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RAPID COMMUNICATION

A Comparison of Buspirone and Chlordiazepoxide in the Shock-Probe/Burying Test for Anxiolytics

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TREIT, D. AND M. FUNDYTUS. *A comparison of buspirone and chlordiazepoxide in the shock-probe/burying test for anxiolytics*. PHARMACOL BIOCHEM BEHAV **30**(4) 1071–1075, 1988.—The effects of chlordiazepoxide (2.5–10.0 mg/kg IP) and buspirone (0.05–1.0 mg/kg SC) were compared by a "blind" observer using the shock-probe/burying test for anxiolytics. Both anxiolytic agents decreased rats' burying behavior toward the continuously electrified (2 mA) shock probe, and increased the number of probe-shocks rats received. These bidirectional, anxiolytic drug effects occurred at doses that did not affect the rats' general activity, and these anxiolytic effects generally increased as a function of drug dose. The relative potency of buspirone was substantially greater than that of chloridazepoxide. These results contrast with those of Craft *et al.* and suggest that inappropriate methodology may have contributed to the inconsistencies in various results. In any case, under the present parameters, this "repeated shock"-probe test appears to have two advantages over the previous, "single shock" procedure. First, increases in probe-shocks and decreases in probe-burying provide two, concurrent measures of anxiolytic drug effects in the same setting. Second, nearly all subjects receive shock in the repeated shock procedure, compared to only 60–80% of subjects in the single shock procedure. Thus, both in terms of behavioral validity and simple economy, the repeated shock-probe procedure warrants further investigation as a selective test of anxiolytic agents.

Anxiolytics Shock-probe test Chlordiazepoxide Buspirone Animal tests Conditioned defensive burying

IN the "shock-probe/burying test" for anxiolytics [cf., (10, 12, 15)], rats are shocked through an electrified probe, and the amount of time that they spend spraying bedding material toward or over the probe (i.e., "burying" behavior) is the measure of fear. A number of studies have shown that low doses of anxiolytic agents (e.g., 5 mg/kg chlordiazepoxide IP) selectively suppress burying behavior elicited by a single, 1-mA shock to the forepaws [e.g., (2, 14, 16)], and this effect can be reversed with the benzodiazepine receptor antagonist Ro 15-1788 (12).

Recently, Craft *et al.* (3,4) used a variation of the shock-probe/burying test in which rats received additional shock each time they recontacted the electrified probe. Although this "repeated-shock shock-probe/burying test" did show some drug-class specificity for anxiolytic agents, the prototypical benzodiazepine anxiolytic, chlordiazepoxide, and the "novel" anxiolytic, buspirone, did not suppress burying behavior in the absence of a conconcurrent suppression of general activity. These results with chlordiazepoxide contrast with the effects of benzodiazepines usually found in the original, "single-shock shock-probe/burying test (2, 12–14, 16), and suggest that procedural variations inherent in the Craft study may have diminished the sensitivity of this test to anxiolytic agents.

In spite of these problems, however, the repeated shock-probe test appears to be relatively easy to administer, with very low subject attrition. Only a few subjects fail to receive shock within 5 min (4), whereas in the single-shock proce-

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dure, 20–40% of all drugged subjects cannot be shocked within a five min criterion, and therefore they cannot be tested (unpublished observations). In view of these economic considerations, and the questionable generality of the shock-probe/burying test for anxiolytic agents, it seemed appropriate to carefully reevaluate the effects of chlordiazepoxide and buspirone under conditions which might enhance the sensitivity of the repeated shock procedure to anxiolytics (i.e., more standard methodology: separate placebo control groups, blind observation controls, 4 days of pretest habituation, and a nonsedating range of anxiolytic doses).

EXPERIMENT 1

The general purpose of the first experiment was to characterize a number of behavioral parameters under which rats reliably show burying behavior to a constantly electrified shock-probe. Characterization of these parameters is necessary in order to provide a baseline against which the effects of anxiolytic agents can be reliably detected. The validity of the Craft *et al.* procedures is questionable because only a single, shocked control group of 8 animals was used to compare multivariate behavioral effects in 176 drugged animals. In addition to the statistical problem of nonindependence of error variance (7), it is difficult to know whether the behavior of this particular sample of 8 animals was representative of the actual population of rats responding under the baseline conditions of the Craft study. On the other hand, the validity of a number of standard procedures used in "traditional" probe-shock studies, such as distributed habituations, limited pretest isolation, and a single shock to the forepaw, has not been systematically established.

Accordingly, the first purpose of the Experiment 1 was to investigate whether the amount of time that rats bury a continously electrified probe varies as a function of the number of pretest habituations [cf., (11)]. A second purpose was to determine the effects of individual housing on rats' burying behavior. A final purpose was to correlate shock intensity with the duration of burying in the repeated shock-probe test.

METHOD

Subjects

One hundred and twenty-eight naive, male (250–350 g) Sprague-Dawley rats served as subjects. The rats were individually housed in wire-mesh cages for 3 to 7 days before the beginning of each test, with the exception of one experimental condition in which rats were either individually housed or group housed for 30 days before the test. Rats had continuous access to rat chow and water, under a 12 hr on/12 hr off light cycle (lights on 6:00 a.m.). All testing took place during the light period, between 9:00 and 16:00 hr.

Apparatus

A separate testing room contained the 40×30×40 cm Plexiglas test chamber. The chamber floor was evenly covered with 5 cm of odor-absorbent kitty litter. The chamber was cleaned of feces after each session and the litter was changed each day. These cleaning procedures seemed at least minimally adequate in the light of a recent study showing that the odor of stressed rats can inhibit the burying response of other rats (17).

In the center of the front wall of the Plexiglas chamber, 2 cm above the bedding material, was a small hole through which a 6.5×0.5×6.5 cm wire-wrapped probe could be inserted. Electric current was administered through the two copper wires wrapped around the probe. Shock intensity could be adjusted with a variable resistor in series with a 1000 V shock source. Shock intensity was confirmed by oscilloscope in a separate group of rats and set at 2 mA, the same intensity as that used by Craft. The behavior of each rat was recorded on video tape via closed circuit television.

Procedures

The rats were randomly assigned to a number of groups which differed with respect to habituation, handling, and housing conditions. First, four different groups (n=15) of individually housed rats were handled for 3 days then habituated in squads of three to the Plexiglas chamber for either 1, 2, 3, or 4 days before the probe test, with the total amount of habituation 120 min for all groups. Second, to examine the possible interactive effects of handling and habituation, two other groups of rats (n=24) were habituated in squads of three for 120 min on either 1 day or 4 days (30 min per day), and half of each of these groups was either handled or not handled on the 3 days just prior to the habituation phase. Finally, to study the effects of isolation, two other groups (n=10) were housed for 30 days before the test, either in groups of five in 65×25×18 cm wire-mesh cages, or individually in 18×20×25 cm wire-mesh cages, and then habituated for 30 min on each of the 4 days before the test.

Just before the test, the shock-probe was inserted 6 cm into the Plexiglas chamber and fixed there. Each animal was placed individually in the chamber facing away from the probe. When the rat touched the constantly electrified probe with either a forepaw or its snout, it received a brief, 2-mA shock, terminated by its withdrawal. The number of shocks each rat received was counted, and the intensity of the behavioral reaction of each animal to each shock was rated on a four-point scale, as previously described (8). Following the first shock, the duration of time that each rat sprayed bedding material toward or over the probe (i.e., "burying behavior") was measured for 10 min [e.g., (15)].

RESULTS AND DISCUSSION

The amount of burying increased as the number of days of habituation increased. Analysis of variance showed that this facilitative effect of habituation was significant, $F(3,56)=3.51$, $p<0.02$, and Newman-Keuls comparisons confirmed that rats given 4 days of habituation (mean=68.7 sec, SEM=20.2) buried significantly more than did rats given 1 day (mean=18.1 sec, SEM=6.45) or 2 days (mean=15.9 sec, SEM=5.9). Although rats habituated for 3 days (mean=48.9, SEM=15.7) buried more than rats habituated for 1 or 2 days, this difference was not significant. Thus, the 1-day habituation procedure used in the Craft study, although time-saving, does not always produce robust baseline burying behavior to a constantly electrified probe [cf., (11)]. The low levels of burying produced by the 1-day habituations could easily result in a baseline against which a suppressive effect of anxiolytic drugs on burying behavior could not be reliably detected.

This facilitative effect of distributed habituations on shock-probe burying appeared to depend on previous handling: The group given 3 days of handling and 4 days of habituations buried the probe more than groups given either

fewer habituations or less handling. These results were confirmed by a 2 by 2 ANOVA, which showed a significant main effect for habituation, $F(1,44)=4.00$, $p<0.051$, a significant main effect for handling, $F(1,44)=4.62$, $p<0.04$, and a significant interaction between habituation and handling, $F(1,44)=4.90$, $p<0.03$. Newman-Keuls comparisons confirmed that the group given both 3 days of handling and 4 days of habituation (mean=77.8 sec, SEM=20.0) buried the probe significantly more than other groups given either less handling (1 day habituation: mean=24.5 sec, SEM=5.7; 4 days habituation: mean=23.7, SEM=9.5), or less habituation (3 days handling: mean=21.7, SEM=10.4).

Although the expectation prior to Experiment 1 was that rats isolated for 30 days would react more to the shock probe than rats housed in groups for 30 days, in fact, the results were just the opposite: individually housed rats (mean=9.9 sec, SEM=5.6) buried the probe significantly less, $t(18)=2.6$, $p<0.02$, than group-housed rats (mean=49.3 sec, SEM=13.9). These preliminary results suggest that more robust baseline levels of burying will occur if rats are not isolated for long periods of time before the shock-probe test.

Finally, the correlation coefficients overall between the duration of burying and shock number (r=.028), and between the duration of burying and the sum of rated shock intensities, (r=.107) were nonsignificant. These results contrast with those found in other studies in which shock intensity has been manipulated independently of the rats' behavior (15), and suggest that variations in the aversiveness of a constantly electrified probe set at 2-mA is not a robust factor in rat burying behavior.

In summary, the results of the Experiment 1 clarify the importance of a number of procedural variables and their effects on baseline burying behavior in the repeated shock-probe procedure. First, rats that have received 30-min habituations on each of 4 consecutive days before the test bury the probe more reliably than rats that have been habituated for the same total amount of time but only on 1 day. Second, the facilitative effect of distributed habituations on probe-burying appears to intereact with pretest handling because the group given 3 days of prior handling appeared to benefit most from four distributed habituation periods. Third, rats that have been isolated for 30 days prior to the shock-probe test bury significantly less than rats that have been group housed for 30 days. Finally, variations in the number or intensity of shocks received by rats in the repeated shock-probe procedure are not linearly related to the amount that rats bury. This result contrasts with previous studies, and suggests that variations in shock aversiveness using a continuous shock procedure may not be so extreme as to obscure true drug effects.

EXPERIMENT 2

The purpose of Experiment 2 was to reassess the effects of chlordiazepoxide and buspirone in the repeated shock-probe test. Of particular interest in this study was whether chlordiazepoxide or buspirone at a moderate range of doses (i.e., 2.5–10 mg/kg; 0.05–1.0 mg/kg, respectively), and under more "optimal" test parameters, could suppress burying behavior in the absence of a suppressive effect on general activity. The doses of chlordiazepoxide and buspirone used in the Craft study (4–32 mg/kg and 8–64 mg/kg, respectively) were well within the range known to produce obvious motor impairments (5, 6, 9). An additional question was whether anxiolytic-induced deficits in passive avoidance of an electrified shock-probe (10,14) could be replicated in the standard "burying" apparatus. Selective decreases in probe-burying concurrent with increases in probe-contacts would provide strong, convergent evidence of "anxiolytic" drug effects.

METHOD

The 120, naive male, 250–350 g rats in Experiment 2 were housed and handled individually for three days immediately after their arrival in the colony, then given 4 consecutive days of 30 min habituations to the Plexiglas test chamber, and then tested on day 5 with the shock probe constantly electrified at 2 mA.

On the test day, the rats were randomized to groups (n=12) and injected with either 0, 2.5, 5, 7.5, or 10 mg/kg of chlordiazepoxide (CDP) intraperitoneally, or 0.0, 0.05, 0.1, 0.5, or 1.0 mg/kg of buspirone subcutaneously [cf., (9)]. Both CDP and buspirone were dissolved in physiological saline (the "0" dose control), and injected at a constant volume of 1 ml/kg. Thirty min after the rats were injected, they were tested in the repeated shock-probe test. The behavior of these rats was measured for 10 min, beginning with the first shock, by two observers who were "blind" with respect to the drug condition of the rats [cf., (4)].

In addition to burying behavior and shock number, the duration of any other behavior (e.g., locomotion, digging, investigation, rearing, grooming, chewing) was measured in order to assess drug effects on rats' general activity. The interobserver reliability coefficients for burying, shock number, and general activity were .98, .99, .97, respectively. Ataxia was noted in only 2 of the 120 animals tested (CDP 10 mg/kg).

RESULTS AND DISCUSSION

As can be seen in Fig. 1 (top panels), CDP produced a decrease in the amount of time rats buried the probe, an increase in the number of shocks rats received, and no apparent change in general activity. These results were confirmed with ANOVAs, which showed a significant effect of CDP on probe-burying, $F(4,55)=7.01$, $p<0.001$, and on probe shocks, $F(4,55)=4.58$, $p<0.003$, but no significant effect on general activity, $F(4,55)=1.92$, $p>0.01$. Newman-Keuls comparisons confirmed that the suppressive effect of CDP on probe-burying was significantly greater than saline at every dose except 2.5 mg/kg. In addition, the three highest doses of CDP produced a significantly greater suppression of burying than did the 2.5 mg/kg dose. A parallel set of Newman-Keuls comparisons of the probe-shock data produced similar, but opposite results. CDP induced a significant increase in the number of probe-shocks, compared to saline control, at 5 and 7.5 mg/kg. The increases at 2.5 and 10 mg/kg were not significantly different from control, and 5.0 and 7.5 mg/kg produced a significantly greater increase than 2.5 or 10 mg/kg CDP. No other differences between means was significant. Thus, at doses of CDP that did not change rats' general activity (5 and 7.5 mg/kg), there was a significant decrease in probe-burying and a significant increase in probe shocks. These bidirectional anxiolytic effects corroborate one another, and strengthen the internal validity of the shock-probe test.

Figure 1 (bottom panels) shows that buspirone produced basically the same results as did CDP: a decrease in burying, and an increase in shocks, at doses that did not change general activity (i.e., 0.1 and 0.5 mg/kg). These results were

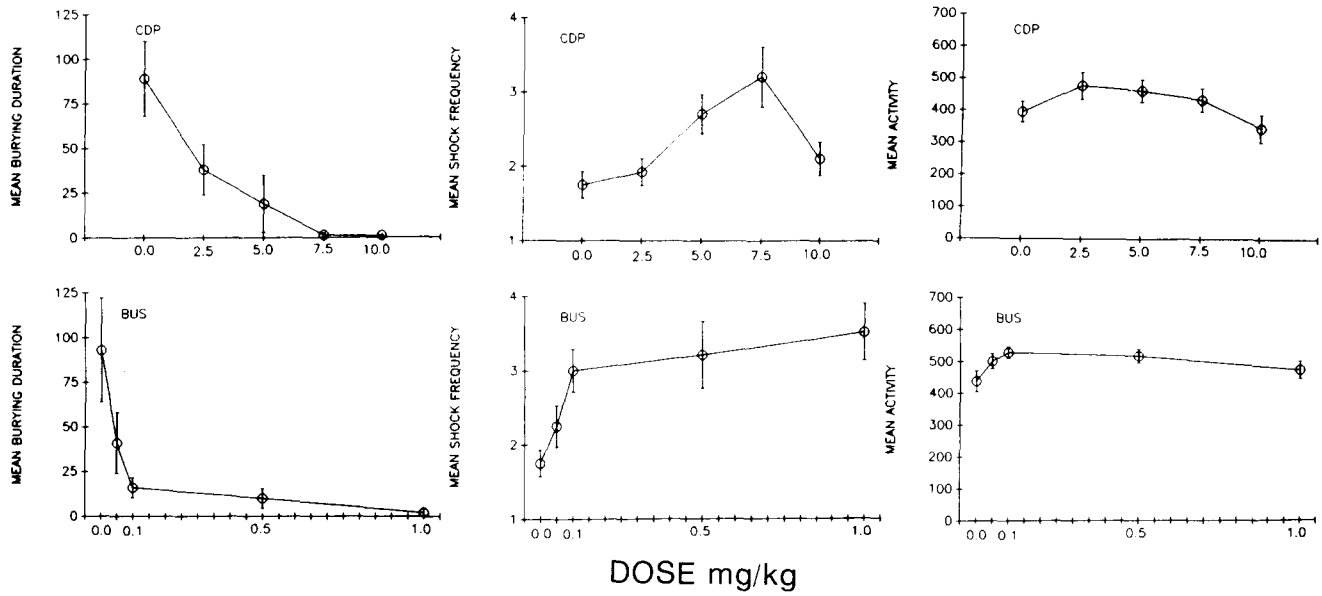

FIG. 1. Mean (±SEM) duration of burying (left panels) and general activity (right panels), and mean frequency of probe-shocks (center panels), as a function of chlordiazepoxide (2.5–10 mg/kg IP, top panels) or buspirone (0.05–1.0 mg/kg SC, bottom panels).

confirmed with ANOVAs, which showed a significant effect of buspirone on probe shocks, $F(4,55)=4.88$, $p<0.002$, and on probe burying, $F(4,55)=5.48$, $p<0.001$, but not on general activity, $F(4,55)=2.02$, $p>0.1$. Newman-Keuls comparisons showed that the suppressive effect of buspirone on burying was significant, compared to saline, at 0.1, 0.5, and 1.0 mg/kg. In addition, the suppressive effect of the two highest doses was significantly greater than that of the two lowest doses. No other effects on burying were significant. Buspirone, like CDP, produced a dose-related increase in probe-shocks, with significance reached at 0.1, 0.5 and 1.0 mg/kg. Furthermore, the three highest doses produced a significantly greater increase in probe-shocks than did the 0.05 mg/kg dose. No other differences between these means were significant.

Although comparisons of relative potency in the present study are complicated by differing routes of drug administration (IP vs. SC), the relative potency calculated (7) for buspirone in suppressing burying ($ED_{50}=0.33$ mg/kg) was substantially greater than that for chlordiazepoxide ($ED_{50}=4.9$ mg/kg). Further studies, in which route of drug administration is held constant, may confirm that buspirone is more potent than CDP in the shock-probe paradigm. Finally, as had been found in the previous experiment, the correlation coefficients between shock number or summed shock intensity and probe-burying were nonsignificant ($r=.105$; $r=.173$).

GENERAL DISCUSSION

These results are inconsistent with those of Craft *et al.* (3,4) and suggest that their failure to show a selective suppressive effect of CDP and buspirone on burying in the shock-probe paradigm, as well as perhaps other anomalous results, were due to a lack of distributed pretest habituations and to a behaviorally sedating range of anxiolytic drug doses. At the same time, the present results suggest that at the parameters used in the present study, the "repeated" shock-probe procedure may ultimately prove to be a far more efficient screen of anxiolytic agents than the original, single shock procedure. In addition to its economy, the test appears to provide convergent behavioral validation of anxiolytic drug effects, since the number of shocks drugged rats incur is increased at the same time as the amount of burying they display is decreased. These results also confirm and extend earlier studies of the effects of anxiolytics on simple spatial passive avoidance of a shock-probe (10,14). In view of these promising initial results, current studies are underway in order to further characterize the drug-class specificity of this "repeated-shock" shock-probe test of anxiolytic agents.

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