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A Sensitive and Selective Monkey Conflict Test

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PATEL, J. B. AND B. MIGLER. *A sensitive and selective monkey conflict test.* PHARMAC. BIOCHEM. BEHAV. 17(4) 645–649, 1982.—A conflict model is described in which clinically effective antianxiety agents exhibit pronounced anticonflict activity. Male squirrel monkeys were trained to depress a bar for 5 sec to obtain food reinforcement. The 6 hr test session was comprised of an initial 3 hr period in which each 5 sec response was punished and then a 3 hr unpunished period. Trained monkeys would rarely be shocked and would make most of their responses during the non-punished period. Both benzodiazepine (chlordiazepoxide and diazepam) and non-benzodiazepine (meprobamate and phenobarbital) anxiolytics produced pronounced and unequivocal increases in punished responding. Other psychoactive agents (d-amphetamine, chlorpromazine, ethanol, morphine, amitriptyline and imipramine) did not produce an increase in punished responding. Sensitivity (i.e., large magnitude effects), selectivity, stable baseline performance and fully automated features make this test useful in identifying potential anxiolytic agents in primates.

Benzodiazepines Meprobamate Phenobarbital Ethanol Monkey Conflict test Anxiolytic agents

THE most commonly used method for evaluating antianxiety effects of drugs in animals is the procedure first described by Geller and Seifter [8]. Briefly, rats trained on a variable interval schedule are intermittently presented with a signaled punishment period in which every response is both rewarded and punished. Clinically effective antianxiety agents (e.g., benzodiazepines, meprobamate and barbiturates) significantly increase responding that was suppressed by punishment [2, 7, 8].

The magnitude of the drug effect in the Geller-Seifter test is sometimes small. Several modifications of the conflict test have been reported for rats [4, 5, 9, 13, 14]. However, there are only a few systematic studies in which monkeys were used to evaluate the effects of anxiolytic agents in conflict tests [3, 10, 11, 12]. We now report a new form of the conflict test for use with monkeys with the advantage over previous forms relating to magnitude of effect, while preserving specificity for anxiolytics.

METHOD

Subjects

Male squirrel monkeys (Saimiri Sciureus, 600–1200 g) obtained from South American Primate Imports (Miami, FL) were used throughout these studies.

Apparatus

The design of the test chambers was such that the chambers also served as the living quarters, thus avoiding daily transportation problems that may contribute to variable results. These test chambers (47×30×35 cm) were made of stainless steel wire with two openings on the back wall for levers and an aperture (5 cm in diameter) centered on the back wall to permit access to a food cup. Each cage was suspended from an overhead rack, with the back wall of each cage adjacent to a test panel consisting of stimulus lights, two response levers and a cup into which 190 mg Noyes nutrient food pellets could be delivered. The levers projected 5 cm into the cage and were covered on the top and sides with a metal hood to insure that only hand and not foot responses on the lever were recorded. Shock was administered to each animal through a chain connected to a metal collar around the neck and the wire cage. Slack in the chain was taken up by a counterweight.

Procedure

The monkeys were first trained to press a lever and hold it depressed until a food reward pellet was delivered. The initial duration required was 0.5 sec, and this was increased over about a week to 5 sec. Punishment (shock) was then introduced during the first 3 hr period by pairing each pellet with a shock. The intensity of shock was increased very gradually and on an individual basis until responses in the punishment period were almost completely suppressed. A well trained monkey would normally make all its food-reinforced responses during the non-punished period and rarely respond during the punished period. For a naive monkey, approximately 8 weeks of training were required before testing could begin.

The 6 hr test session was comprised of an initial 3 hr period of punishment and then a 3 hr period with no punishment. The onset of the punishment period was signaled by a green cue light over the right lever; during this period, a bar press response for 5 sec on the right lever was rewarded with a food pellet and also punished with an electric shock. The onset of the unpunished period was signaled by the green cue

TABLE 1

EFFECT OF SELECTED ANXIOLYTIC AGENTS ON PUNISHED RESPONDING

Treatment	Dose mg/kg, po	Monkey Number	Pre-Drug Day Completed Responses*	Drug-Day Completed Responses*
Chlordiazepoxide	1.25	1	0	0
		18	0	1
		19	1	1
		21	0	0
		Median	0	0
	2.5	1	2	49
		4	0	128
		6	0	0
		8	6	106
		Median	1	78†
	5	1	0	131
		2	0	1
		3	0	1
		4	0	95
		7	0	1
		8	1	121
		9	0	42
		10	0	10
		18	0	10
		14	0	218
		6	0	275
		13	0	298
		15	0	79
		16	0	151
		Median	0	87†
	10	4	0	99
		8	0	111
		9	0	1
		10	1	14
		18	0	81
		Median	0	81†
Diazepam	0.625	16	0	0
		18	0	0
		19	1	4
		Median	0	0
	1.25	1	0	0
		16	0	12
		18	0	7
		19	3	20
		Median	0	10†
	2.5	1	0	239
		2	0	7
		3	0	3
		4	0	56
		6	1	118
		7	0	0
		8	1	292
		9	0	223
		10	0	6
		Median	0	56†

TABLE 1

EFFECT OF SELECTED ANXIOLYTIC AGENTS ON PUNISHED RESPONDING
(Continued)

Treatment	Dose mg/kg, po	Monkey Number	Pre-Drug Day Completed Responses*	Drug-Day Completed Responses*
Meprobamate	50	1	0	41
		13	0	0
		16	0	69
		18	2	61
		20	0	0
		Median	0	41†
	100	1	1	194
		5	0	8
		6	0	151
		15	0	18
		16	1	36
		20	0	161
		Median	0	93†
Phenobarbital	10	6	0	0
		13	0	0
		15	0	0
		16	0	2
		Median	0	0
	20	4	0	1
		13	0	0
		15	0	0
		Median	0	0
	40	16	0	0
		1	0	83
		13	0	231
		15	0	36
		Median	0	59.5†

*Responses of ≥5 sec: Food and shock delivered.
†Significantly ($p<0.5$) different from pre-drug day as determined by Mann-Whitney U test [16].

light switching over to the left lever for 3 hr, and during this period a response on the left lever was rewarded with a food pellet but no shock. A lever press on the wrong side (no green light over the lever) or of less than 5 sec duration on the correct side was not rewarded. A lever press on the correct lever also turned on a red light (located above the lever) which stayed on until the lever was released and served as a feedback stimulus to indicate that the lever was fully depressed. The total number of bar presses and reinforcements received during both periods were recorded. Water was available at all times. Occasional supplements of fruit were given to the monkeys; otherwise they obtained all of their food from the food pellets they earned during the daily test. The test has been conducted on a fully automated, seven days per week schedule providing for the testing of up to ten monkeys simultaneously.

Drugs

All drugs were administered orally 10–15 min prior to test sessions. Drugs were suspended in HPMC suspension (0.1% TWEEN 80, 0.5% hydroxylpropylmethylcellulose in 0.9% NaCl) and the volume of each injection was 5 ml/kg of body weight. All doses were calculated in terms of the free base. Drugs used were chlordiazepoxide and diazepam (Roche Laboratories), sodium phenobarbital (J. T. Baker), morphine sulfate (Mallinckrodt), amitriptyline (Merck Sharp and Dohme), imipramine (Geigy), meprobamate (Wallace), d-amphetamine (Sigma), and chlorpromazine (Smith Kline).

RESULTS

Table 1 summarizes the effects of four anxiolytic agents upon punished responding; the number of shocks taken during pre-drug and drug sessions are shown for comparative purposes. As a result, individual monkey data are presented for each dose and medians were analyzed using Mann-Whitney U test [16]. As seen in Table 1 the number of completed, i.e., punished, responses during pre-drug sessions for all the monkeys tested was usually zero.

Diazepam produced a dose-related increase in the median number of shocks taken. The lowest dose of diazepam (0.625

mg/kg) produced no change in median number of shocks taken, whereas the 1.25 mg/kg dose resulted in a significant increase (from 1 to 10) and the 2.5 mg/kg dose produced a highly significant increase (from 0 to 56) in the median number of shocks taken. One third of the diazepam-treated subjects at 2.5 mg/kg took more than 200 shocks during the punishment period (Table 1). Similarly, chlordiazepoxide (CDP) exhibited a pronounced increase in the median number of shocks taken; e.g., at 2.5 mg/kg from 1 to 78, 5.0 mg/kg from 0 to 87, and at 10 mg/kg from 0 to 81.

Meprobamate, a non-benzodiazepine anti-anxiety agent at doses of 50 and 100 mg/kg produced a significant increase in the median number of shocks taken (Table 1). All six monkeys were disinhibited following meprobamate at 100 mg/kg.

Phenobarbital, another non-benzodiazepine antianxiety agent showed anticonflict activity at the highest dose (40 mg/kg) tested. The two lower doses of 10 and 20 mg/kg did not produce a significant increase in number of shocks taken (Table 1). Both meprobamate (50 and 100 mg/kg) and phenobarbital (40 mg/kg) produced a large increase in the number of aborted responses in selected monkeys, but this was not statistically significant for the group.

Table 2 presents the comparative effect of test agents on total food pellets earned during entire 6-hr sessions during the pre-drug and drug days. As indicated in Table 2, none of the drugs tested produced any significant ($p>0.05$) increase in the total number of food pellets earned.

Table 3 summarizes the results found with other psychoactive agents on punished responses. Chlorpromazine (an antipsychotic), morphine (an analgesic), d-amphetamine (a stimulant), ethanol (a depressant), amitriptyline and imipramine (antidepressants) all failed to produce any significant increase in the number of shocks taken over the range of doses tested. In fact, the median pre-drug and drug-day punished responses were always zero. In all cases the highest dose tested was the dose that produced either a decrease in responding during the non-punished period or gross behavioral effects.

TABLE 2

MEDIAN TOTAL NUMBER OF FOOD PELLETS EARNED DURING ENTIRE 6 HOUR SESSION (PUNISHED PLUS NONPUNISHED PERIODS)

Treatment mg/kg, PO	Dose mg/kg, PO	Pre-drug Day	Drug Day	p Value
Chlordiazepoxide	1.25	161	182	NS*
	2.5	211	187	NS
	5.0	201	195	NS
	10.0	176	198	NS
Diazepam	0.625	179	196	NS
	1.25	165	169	NS
	2.5	176	186	NS
Meprobamate	50.0	241	242	NS
	100.0	219	255	NS
Phenobarbital	10.0	189	256	NS
	20.0	258	292	NS
	40.0	226	192	NS

*NS—Not significant ($p>0.05$) as compared to pre-drug day as determined by the Mann-Whitney U test.

TABLE 3

EFFECTS OF OTHER PSYCHOACTIVE AGENTS IN THE CONFLICT TEST

	Dose Range Tested* mg/kg, PO	Median Number Punished Responses	
		Pre-Drug Day	Drug Day†
Chlorpromazine	1.0 - 4.0	0	0
Morphine sulfate	2.5 -20.0	0	0
d-amphetamine sulfate	0.25- 0.5	0	0
Ethanol	800-1200	0	0
Amitriptyline	2.5 - 5.0	0	0
Imipramine	2.5 - 5.0	0	0

*Two to four monkeys were tested at each dose.
†Maximum effect of any dose tested.

DISCUSSION

The present paper describes a modified conflict test which appears to be able to reveal the disinhibitory properties of clinically effective anxiolytic agents more clearly than previously described conflict tests. Chlordiazepoxide, diazepam, meprobamate and phenobarbital all greatly elevated the number of punished responses from a baseline of approximately zero, providing unequivocal evidence of activity. It should be emphasized that the anxiolytic agents increased punished responding without significantly increasing total food intake during the 6 hour session (Table 2), suggesting that the disinhibitory effect was not due to an increase in motivation for food. Since psychoactive agents that are not used clinically as anxiolytic agents did not produce disinhibitory effects (Table 3), it appears that the test is selective for anxiolytic agents.

In general our results are in agreement with effects reported by others in monkeys [3, 10, 11, 15, 17]. The main differences are given below.

Stein and Berger [17] reported that lorazepam and diazepam increased the mean rate of punished responses up to a maximum of 50-fold. In our procedure we observed this magnitude or greater with every agent. Also, in their procedure each punishment period lasted only 3 minutes and was repeated 7 times during the session with alternate non-punished periods. This procedure would appear to be useful for agents which have a powerful releasing effect on responses suppressed by punishment, but whether it is sensitive enough to detect the activity of agents with weak effects is questionable. In contrast, the present procedure allows a 3-hr punishment period, and the introduction of partial or aborted responses adds the potential for even greater sensitivity to weaker agents.

Hanson, *et al.* [10], using a modified Geller Conflict procedure, demonstrated that CDP (36 mg/kg PO) and meprobamate (120 mg/kg PO) increased the rate of punished responses from about 20/hr to 105/hr. These high doses and the relatively small magnitude of effect suggest that their procedure may not be sensitive enough for use in determining potential anxiolytics.

Finally, in the report by Miczek [12] squirrel monkeys were punished when they drank dextrose from a drinking tube. CDP increased the amount of dextrose consumed in

this situation, but amphetamine and scopolamine did also. Thus, their procedure lacks specificity for anxiolytics.

A practical advantage of this conflict test is that the period of nonpunished responding permits sufficient food intake to maintain normal body weight. Thus, this test does not require any specific deprivation (i.e., reduced body weight) schedule which might affect the results. Normal responding during the nonpunished and punishment periods also serves to indicate that the drug being tested has not produced side effects which are not visible by gross inspection of the monkeys.

Other useful features of this test are: use of a long (3 hr) punishment period immediately after drug administration enables one to detect activity in an agent with a very slow onset of activity or an active metabolite. The use of a requirement that the lever be held depressed for 5 sec for reinforcement enables one to determine the effects of agents on aborted (i.e., less than 5 sec) responses. With low doses of diazepam, for example, an increase is sometimes observed in the number of aborted responses without any increase in the number of shocks taken (Migler, unpublished). In the present experiment anxiolytic agents increased, though not significantly, the number of aborted responses as well as the number of punished responses. Amphetamine did not produce a significant increase in aborted responses as reported by Fowler and Price [6], possibly due to the use of a metal hood over the lever preventing responses produced by the monkey walking on the lever during periods of hyperactivity.

The conflict test described here has been adapted for rats (Clody, unpublished, Patel unpublished) and found to produce similar results. A preliminary description of this test has been reported [1].

In summary, the unequivocal disinhibitory effect observed only with antianxiety agents, and the stable baseline performance (as well as the fully automated feature that provides simultaneous testing of the monkeys) make this test useful in identifying potential antianxiety agents.

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