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Neuropharmacologic Specificity of a Simple Animal Model for the Behavioral Actions of Benzodiazepines

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CRAWLEY, J. N. *Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines.* PHARMAC. BIOCHEM. BEHAV. 15(5) 695–699, 1981.—A simple model system for the behavioral actions of benzodiazepines is analyzed for its dose-response predictiveness, using several benzodiazepines, and for its pharmacological specificity, using other non-anxiolytic classes of psychoactive drugs. The model demonstrates an increase in mouse exploratory activity between a brighty-lit open field and a small, dark compartment by clonazepam, diazepam, flurazepam, chlordiazepoxide and meprobamate, but not by the peripheral benzodiazepine R05-4864, nor by clorgyline, butriptyline, or chlorpromazine.

Benzodiazepines Anxiolytics Antidepressants Neuroleptics Exploration Locomotion Sedation Mouse behavior

THE mechanism of the anxiolytic action of the benzodiazepines has acquired renewed attention with the recent discovery of specific benzodiazepine receptor sites in brain [26,34] followed by reports of putative endogenous ligands or modulators of these receptors [2, 14, 17, 22, 23, 27, 28, 29]. The behavioral effects of benzodiazepines have been modeled in several well documented animal behavior paradigms, including conflict procedures [13, 18, 24, 31, 39] punished crossings [1] social interactions [36], especially under varied levels of illumination [9,12] exploration of novel environments [5, 25, 30] and isolation-induced male mouse fighting [21,37]. These paradigms have the advantages of sensitivity and specificity, but the disadvantages of requiring lengthy animal training procedures and/or considerable human observation time. Pharmacological studies of benzodiazepine actions, using large sample sizes and many drug dosages, would be greatly aided by a single-trial, single index, automated paradigm. We have recently proposed such a paradigm which appears to reflect benzodiazepine activity on a simple and naturalistic rodent behavior [6]. Mice tend to explore a novel environment, but to retreat from the aversive properties of a brightly-lit open field. In a two-chambered system, where mice could freely move between a brightly-lit open field and a dark corner, mice showed more crossings between the two chambers and more locomotor activity after treatment with the benzodiazepines, clonazepam, diazepam, and chlordiazepoxide [6,7]. When mice were tested in an empty, clear polypropylene cage, there was no significant difference between benzodiazepine-treated and vehicle-treated mice [6]. The benzodiazepine effect on activity was not manifested when the empty cage was placed in either a lighted room or a darkened room [6]. Thus, the two-chambered apparatus appears to promote an increase in exploratory activity with benzodiazepine treatment which is not a generalized motor effect, but may be a function of the novelty of two connecting chambers with different characteristics [6].

A more thorough analysis of the pharmacologic specificity of this behavioral model for benzodiazepines is presented herein. A wide dose range for five benzodiazepines of varying potencies and sites of action is tested, with particular attention to the dose-window for changes in behavioral activity as opposed to the sedative-hypnotic doses. Other categories of psychoactive drugs are tested, including non-benzodiazepine anxiolytics, antidepressants and an antipsychotic. Correlations are described between the increase in transitions between the two chambers and the increases in exploratory rearings and locomotor activity. The results of these studies support the possibility that a single, automated parameter may be sufficient to describe the increase in exploratory-like behavior in mice as a model for the behavioral effects of benzodiazepines.

METHOD

Male NIH(s) albino general purpose mice, 18–25 g, were individually tested in ten minute sessions in apparatus previously described [6]. In brief, a polypropylene animal cage was divided into two compartments. One compartment consists of one third of the cage blackened on all surfaces; the other compartment being the two thirds of clear polypropylene, highly illuminated by a fluorescent lamp. A black Plexiglas partition containing a 13 m long × 5 cm high opening divides the two compartments. Four sets of photocells

across the partition opening are connected to an electronic system which counts transitions across the partition. The four sets of photocells are sequentially programmed to exclude head pokes and to distinguish transitions entering and exiting the dark chamber. The entire cage rests on an Animex activity monitor (Type M, Farad Electronics, LKB, Hagersten, Sweden), which counts total locomotor activity. Mice were placed in the lighted compartment to initiate the test session. Testing was performed between 1 p.m. and 5 p.m. Mice were naive to the apparatus and had no previous drug treatment. Six animals per group were used for each drug at each dose. All drugs were administered intraperitoneally, 30 minutes before testing. The benzodiazepines, clonazepam, diazepam, chlordiazepoxide, and R05–4864 (Hoffman LaRoche, Nutley, NJ) and meprobamate (Wallace Laboratories, Cranbury, NJ) were dissolved in a vehicle consisting of 2% ethyl alcohol, 4% propylene glycol in phosphate buffered saline pH 7.2. Flurazepam (Hoffman LaRoche, Nutley, NJ) pentobarbitol (Diamond Labs, Inc., Des Moines, IA), clorgyline (May and Baker Pharmaceuticals, Dagenham, U.K.) butriptyline (Ayerst Laboratories, Montreal, Canada), and chlorpromazine (Smith, Kline, Philadelphia, PA) were dissolved in 0.9% saline. All drugs were injected in volumes of 5 ml/kg.

RESULTS

The benzodiazepines clonazepam, diazepam, flurazepam and chlordiazepoxide increased both the number of transitions across the partition and total locomotion, as seen in Table 1. Observation of these mice during the test session revealed well coordinated locomotion, rearing, and sniffing of all cage walls and surfaces of the partition. The lowest dose producing the behavioral increases was 0.1 mg/kg for clonazepam, 0.5 mg/kg for diazepam, 1.0 mg/kg for flurazepam, and 5.0 mg/kg for chlordiazepoxide. These doses are the same as or less than the mean effective doses for the benzodiazepines on conflict tests in rats [19], the social interaction test under varied conditions of illumination [11], an acoustic startle paradigm [8], and hole-board exploration [31]. The rank-order potency relationship is consistent with the receptor binding and clinical rank-order potencies of the benzodiazepines: clonazepam > diazepam > flurazepam > chlordiazepoxide.

The sedative effect, consisting of a significant reduction in transitions and locomotion, occurred at doses of 25 mg/kg for diazepam, 50 mg/kg for chlordiazepoxide, and 20 mg/kg for R05–4864, in this paradigm, consistent with other reports [35]. At high doses, mice were observed to move very slowly and to show long pauses without movement, often accompanied by piloerection and partially closed eyes. These signs were variably present beginning at dose levels above 5.0 mg/kg for clonazepam, at 10.0 mg/kg for diazepam, at 20.0 mg/kg for flurazepam, and at 30.0 mg/kg for chlordiazepozide, with larger standard errors often seen at these borderline doses. The sedative doses provided an upper limit for each dose-response curve, ensuring that the behavioral effects of interest were obtained at doses well below the sedative-hypnotic range.

The benzodiazepine R05–4864, which binds to peripheral but not to brain benzodiazepine receptors [4] did not increase locomotion or transitions at 5.0 and 10.0 mg/kg, suggesting that the behavioral effects observed with clonazepam, diazepam and chlordiazepoxide are related to a central nervous system action rather than a generalized response to peripheral benzodiazepine actions.

TABLE 1

INCREASES IN MOUSE EXPLORATORY AND LOCOMOTOR BEHAVIOR AFTER TREATMENT WITH THE ANXIOLYTIC BENZODIAZEPINES CLONAZEPAM, DIAZEPAM, FLURAZEPAM, AND CHLORDIAZEPOXIDE BUT NOT WITH RO5-4864, WHICH BINDS TO THE PERIPHERAL BENZODIAZEPINE RECEPTOR

Dose (mg/kg)	Exploratory Transitions (Mean ± S.E.M.)	Animex Locomotion (Mean ± S.E.M.)
Vehicle	35.0 ± 4.0	226 ± 24
Clonazepam		
0.01	32.0 ± 2.6	257 ± 10
0.1	50.4 ± 2.8*	280 ± 16*
0.5	69.2 ± 6.0‡	315 ± 30*
5.0	65.6 ± 20.4	301 ± 83
Diazepam		
0.5	38.3 ± 3.8	186 ± 28
0.5	60.5 ± 6.4	192 ± 27
2.0	68.2 ± 4.1†	390 ± 25†
5.0	95.4 ± 15.6†	295 ± 35*
10.0	28.0 ± 20.0	128 ± 90
25.0	17.8 ± 6.6*	80 ± 5†
Flurazepam		
0.5	34.7 ± 5.2	235 ± 26
1.0	52.2 ± 4.2†	308 ± 28*
5.0	50.3 ± 3.2†	450 ± 22‡
10.0	55.0 ± 5.1†	368 ± 43*
20.0	61.2 ± 11.8*	309 ± 66
Chlordiazepoxide		
0.5	39.8 ± 3.3	230 ± 38
2.5	51.2 ± 9.8	323 ± 37*
5.0	54.8 ± 2.5†	301 ± 20*
10.0	67.8 ± 6.9†	332 ± 30*
30.0	33.6 ± 11.1	146 ± 31.7*
50.0	<1‡	<14.5‡
RO5-4864		
5.0	32.0 ± 7.1	239 ± 51
10.0	35.2 ± 2.9	224 ± 19
20.0	6.0 ± 1.3‡	49 ± 6‡

N=6 for each dose of each drug.
*$p<0.05$; †$p<0.01$, ‡$p<0.001$.

Meprobamate, a non-benzodiazepine anxiolytic, increased transitions and locomotion in the dose range 50–100 mg/kg, with sedation beginning above 150 mg/kg (Table 2). This dose range is consistent with the meprobamate doses showing increases in punished behavior [1, 3, 19].

Pentobarbitol, a sedative-hypnotic, increased both locomotion and transitions at one dose, 40 mg/kg. This response was similar to findings with phenobarbitol in the social interaction test [12]. Observation of ongoing behaviors during the test session at 40 mg/kg found these mice moving in quick bursts with considerable stumbling, suggesting the beginning of the stage preceding sedation. Unequivocal sedation was seen at doses above 50 mg/kg. The sedative doses for pentobarbitol and for meprobamate are somewhat high in comparison to some reports. This variation may be partly a

TABLE 2

INCREASES IN MOUSE EXPLORATORY AND LOCOMOTOR BEHAVIOR AFTER TREATMENT WITH THE ANXIOLYTIC MEPROBAMATE AND PENTOBARBITOL, BUT NOT WITH THE ANTIDEPRESSANTS CLORGYLINE AND BUTRIPTYLINE, NOR WITH THE NEUROLEPTIC, CHLORPROMAZINE

Dose (mg/kg)	Exploratory Transitions (Mean ± S.E.M.)	Animex Locomotion (Mean ± S.E.M.)
Vehicle	35.0 ± 4.0	226 ± 24
Meprobamate		
20.0	36.0 ± 5.2	254 ± 42
50.0	71.2 ± 6.6†	478 ± 119‡
100.0	70.0 ± 5.0†	487 ± 74*
150.0	41.8 ± 20.2	277.2 ± 118.3
200.0	<1‡	<10‡
Pentobarbitol		
10.0	42.0 ± 4.2	221 ± 19
20.0	44.3 ± 4.7	255 ± 7
30.0	29.7 ± 12.0	195 ± 66
40.0	69.5 ± 10.0*	350 ± 43*
50.0	28.6 ± 23.3	152 ± 105
60.0	0.3 ± 0.3†	25 ± 23†
Clorgyline		
1.0	34.8 ± 5.8	194 ± 43
10.0	34.0 ± 3.9	223 ± 8
Butriptyline		
5.0	40.4 ± 3.8	230 ± 23
30.0	45.8 ± 5.0	212 ± 17
Chlorpromazine		
0.5	33.0 ± 5.7	225 ± 10
1.0	24.4 ± 5.4	187 ± 42
5.0	3.8 ± 1.7‡	11 ± 7‡

N=6 for each dose of each drug.
*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

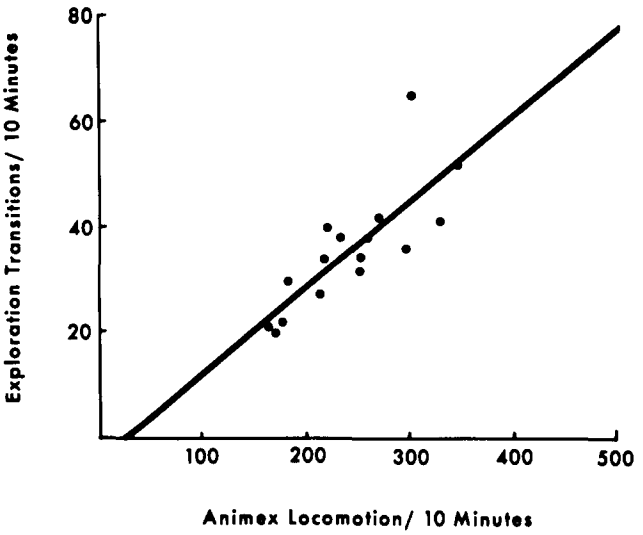

FIG. 1. Correlation between locomotor behavior as measured by an Animex activity monitor and transitions between the light and the dark compartments of a two-chambered arena. r=0.706, $p<0.005$.

species difference between mice and rats [11] and a documented strain difference in mice [25] to sedative-hypnotics. It may also reflect some behavioral arousal stimulated by the novel environment, which could shift the dose-response curve for sedation to the right.

Clorgyline, a monoamine oxidase inhibitor with antidepressant properties in humans [20] produced no behavioral changes in this paradigm at doses of 1.0 and 10.0 mg/kg. Butriptyline, a tricyclic antidepressant, [32] also produced no behavioral changes at doses of 5.0 and 30.0 mg/kg, which are known to potentiate amphetamine-induced hyperthermia [15]. The neuroleptic, chlorpromazine, which alters discriminative control in several operant schedules [16] produced no changes at doses of 0.5 and 1.0 mg/kg , above which sedation was seen at 5.0 mg/kg. The lack of behavioral changes after acute treatment with these three psychoactive compounds, whose neuropharmacologic mechanisms of action are thought to differ considerably from the benzodiazepines, supports a degree of pharmacological specificity of this proposed paradigm for increased exploration by benzodiazepines.

Figure 1 illustrates the correlation seen between the two behavioral parameters, transitions across the partition between light and dark chambers, and total locomotion over the cage surface, in vehicle-treated mice. Similarly, correlations between these two parameters were seen with the drug treatments (Tables 1 and 2), which increased both transitions and locomotion in the cases of clonazepam, diazepam, flurazepam, chloridazepoxide, meprobamate, and pentobarbitol. The interrelationship of the light ⇄ dark transitions and the Animex locomotion score suggests that either of these parameters could effectively represent the effects of these drugs in the two-chambered apparatus.

As demonstrated previously, the increase in locomotion in the two-chambered apparatus is not a generalized motor effect, as it was not seen in a bare cage but only in the differentiated, two-chambered apparatus. Increased locomotion is integral to this and other paradigms requiring ambulation [5, 25, 30, 33]. Stimulants must be distinguished from anxiolytics by a separate motor test, to demonstrate a dose which increases the paradigm behavior without increasing spontaneous locomotor activity [1]. Since benzodiazepines increased locomotion and transitions in the light⇄dark apparatus, but did not increase locomotion in a bare, undifferentiated cage [6] the increased locomotion appears to be function of increased exploratory tendencies.

Figure 2 illustrates the correlation between number of transitions and frequency of occurrence of rearings, as scored by a human observer. Rearing is a vertical upright posture, in which both forepaws are lifted and the mouse sniffs at the upper reaches of the cage. This behavior is a characteristic of exploration in mice [10,38]. The significance of this correlation (Fig. 2, controls $p<0.001$, for combined benzodiazepines, $p<0.01$), implies that the number of transitions between the light and dark chambers is linked to the movements and postures which characterize exploratory behavior in mice. This interrelationship strengthens the concept that number of transitions is an index of exploratory behavior.

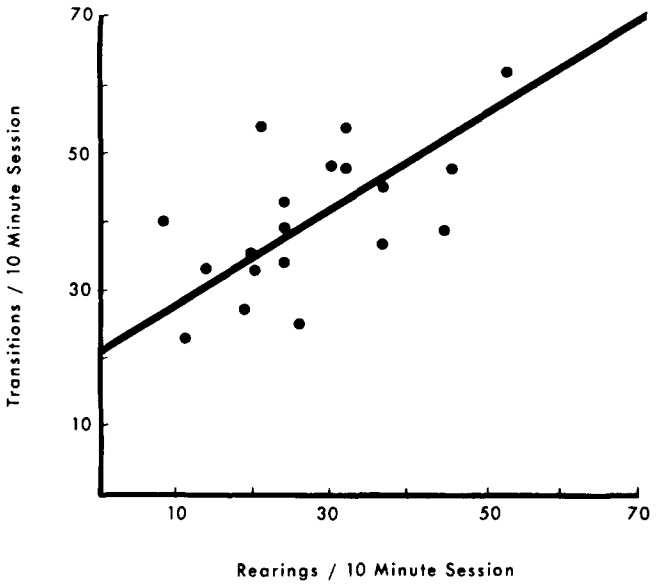

FIG. 2. Correlation between light⇄dark transitions and frequency of rearing postures associated with exploratory sniffing of the cage. $r=0.720$, $p<0.005$.

DISCUSSION

The introduction of a new animal model for the behavioral effects of benzodiazepines must be justified by advantages which more closely meet the criteria for an ideal screening test [3]. These criteria include: (1) High efficiency, high speed, simplicity; (2) Reproducibility; (3) Specifity, a given drug effect being characteristic of a well-defined class of chemicals and indicative of a specific mode of action; (4) Adequate design, adequate data processing, potential for statistical analysis; (5) Good correlation with tests in man. The two-chambered exploration model proposed herein appears to meet these criteria.

The single, automated parameter of transitions between the compartments yields efficiency, simplicity, and potential for statistical analysis. The benzodiazepine effect is highly reproducible, at doses similar to those used in other models. Rank order potencies correlate with doses used in treating human anxiety. Psychopharmacological specificity is evidenced by the lack of increase in transitions by non-anxiolytic classes of psychoactive drugs. The equipment is simple and inexpensive. There is no baseline training and no human observation required.

The procedure is comparable in simplicity and economy to the suppression of licking test [31,39]. However, it does not employ shock, and may therefore bypass assumptions about changes in pain threshold, appetite or thirst. The proposed model, like all animal models in current use, requires further analysis of its relevance to "anxiety" in mice, and its relationship to the anxiolytic action of benzodiazepines in man.

Tolerance does not develop to the antianxiety effects of benzodiazepines in humans, raising the issue of development of tolerance to the exploratory effects in mice. Preliminary unpublished observations suggest that no tolerance develops when diazepam, 2 mg/kg IP, is administered at intervals of 3 days. More complete time course studies with chronic administration will further test the apparent lack of tolerance to antianxiety drugs in the proposed model.

In conclusion, benzodiazepines appear to increase exploratory behavior of mice in a two-chambered system, at dose ranges well below the sedative levels. Effective doses of four benzodiazepines show rank-order potencies qualitatively analogous to receptor binding and clinical potencies. A peripherally active benzodiazepine and psychoactive drugs of other classes did not influence behaviors in this paradigm, indicating some degree of neuropharmacological specificity. The increase in transitions across the partition in a light ⇄ dark two-chambered apparatus appears to be a rapid, simple, automated, one-parameter test for studies of the behavioral actions of benzodiazepines.

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