vehicle conditions. Rats in the drug conditions (n=6) received an intraperitoneal injection of either picrotoxin (0.5 mg/kg), d-amphetamine (1 mg/kg), morphine sulfate (1.5 mg/kg), chlorpromazine (4 mg/kg), or diazepam (1 mg/kg) 30 min before being placed in the test chamber; rats given pentylenetetrazol were injected (20 mg/kg) 1 min before the test. The intervals between injection and testing and the dose of each drug had been shown to produce reliable pharmacological effects in previous studies (cf. [17]. Immediately after receiving a 1-mA shock, the drug-injected rats were observed for 15 min. Rats in the six vehicle conditions (n=6) were treated in exactly the same manner, except that instead of drugs, these rats received an equivalent volume of the appropriate vehicle.

RESULTS AND DISCUSSION

The results of Experiment 3 shown in Table 1 indicate that the suppression of conditioned defensive burying is not a reliable effect of all psychoactive agents. Planned orthogonal comparisons between the duration of conditioned defensive burying by the drug-injected and the vehicle-injected rats showed that there was no significant suppression of conditioned burying by the CNS stimulants, picrotoxin, $t(10)=0.06$, $p>0.05$, pentylenetetrazol, $t(10)=1.12$, $p>0.20$, and d-amphetamine, $t(10)=1.27$, $p>0.20$, or by the narcotic analgesic, morphine, $t(10)=0.15$, $p>0.5$; whereas, the anxiolytic, diazepam, $t(10)=2.86$, $p<0.02$, and the 'major' tranquilizer, chlorpromazine, $t(10)=2.94$, $p<0.01$ both produced a significant suppression of burying behavior.

The present results suggest that the suppression of conditioned defensive burying is not a reliable effect of all psychoactive agents at nontoxic doses; only chlorpromazine and diazepam produced a significant suppression of burying (cf. [10]). However, in view of the suppressive effect of chlorpromazine, it cannot be concluded that minor tranquilizers are the only psychoactive drugs that disrupt conditioned defensive burying. Although chlorpromazine has been used clinically to treat acute anxiety (e.g., [12]) and has effects on a number of animal screening tests that are qualitatively similar to those produced by standard anxiolytic compounds [28], it is primarily used to treat psychoses [19], and its effects in some animal tests (e.g., the Geller conflict test) can be clearly dissociated from those of standard anxiolytic compounds. Thus, a more detailed investigation of the suppressive effects of chlorpromazine and diazepam on conditioned defensive burying was warranted.

TABLE 1

MEAN DURATION OF BURYING (±SD) BY THE DRUG-INJECTED AND THE VEHICLE-INJECTED RATS IN EXPERIMENT 3

	Drug	Vehicle
picrotoxin	83.4(± 71.8)	81.4(±58.7)
pentylenetetrazol	68.9(± 56.3)	108.7(±66.0)
d-amphetamine	165.2(±103.9)	105.1(±50.1)
morphine	74.8(± 49.3)	80.3(±72.6)
chlorproxmazine	19.9(± 30.0)	132.0(±88.2)
diazepam	36.6(± 24.0)	148.5(±92.6)

EXPERIMENT 4

The results of a number of studies have suggested that neuroleptics such as chlorpromazine can impair fear-motivated behavior by disrupting the animals' ability to perform coordinated motor responses, whereas anxiolytics such as diazepam appear to modulate fear-motivated behavior without disrupting motor performance per se (e.g., [2, 41, 45]). Furthermore, the motor deficits that are produced by moderate doses of neuroleptic agents are usually not affected by increases in the severity of the unconditioned aversive stimulus, whereas the effects of moderate doses of anxiolytic agents may be substantially diminished by increases in the severity of the unconditioned aversive stimulus (e.g., [45]). Thus, it seemed possible that the effects of diazepam and chlorpromazine in the conditioned defensive burying paradigm could be dissociated simply by exposing rats to shocks of different intensity. Experiment 4 was designed to assess this possibility.

METHOD

On day 5, 160 rats were randomly assigned to one of two basic conditions. Rats in one condition (chlorpromazine) were injected with one of three different doses of chlorpromazine (1,2, or 3 mg/kg) or 0.5 ml of its vehicle (0 mg/kg) 30 min before receiving either a 1-mA or a 10-mA shock from the prod. Rats in the other condition (diazepam) were treated in exactly the same manner, except that they were injected with 1,2, or 3 mg/kg of diazepam or 0.5 ml of its vehicle. All rats in each of the 16 groups (n=10) were tested for 15 min.

RESULTS AND DISCUSSION

It can be seen from Fig. 3 that in the low-intensity shock condition, both diazepam and chlorpromazine produced a dramatic suppression of conditioned defensive burying, whereas in the high-intensity condition, only chlorpromazine suppressed burying. Thus, the suppressive effects of chlorpromazine and diazepam on conditioned defensive burying were dissociated by varying the intensity of the electric shock.

A 3-way analysis of variance confirmed that the intensity of shock, $F(1,144)=20.84$, $p<0.0001$, the type of drug, $F(1,144)=6.73$, $p<0.01$, and the dose of the drug,

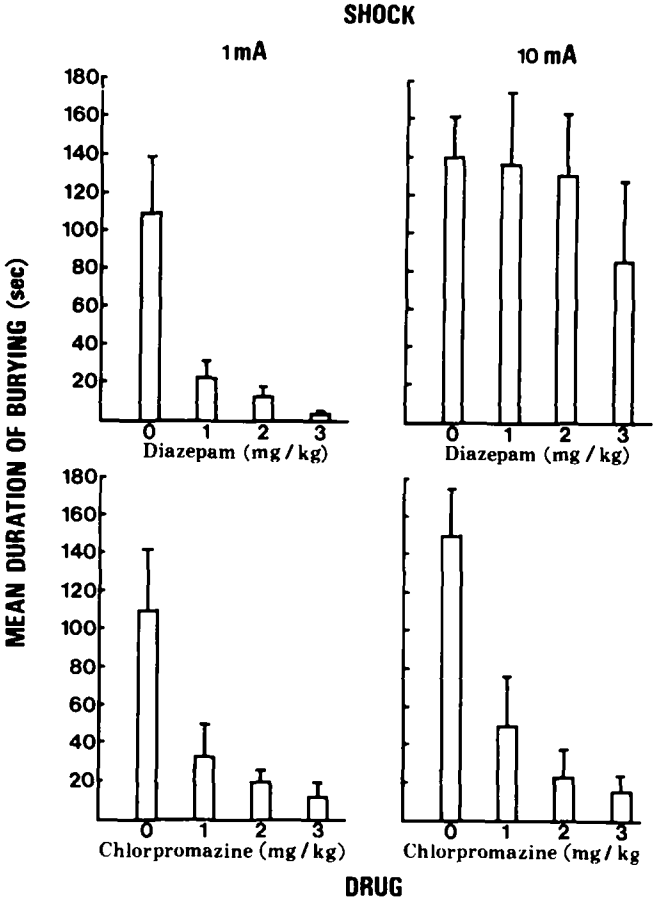

FIG. 3. Mean duration (± S.E.M.) of burying by diazepam-injected rats shocked with 1 mA (upper left panel) or 10 mA (upper right panel) and by chlorpromazine-injected rats shocked with 1 mA (lower left panel) or 10 mA (lower right panel) at each of four dose levels in Experiment 4.

$F(3,144)=15.91$, $p<0.0001$) each had a significant effect on the amount of time rats spent burying the prod. In addition, there was a significant 2-way interaction between the intensity of shock and the type of drug, $F(1,144)=10.76$, $p<0.001$. None of the other interaction effects reached the 0.05 level of significance.

Subsequent Duncan's multiple comparison tests ($p=0.05$) of the overall effects of the drug, dose, and shock intensity showed that across dose and shock conditions the suppressive effect of chlorpromazine on burying behavior was significantly greater than that of diazepam, that across drug and shock conditions the 3 and 2 mg/kg doses produced a significantly greater suppression than did either the 1 or 0 mg/kg doses, and that across dose and drug conditions the 10-mA shock produced significantly more conditioned burying than did the 1-mA shock. These overall differences were analyzed into their constituent parts using all possible pair-wise comparisons (Duncan's $p=0.05$). The results of this analysis showed that there were no significant differences between mean durations of burying behavior of rats in the four control conditions; however, in the 1-mA shock condition, animals treated with 1, 2, and 3 mg/kg of diazepam buried the prod significantly less than did rats in their vehicle control group, as did rats treated with 1, 2, and 3 mg/kg of chlorpromazine. In contrast, rats in the 10-mA condition that were treated with diazepam were not significantly different from their vehicle-injected control group at any dose level, whereas every dose of chlorpromazine produced a significant suppression of burying behavior. Thus, the significant drug-by-shock interaction was due to the fact that chlorpromazine but not diazepam disrupted conditioned burying at high shock intensities. This generalization was also supported by pair-wise comparisons (Duncan's, $p=0.05$) between the means of the drug-injected experimental groups at each of the two shock levels and three drug-dose levels. As expected, the diazepam treated rats in the 1-mA condition buried significantly less than did diazepam treated rats in the 10-mA condition at each of the three dose levels; whereas, there was no significant differences in the burying behavior of chlorpromazine treated rats in the 1-mA amd 10-mA conditions.

The same pattern of results was obtained when comparisons were made between the duration scores in both drug conditions. Although there were no significant differences between the diazepam-treated rats and the chlorpromazine-treated rats in the suppression of rats' conditioned burying at 1-mA, at 10-mA, chlorpromazine produced a significant suppression compared to diazepam at every dose except 6 mg/kg. These results clearly show that the suppressive effects of chlorpromazine and diazepam on conditioned defensive burying can be dissociated by varying the intensity of the electric shock.

In addition to providing evidence for the selectivity of the conditioned defensive burying test, these results indicate that chlorpromazine and diazepam may suppress burying by acting on different mechanisms. Because neuroleptics have been shown to have powerful disruptive effects on motor behavior at the doses used in the present study [41], it is possible that such motor impairment was responsible for the disruption of conditioned burying in the rats injected with chlorpromazine, who were generally immobile during the test. On the other hand, anxiolytics such as diazepam generally do not suppress motor behavior except at doses much higher than those used in the present study (cf. [28]), which is consistent with the fact that the diazepam-treated rats in the high-intensity shock condition buried the prod in a manner that was indistinguishable from that of vehicle-injected controls. Thus it seems more likely that diazepam inhibited conditioned burying by interfering with processes that may underlie the rat's reactions to aversive stimuli rather than by disrupting motor behavior per se.

GENERAL DISCUSSION

Taken together, the results of the present investigations suggest that the conditioned burying paradigm may be able to fulfil three major criteria of an animal test of anxiolytic agents: dose-dependent sensitivity, relative potency, and selectivity. The results of Experiment 1 clearly demonstrated that the conditioned burying response is sensitive in a dose-dependent manner to the effects of a known anxiolytic agent. Every dose of diazepam over 0.1 mg/kg produced a significant suppression of conditioned burying, and the magnitude of this suppressive effect increased significantly as the dose of diazepam was increased, without rendering the animals ataxic or somnolent. The generality of this effect was established in Experiment 2. It was found that three

anxiolytics, diazepam, chlordiazepoxide, and pentobarbital, each suppressed conditioned burying. Furthermore, at 1 mg/kg, diazepam produced a significantly greater suppression of burying behavior than did either chlordiazepoxide or pentobarbital, and at 3 mg/kg, a significantly greater suppression than chlordiazepoxide. Thus, the relative potencies of these three drugs in the conditioned burying paradigm compare favorably with their relative potencies in clinical settings. However, it is quite clear that the effects of many more anxiolytics on conditioned defensive burying must be assessed before it is reasonable to conclude that the conditioned defensive burying paradigm satisfies the criterion of relative potency.

In Experiments 3 and 4, the selectivity of the conditioned defensive burying test was assessed by observing the effects on conditioned burying of anxiolytic and nonanxiolytic compounds. The finding that neither CNS stimulants (i.e., picrotoxin, pentylenetetrazol, d-amphetamine) nor a narcotic analgesic, (i.e., morphine) had a significant effect on conditioned defensive burying at nontoxic doses indicated that the burying paradigm possesses some degree of selectivity. However, in Experiment 3 the effects of the major tranquilizer, chlorpromazine, were comparable to those of diazepam; both drugs produced a comparable suppression of conditioned burying. Accordingly, Experiment 4 was designed to differentiate the effects of chlorpromazine and diazepam. Both drugs were administered to rats exposed to one of two shock conditions that differed in severity. In the less severe shock condition (i.e., 1 mA), both drugs again produced an equivalent suppression of conditioned burying, but in the more severe condition (i.e., 10 mA), only chlorpromazine produced the suppression. Thus, even when anxiolytics and nonaxiolytics have comparable effects on burying conditioned at moderate shock intensities, their effects can be dissociated at higher shock intensities.

In spite of these promising results, however, other interpretations of these data must be ruled out before it can be firmly concluded that the conditioned defensive burying test is selective. For example, it is possible that the results of Experiment 4 were due to quantitative differences in the relative potency of diazepam and chlorpromazine, rather than to qualitative differences in their basic effects. If this were the case, very low doses of chlorpromazine might result in a suppression of conditioned burying similar to that of diazepam: i.e., only at the low shock intensity. Conversely, very high doses of diazepam might result in a suppression of conditioned burying equivalent to that of chlorpromazine: i.e., at both the low and high shock intensities. The pattern of suppression found with other neuroleptics whose extrapyramidal side-effects are less severe than those of chlorpromazine might also clarify this issue. Whether or not the suppressive effects of a wide range of doses of a variety of nonanxiolytic agents can be dissociated from those of anxiolytic agents in this particular paradigm thus remains to be determined. Nevertheless, the results of Experiment 4 suggest that this strategy could prove useful in dealing with comparable cases.

In addition to its apparent ability to meet the criteria of sensitivity, selectivity, and relative potency, the conditioned defensive burying test possesses a number of practical attributes that could facilitate the screening of potential anxiolytic compounds, which now number in the thousands [21]. The speed and simplicity of the burying test make it possible to screen large numbers of compounds in a relatively short period of time. The test response occurs reliably after only a single exposure to an aversive stimulus, and it is suppressed shortly after a single injection of an anxiolytic compound. In addition, because the burying reponse can be produced without positive reinforcement, antianxiety effects in this paradigm are not confounded with effects on appetitively motivated behavior, as they are in other learning paradigms (e.g., the conflict test). Although there are other animal tests of anxiolytic compounds that are reasonably reliable (e.g., the conflict test), they often require lengthy periods of pretraining, expensive instrumentation, and repeated exposures to the test compound. Moreover, the complexity of these paradigms often complicates interpretation of a drug's effect. In contrast, the suppression of conditioned buying appears to be a readily quantified, unambiguous measure of anxiolytic action. It must be emphasized, however, that although the conditioned defensive burying test appears to have a number of practical advantages over current behavioral tests of anxiolytic action, any meaningful comparisons must wait until the burying paradigm has been more extensively studied.

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