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Preliminary Report of a Simple Animal Behavior Model for the Anxiolytic Effects of Benzodiazepines

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CRAWLEY, J. AND F. K. GOODWIN. *Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines*. PHARMAC. BIOCHEM. BEHAV. 13(2) 167–170, 1980.—A simple system is described to analyze the possibility that increased exploratory behavior is an index for the anxiolytic effects of benzodiazepines in laboratory rodents. Mice were allowed free run in a two-chambered arena, where two-thirds of the area was illuminated and one-third was darkened. The two chambers were separated by a black partition equipped with photocells across the opening, and the entire cage rested on an Animex activity monitor. Transitions across the partition between the light and dark chambers, and total Animex locomotor activity, were increased by clonazepam and chlordiazepoxide, in dose-dependent ranges consistent with previously reported behavior models. The increased exploratory activity with benzodiazepines does not appear to be a non-specific increase in general motor activity, as locomotion in clonazepam and chlordiazepoxide treated mice placed in a bare, undifferentiated cage was not significantly different from vehicle treated mice.

Benzodiazepines Locomotion Anxiolytic Sedation Mouse behavior
Light⇌dark chamber transitions Exploration

THE recent discovery of specific benzodiazepine receptor sites in brain [16,19], along with reports of substances which may be endogenous ligands for, or modulators of, these receptors [1, 6, 7, 12, 13, 17], has stimulated a renewed interest in the mechanism of action of the benzodiazepines. Currently available animal behavior models for the evaluation of the anti-anxiety properties of benzodiazepines and related compounds include the conflict test [5, 8, 14, 23], social interaction under varied levels of illumination [3, 4, 20], and isolation-induced male mouse fighting behavior [11,22]. While these are sensitive and specific, the conflict test requires considerable individual animal training procedures, and the social behavior tests involve extensive human observation time. A single-trial, single-index, automated paradigm would be of advantage for pharmacological studies of benzodiazepine actions, where large sample size for a series of drugs and dosages is needed. The new model described here is based on the ethological view of shifting relative propensities to explore and to retreat from an unknown space [10]. It appears to be rapid and simple, with the advantages of quick and accurate assessment, simplicity of instrumentation, no requirements for prior animal training protocols, and lack of assumptions about the animal's pain threshold or appetite. The model is based on observations that, although nocturnal rodents such as mice and rats will naturally tend to explore a novel environment, open fields appear to have aversive properties which inhibit rodent exploratory behavior [2, 18, 21]. We find that benzodiazepines produce a dose-dependent facilitation of exploratory behavior between a lighted open field and a dark enclosure, presumably by inhibiting avoidance.

Figure 1 illustrates the testing apparatus, consisting of light and dark chambers divided by a photocell-equipped border. A polypropylene animal cage, 44×21×21 cm, was darkened with black spray paint over one-third of its surface. A partition containing a 13 cm long×5 cm high opening separates the dark third from the bright two-thirds of the cage. Fluorescent light from a desk lamp above the cage provides the only room illumination. The cage rests on an Animex activity monitor (Type M, Farad Electronics, LKB, Hagersten, Sweden) which counts total locomotor activity. An electronic system automatically counts transitions across the partition and clocks the amount of time spent in the light and dark compartments, using four set of photocells across the partition.

Male NIH(s) albino general purpose mice, 18–25 g, were individually tested in ten minute sessions in the apparatus described above. All mice were placed in the light compartment to initiate the test session. Testing was performed between 2 p.m. and 6 p.m. Mice were naive to the apparatus, and had no previous drug treatment. Drugs were administered intraperitoneally, 30 min before testing, five mice per

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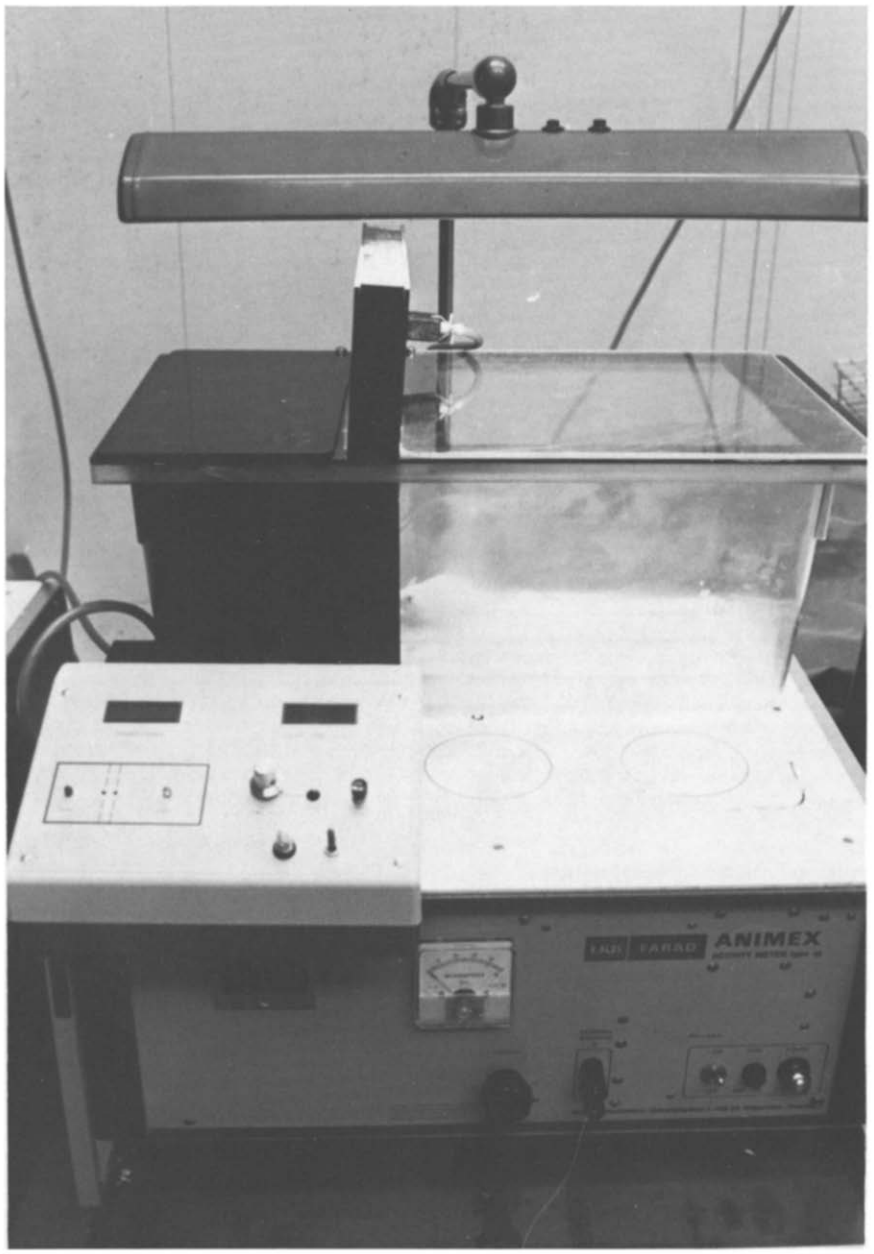

FIG. 1. Electronic system for monitoring locomotor and exploratory behavior of small rodents. A polypropylene animal cage, 44×21×21 cm, darkened with black spray paint over one third of its surface, illuminated by a fluorescent desk lamp over the lit two-thirds of the cage, sits on an Animex activity meter, which counts total locomotor activity over the entire cage. The dark chamber is separated from the lighted chamber by a black partition, 3 cm wide, with an opening 13 cm long and 5 cm high. Photocells across the opening detect number of times the animal crosses the partition (transitions), and control timers for length of time spent in each compartment.

dose for each drug. Clonazepam and chlordiazepoxide (Hoffmann LaRoche, Nutley, NJ), were dissolved in a vehicle consisting of 2% ethyl alcohol, 4% propylene glycol in phosphate buffered saline pH 7.2.

A separate group of mice were individually tested for Animex locomotion in ten minute sessions in an unpainted, unpartitioned animal cage, 44×21×21 cm, resting on the Animex activity monitor. Clonazepam, 0.2 mg/kg, chlordiazepoxide, 5.0 mg/kg, or vehicle was administered intraperitoneally 30 min before testing. Half of each group was tested in the bare cage with the room lights on, and half were tested in the bare cage with the room lights off.

Figures 2 and 3 demonstrate a dose-dependent increase in total locomotor activity and in transitions across the partition between the light and dark compartments with both clonazepam and chlordiazepoxide as compared to vehicle controls, using two-tailed t-test statistical analysis. The significant dose range for clonazepam was 0.05–1.0 mg/kg. The

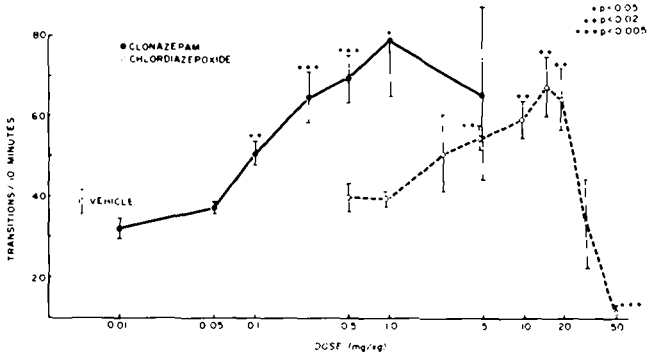

FIG. 2. Total number of transitions across the partition between the light and dark chambers of Fig. 1. Clonazepam in the dose range 0.1–1.0 mg/kg, administered intraperitoneally 30 min before testing, significantly increased the number of transitions in a ten-minute session. Chlordiazepoxide in the dose range 5–20 mg/kg intraperitoneally 30 min before testing significantly increased the number of transitions in a ten-minute test session.

TABLE 1

TIME SPENT IN THE DARK ONE-THIRD OF THE TEST APPARATUS

Treatment	Time in dark chamber per ten-minute session (minutes ± SEM)
Vehicle	5.2 ± 0.6
Clonazepam (mg/kg IP)	
0.001	4.2 ± 0.2
0.05	5.2 ± 0.5
0.1	4.2 ± 0.2
0.25	4.3 ± 0.7
0.5	4.3 ± 0.7
1.0	5.9 ± 0.6
5.0	4.5 ± 0.4
Chlordiazepoxide (mg/kg IP)	
0.5	4.8 ± 0.2
1.0	4.9 ± 0.3
5.0	5.0 ± 0.5
10.0	5.2 ± 0.8
15.0	4.7 ± 0.6
20.0	5.0 ± 0.6
50.0	7.6 ± 0.8*

Vehicle control values were 5.2 ± 0.6 min in the dark per ten-minute session. Dark times for vehicles and for all drug doses were significantly greater than 3.3 min dark/10 min session, which would be the predicted dark time if no preference existed for the dark one-third. (*$p<0.05$).

significant dose range for chlordiazepoxide was 2.5–20 mg/kg. These doses are consistent with the effective dose for increased punished responding in the conflict test [8,23], and well below the sedative range [9]. Sedation was seen in the present model at chlordiazepoxide doses of 20–50 mg/kg, where locomotion and exploration significantly decreased and time spent in the dark chamber significantly increased. Clonazepam appeared to be at least twenty times as potent

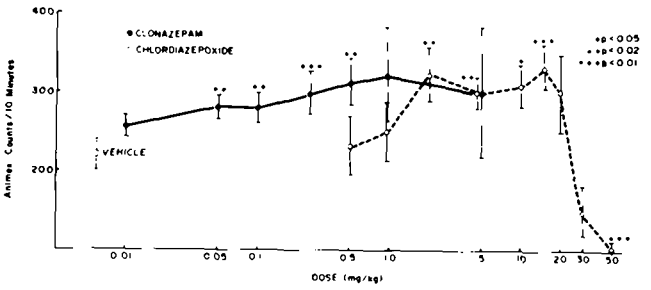

FIG. 3. Total Animex locomotor activity counts during the ten-minute session in the apparatus pictured in Fig. 1. Clonazepam in the dose range 0.05–1.0 mg/kg and chlordiazepoxide in the dose range 2.5–20 mg/kg significantly increased locomotor activity.

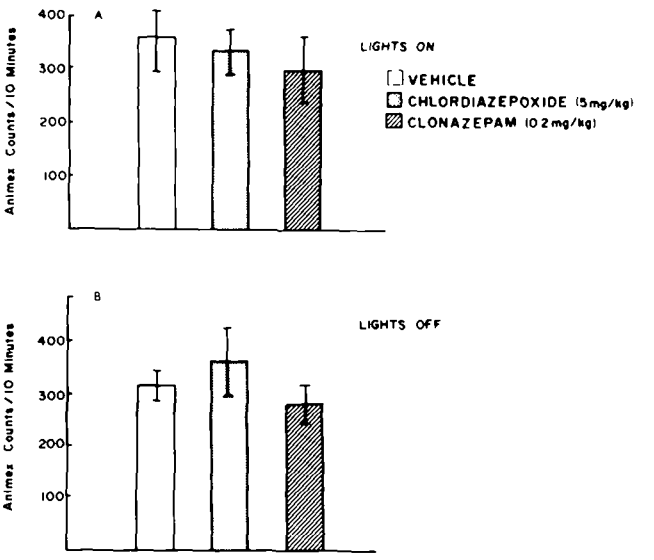

FIG. 4. Total Animex locomotor activity counts during a ten-minute test session in a bare, undifferentiated cage. A: house lights on; B: house lights off. Clonazepam and chlordiazepoxide were not significantly different from vehicle controls at doses which increased exploration and locomotion in the two-chambered apparatus.

as chlordiazepoxide in increasing exploration and locomotion, a relationship relatively consistent with the ranking of these compounds in their benzodiazepine receptor binding affinity [19] and in their therapeutic effects.

The clonazepam and chlordiazepoxide doses which increased locomotor activity and light⇌dark transitions in Figs. 2 and 3 did not appear to increase locomotor activity in a bare and undifferentiated cage (Fig. 4), which was either uniformly illuminated or uniformly darkened. No significant increase in total Animex locomotion was found with chlordiazepoxide 5 mg/kg or clonazepam 0.2 mg/kg, doses found to increase exploration and locomotion in the two-chambered apparatus. These results suggest that in the proposed model, benzodiazepines may be specifically affecting exploratory behavior rather than having a generalized effect on motor activity.

Table 1 shows no significant differences in amount of time spent in the dark third of the arena at any of the effective

drug doses. All mice used in this study displayed a preference for the dark chamber, with amount of time in the dark ranging from 4.2 to 5.9 min in the dark per ten minute session. All values were significantly above the predicted 3.3 min in the dark per 10 min session, hypothesized on the basis of random activity over the dark one third and the light two-thirds of the arena. A blind strain of mice, C3H/HeN, showed exactly this 1/3 : 2/3 dark : light time ratio (unpublished observation). Therefore, while benzodiazepines do not appear to change the dark preference, they do appear to increase locomotor and exploratory behavior in response to some property of the two-chambered apparatus.

The significant property may be the novelty of two communicating chambers with different characteristics, promoting exploratory behavior in the presence of benzodiazepines. Further investigation is needed to clarify whether increased light⇌dark transitions and locomotion with benzodiazepine treatment represents true exploratory behavior, e.g., will benzodiazepines increase exploratory behavior to a variety of novel stimuli such as pheromone markings, or unusual foodsources? Increased exploratory tendencies over many stimulus modalities would be consistent with the benzodiazepine-induced increase in the social interaction test [4], and the hole-board exploratory test [21]. Studies are in progress to further test these behaviors for their specificity to animal "anxiety."

The model proposed herein may provide a rapid, simple test for pharmacological studies of drug effects on exploratory activity. The single measure of transitions across the barrier between a two-chambered apparatus might be a simple index of such an increase in exploratory tendencies of benzodiazepines. Studies are in progress to test other benzodiazepines along the potency spectrum, as well as other psychoactive drugs, to determine the pharmacological specificity of this proposed "exploratory" animal behavior model for the anxiolytic action of benzodiazepines.

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