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Conditioned Defensive Burying as a Model for Identifying Anxiolytics

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CRAFT, R. M., J. L. HOWARD AND G. T. POLLARD. *Conditioned defensive burying as a model for identifying anxiolytics*. PHARMACOL BIOCHEM BEHAV 30(3) 775–780, 1988.—Rats exposed to a presumably aversive stimulus such as electric shock respond by heaping litter on the source, a behavior known as conditioned defensive burying (CDB). Because some anxiolytics suppress this behavior, CDB has been proposed as a screening method for anxiolytics. We tested the effects of the conventional anxiolytics chlordiazepoxide (4–32 mg/kg) and meprobamate (75–125 mg/kg), the novel anxiolytic buspirone (8–64 mg/kg), the antidepressant imipramine (4–16 mg/kg), the opiate analgesic morphine (2–8 mg/kg), and the antipsychotic chlorpromazine (1–16 mg/kg) on CDB. Chlordiazepoxide, meprobamate, imipramine, and morphine significantly suppressed CDB, but chlordiazepoxide did so only at a dose that reduced general activity. Buspirone and chlorpromazine did not suppress CDB at doses that reduced activity. There were some methodological differences from previous studies. We concluded that the test as constituted in this study lacks drug-class specificity. The necessity of distinguishing between specific reduction of burying and general reduction of activity is emphasized.

Conditioned defensive burying Anxiolytic Chlordiazepoxide Meprobamate Buspirone Chlorpromazine Imipramine Morphine Rat

IN an early monograph, Hudson [10] described "pushing of wood shavings" as a typical avoidance response of the rat to aversive stimulation. This response, which is characterized by pushing and spraying of bedding with forepaws and snout toward an object previously paired with an aversive stimulus—e.g., a prod that delivered electric shock—has been termed conditioned defensive burying (CDB) [12]. The response is robust and has been studied in detail (for reviews see [13, 14, 17]).

Several investigators, having shown that anxiolytics suppressed burying, suggested that CDB might have advantages as a preclinical screening method for anxiolytics [13, 17, 18]. Burying is part of the rat's natural repertoire [12], it requires no training, and it is elicited by several stimuli [16,24]. Moreover, it does not require food or water deprivation, which can be confounding factors in the commonly used conflict tests [9,22].

Table 1 summarizes results from studies of the effects of drugs on burying. Inherent in any test in which the reduction or elimination of a behavior is considered a positive result is the difficulty of distinguishing between drug effect on the behavior of interest and drug effect on activity in general. Treit *et al.* [18] reported that the anxiolytics diazepam, chlordiazepoxide, and pentobarbital suppressed burying at doses below those that caused visible motor deficit, and Blampied and Kirk [4] found that diazepam did not significantly affect activity scored as ultrasonic field disruption and gross movement. Treit *et al.* did observe that the antipsychotic chlorpromazine reduced motility as well as CDB, and Beninger *et al.* [2] suggested that the suppressive effect of the antipsychotic pimozide on burying was probably due to a "concomitant reduction in general activity" measured as the number of squares traversed during the test session. In other investigations of drug effect on CDB, general activity was not reported quantitatively [7, 8, 18].

Our objective was to assess the specificity and sensitivity of CDB as a screening test for anxiolytics. We tested the benzodiazepine chlordiazepoxide as a typical anxiolytic, the propanediol carbamate meprobamate as a conventional anxiolytic of another chemical class, and buspirone as a novel anxiolytic that seems to be more anxiospecific than earlier drugs in that it is free of such side effects as sedation and euphoria. To examine the question whether CDB rejects nonanxiolytics, we tested the tricyclic antidepressant imipramine, the opiate analgesic morphine, and the antipsychotic chlorpromazine. We chose doses on the basis of published studies showing effects in CDB or other behavioral measures in rats. Two of the three anxiolytics and two of the three nonanxiolytics suppressed burying. We concluded that our version of the test, which differs somewhat in methodology from other versions, lacks therapeutic-class specificity.

METHOD

Subjects

Naive male Long-Evans rats weighing 325–500 g from

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TABLE 1
SUMMARY OF DRUG EFFECTS IN THE CONDITIONED DEFENSIVE BURYING TEST

Drug	Dose* (mg/kg)	Time†	Burying‡	General Activity‡	Reference
Diazepam	0.1	30′	→	no deficit	18
	0.5		↓	noted	
	1		↓		
	2		↓		
	0.5	20′	↓	→	4
	1		↓		
Chlordiazepoxide	1	30′	→	no deficit	18
	3		→	noted	
	6		↓		
Pentobarbital	1	30′	→	no deficit	18
	3		↓	noted	
	6		↓		
Chlorpromazine	1	30′	↓	"rats	18
	2		↓	generally	
	3		↓	immobile"	
	2	60′	↓	not reported	8
Pimozide	1	240′	↓	→	2
Amitriptyline	2	10′	↑	not reported	8
Morphine	1.5	30′	→	not reported	18
d-Amphetamine	1	30′	→	not reported	18
Oxprenolol	10	20′	↓	→	4
	20		↓		
Physostigmine	2	30′	→	not reported	7
Pentylenetetrazol	20	30′	→	not reported	18
Picrotoxin	0.5	30′	→	not reported	18
Diazepam 1 30′ + Picrotoxin 1 15′			→	not reported	20
Naloxone	3		→	not reported	23
	10	10′	→	not reported	21
Diazepam 1 30′ + Naloxone 10 10′			↓	not reported	21

*Route of administration was IP.
†Time is min between injection and testing.
‡↑=increase, ↓=decrease, →=no change.

Charles River Breeding Laboratories, Wilmington, MA, were housed in groups of six or seven in 65×25×18 cm wire mesh cages and tested in the light part of the light/dark cycle (lights on 0600–1800). Food and water were available continuously in home cages.

Apparatus

Habituation, shocking, and testing were done in a 35×35×35 cm Plexiglas chamber. The floor was covered with ground corncob bedding (Bed o'Cobs, Andersons Cob Division, Maumee, OH) to a depth of 5 cm. A piece of Plexiglas 36×36 cm covered the top of the chamber. In the center of one wall, 2 cm above the bedding, was a small hole through which a shock prod could be inserted. The shock prod was a 1-cc plastic syringe (7.0×0.6×0.6 cm) wrapped with 24-gauge copper wire that was connected to a two-pole shocker (Coulbourn Instruments, Lehigh Valley, PA).

Procedure

Experimental design. Subjects were randomly assigned to the following treatment groups (N=8 in each group): buspirone (Bristol-Myers) 8, 16, 32, 64 mg/kg; chlordiazepoxide (Sigma) 4, 8, 16, 32 mg/kg; chlorpromazine (Smith Kline & French) 1, 2, 4, 8, 16 mg/kg; imipramine (CIBA-GEIGY) 4, 8, 16 mg/kg; meprobamate (Wallace) 75, 100, 125 mg/kg; morphine (Mallinckrodt) 2, 4, 8 mg/kg; vehicle-no-shock; and vehicle-shock. Vehicle-shock was compared to vehicle-no-shock to determine the effects of shock on the behaviors measured. Each dose of each drug was compared to vehicle-shock. Because treatment group variances were not homogeneous, the Mann-Whitney U-test was used for all comparisons.

Habituation. Each home-cage group was placed in the test chamber without the shock prod for 2 hr approximately 24 hr before testing. The bedding was cleaned of feces and smoothed to a uniform depth of 5 cm after each habituation period.

Drug administration. Meprobamate was suspended in 0.5% methyl cellulose and injected 60 min before testing. All other drugs were dissolved in 0.9% saline and injected 30 min before testing. Volume of injection was 1 ml/kg body weight.

TABLE 2

EFFECT OF SHOCK ON SIX CATEGORIES OF BEHAVIOR IN THE CONDITIONED DEFENSIVE BURYING TEST

Treatment	N	Mean (± SE) Seconds Engaged in Behavior					
		BURY	DIG	LOC	EAT	GROOM	REST
Vehicle-no-shock	8	2 ± 1*	17 ± 2*	330 ± 25	146 ± 38	84 ± 26*	20 ± 10
Vehicle-shock	8	90 ± 21	51 ± 6	315 ± 19	88 ± 24	35 ± 11	21 ± 9

*$p<0.05$, Mann-Whitney U, vehicle-no-shock vs. vehicle-shock.

Morphine was injected intraperitoneally; vehicle (saline) and all other drugs were injected by gavage.

Shock administration. The shock prod was inserted into the chamber before the test session. The prod delivered a 2-mA shock each time the subject touched it (except for the no-shock group). The subject was placed in the test chamber on the side opposite the shock prod. When the subject crossed back to the side of the chamber opposite the prod after receiving a single shock to its nose, or after the subject received two shocks to its nose without crossing back, the current was turned off and the shock administration period ended. A subject that was not shocked, or was shocked other than on its nose, or appeared not to receive shock upon touching the prod (no flinch response) within 3 min was discarded and replaced.

Behavioral observations. Behavior during the 10 min following shock administration was divided into six mutually exclusive categories and timed with a Radio Shack Model 100 portable computer (Tandy Corporation, Fort Worth, TX): (1) BURY: moving bedding toward the prod with front paws or snout, as described by Treit *et al.* [13,18]; (2) DIG: other behaviors that caused bedding to be displaced (primarily forelimb digging and hindlimb spraying); (3) LOC: continuous walking or rearing, with pauses of less than 2 sec; (4) EAT: chewing on bedding; (5) GROOM; and (6) REST: completely still, or sniffing without locomotion. Ataxia (uncoordinated locomotion, stumbling, falling) was scored as present or absent during the trial. The observer was hidden behind a cardboard blind with a small viewing slit. The test chamber bedding was cleaned of feces and smoothed to a uniform depth of 5 cm after each trial.

RESULTS

Effects of Shock

Vehicle-treated subjects that received shock displayed significantly more BURY and DIG and less GROOM than subjects that did not receive shock. LOC, EAT, and REST were not significantly different between the vehicle-shock and vehicle-no-shock groups (Table 2).

Effects of Drugs

Figure 1 shows the effect of each drug tested on each of the six behaviors.

Anxiolytics. Chlordiazepoxide and meprobamate suppressed BURY and DIG in a dose-related manner, but only meprobamate significantly suppressed BURY at doses that did not also reduce LOC or increase REST. Meprobamate caused no obvious motor disruption. Chlordiazepoxide 16 mg/kg produced ataxia in 9 of 12 subjects, and 32 mg/kg produced ataxia in all subjects. Buspirone 32 mg/kg significantly reduced LOC and GROOM, and 64 mg/kg reduced DIG and LOC and increased REST, but no dose significantly affected BURY. All three anxiolytics significantly increased EAT at two or more doses.

Nonanxiolytics. Imipramine suppressed BURY significantly at the intermediate dose, 8 mg/kg, which did not reduce LOC or increase REST. Morphine suppressed BURY at doses that also reduced DIG, LOC, and GROOM, and in increased REST; four subjects in the 8 mg/kg group did not meet shock criterion and had to be replaced (no more than two subjects from any other group were replaced for this reason). Chlorpromazine did not significantly suppress BURY even at doses that reduced LOC (4 and 16 mg/kg) or increased REST (8 and 16 mg/kg). Of the nonanxiolytics, only the lowest dose of morphine significantly affected EAT.

DISCUSSION

The CDB test for anxiolytics could yield the following results: A true positive is suppression of burying by a clinically effective anxiolytic, a false positive is suppression of burying by a nonanxiolytic, a true negative is no suppression of burying by a nonanxiolytic, and a false negative is no suppression of burying by an anxiolytic. By these criteria, two of three anxiolytics and two of three nonanxiolytics that we tested were positive: Chlordiazepoxide and meprobamate were true positives, and imipramine and morphine were false positives. Chlorpromazine was a true negative and buspirone was a false negative.

However, any drug in sufficient amount will suppress burying. Drugs that cause sedation (e.g., reduce locomotion or increase resting) in addition to suppressing burying cannot thereby be reliably classified as positive, since suppression of burying might be merely a correlate of reduction of activity. If the criterion for true positive is altered to require suppression of burying with no reduction of general activity, the results would change as follows: Chlordiazepoxide, which reduced not only burying but digging, locomotion, and grooming, and increased resting, would be a false negative. Morphine (significant at 4 and 8 mg/kg) would be a true negative, but imipramine, because it suppressed burying without affecting any other behavior (significant at 8 mg/kg), would remain a false positive (unless imipramine were classified as an anxiolytic on the basis of its effectiveness in phobic and obsessive compulsive disorders).

At least two investigators have shown that chlordiazepoxide and diazepam suppress burying at much lower doses than were required to suppress it in this study [4,18]. It

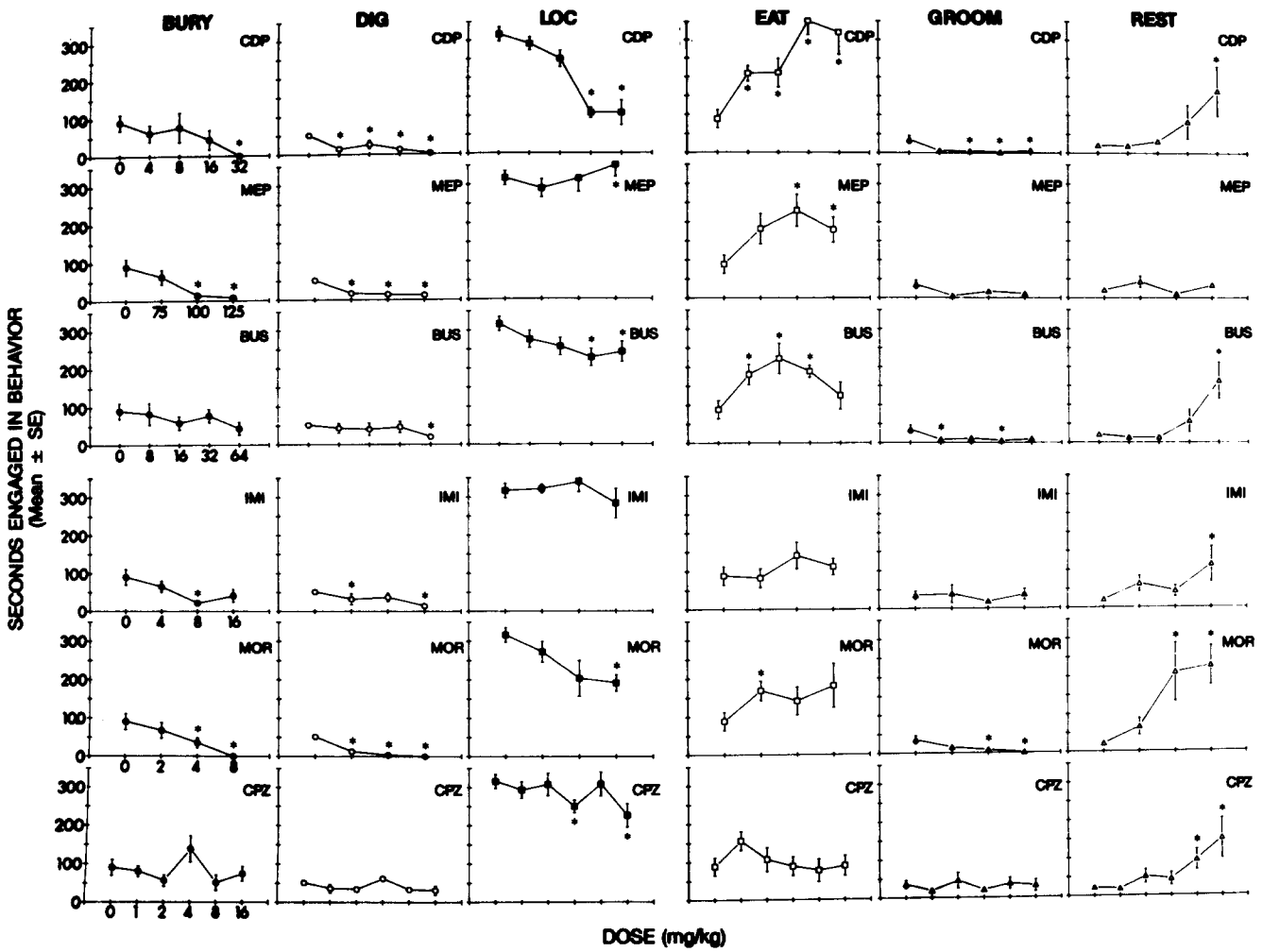

FIG. 1. Effects of anxiolytic and nonanxiolytic drugs on six behaviors in the conditioned defensive burying test. CDP=chlordiazepoxide, MEP=meprobamate, BUS=buspirone, IMI=imipramine, MOR=morphine, CPZ=chlorpromazine. *$p<0.05$, Mann-Whitney U.

is possible that differences in shock method led to the different results. While rats in other studies were reported to have received a single paw shock, nearly all of our subjects contacted the shock prod with the nose, and approximately 75% of the subjects contacted the prod twice rather than once. Treit *et al.* [18] demonstrated that increased shock intensity can override the suppressive effect of chlordiazepoxide on burying. Anderson *et al.* [1] showed that multiple shocks elicit more burying than does a single shock, and Treit *et al.* [19] demonstrated a positive relationship between shock intensity and duration of burying. Although we did not measure actual shock intensity received by each subject, it is possible that our rats received more aversive stimulation than rats in other studies, so that low (nonsedative) doses of chlordiazepoxide failed to suppress burying. However, as noted under "(d) Shock procedure" below, it can be argued that these methodological differences may not explain the different results.

Our data reveal a distinct relationship between burying and digging, which was not unexpected since burying was often immediately preceded by forelimb digging and the associated hindlimb (backward) spraying of bedding on the side of the chamber opposite the prod. Although subjects that were not shocked displayed a little digging, the amount increased significantly with shock; digging may be considered another aspect of the defensive response, as suggested by Hudson [10]. Digging was in fact more sensitive to drugs than burying. Digging was the only behavior measured that was significantly reduced by every drug (except chlorpromazine) at one or more doses. The lowest doses of chlordiazepoxide, meprobamate, imipramine, and morphine significantly reduced digging without affecting burying.

Eating was increased by all three anxiolytics at two or more doses but not by the other drugs (except the lowest dose of morphine). While corncob bedding is not food per se, rats in the test appeared to be ingesting it, and it is likely that the same mechanism by which many anxiolytics enhance food consumption may stimulate ingestion of other substances in the absence of normal food. Benzodiazepine-induced hyperphagia is well documented [3,5], and three nonbenzodiazepine anxiolytics (Meprobamate, zopiclone, and CL 218,872) and a barbiturate (phenobarbital) have been found to increase food consumption [6,15]. Hudson [10] also noted that time spent eating was negatively correlated with

time spent engaged in avoidance behaviors. Oral behavior in the CDB test or in some other environment may be selectively sensitive to moderate doses of anxiolytics.

There were several methodological differences between this and previous studies. (a) Route of administration: Except for morphine, we injected drugs PO (to match the most common clinical route); other investigators have used IP. However, the drugs in the dose ranges we tested are known to produce behavioral effects in rat after PO injection. In a preliminary study, we found that chlorpromazine 2.0 and 4.0 mg/kg IP did not affect burying, although Treit *et al.* [18] found suppression at 1.0 mg/kg IP with the same pretreatment time, 30 min. (b) Origin of subjects: We used rats of the same sex, strain, age, and weight as Treit *et al.*, but ours came from Charles River Breeding Laboratories whereas theirs came from Canadian Breeding Farm. Strain can be important in behavioral work. In a preliminary experiment we found that Blue Spruce rats tended to bury less than Charles River rats—77±9 sec vs. 120±20 sec (mean±standard error). Seasonal variation is also possible. (c) Habituation: We habituated for 24 hr before testing. Treit *et al.* [18] used four daily 30-min periods, Davis *et al.* [8] a single 4-hr period 48 hr before testing, Whiteside and Devenport [23] a single 2-hr period 24 hr before testing, Blampied and Kirk [4] four daily 15-min periods. In a preliminary experiment, we compared one 2-hr period to four daily 30-min periods and found no difference. Within limits, amount of habituation seems not to be critical. (d) Shock procedure: Treit *et al.* [18] used 1-mA paw shock, we used 2-mA nose shock. Burying has been shown to increase with higher shock (3.5, 6.5, and 10 mA, with maximum effect at 6.5 mA [19]). Intensities of 1 and 2 mA could be considered moderate, but it is possible that the difference is great enough to produce a difference in the effect of chlordiazepoxide. However, our control values for burying were similar to those of other investigators using lower shock intensities: furthermore meprobamate at a modest dose, 100 mg/kg, reversed suppression by 2 mA. Whether paw shock and foot shock differ with respect to drug effect is problematic; we found that about 95% of undrugged rats touched the prod with the nose and did not touch it with the paw in the first 5 min of a trial; given that other aversive events induce burying (e.g., light flash, air blast), the behavior seems to have some generality, but it is possible that route of shock alters drug effect.

One unexpected result was the failure of chlorpromazine to suppress burying significantly at doses up to 16 mg/kg PO in our hands, whereas 1.0 mg/kg IP suppressed burying in two other sudies [8,18]. Methodological differences may be at least partly responsible. Doses of 4 and 16 mg/kg did reduce locomotion, and 8 and 16 mg/kg increased resting, so the doses were in the behaviorally active range.

The separation of reversal of burying and reduction of general activity is a critical issue in this model. It is avoided in a recently published procedure called shock probe conflict [11]. Rats were placed in a novel environment containing an electrified probe similar to that used in CDB. Contact with the probe reduced exploration, and conventional anxiolytics reversed this reduction. The nonanxiolytics tested were less effective or ineffective. Buspirone was ineffective.

We concluded that the CDB configuration we used does not have sufficient therapeutic-class specificity as a primary screen for anxiolytics. Methodology, the separation between reversal of burying and reduction of general activity, and the conceptual difficulty of establishing therapeutic-class specificity preclinically and clinically need especially close consideration.

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