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Cork Gnawing in the Rat as a Screening Method for Buspirone-Like Anxiolytics

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ABSTRACT

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Male Long-Evans rats were individually allowed access to No. 11 corks for 30 min per day. After 30 sessions, the mean amount gnawed away, 0.10 g per session, was stable enough to allow drug testing; it reached asymptote at 0.03 g after 140 sessions. Drugs were injected PO 30 min before testing, except as noted. On the asymptotic baseline, the novel anxiolytic buspirone (8–32 mg/kg) and its congener gepirone (8–32 mg/kg) produced large, dose-related increases in cork gnawing. The standard anxiolytics chlordiazepoxide (16–32 mg/kg) and meprobamate (128 mg/kg, 60 min pretreatment) and the new sedative zopiclone (4–32 mg/kg) also produced substantial increases. Diazepam, oxazepam, and alprazolam produced marginal increases, and pentobarbital had no effect at behaviorally relevant doses. The non-anxiolytics *d*-amphetamine, chlorpromazine, acute imipramine, morphine (IP), and valproic acid either decreased or did not change cork gnawing. Phencyclidine (IP) and scopolamine produced marginal increases. Apomorphine (5 mg/kg SC) produced intense stereotyped gnawing of cage mesh but abolished gnawing of cork. Cork gnawing is proposed as a simple, economical behavioral method to identify buspirone-like anxiolytics.

Key words: oral behavior

INTRODUCTION

Standard anxiolytics are characterized behaviorally in animals by their capacity to increase responding that has been suppressed by punishment. Benzodiazepines, propanediol carbamates, barbiturates, and some other sedatives increase shock-suppressed lever pressing

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[Geller and Seifter, 1960] and shock-suppressed drinking [Vogel et al., 1971] in rat. The literature of this phenomenon has been reviewed by Pollard and Howard [1990].

The structurally novel anxiolytic, buspirone, differs clinically from standard anxiolytics in that it lacks sedative properties and abuse liability and is effective only with chronic dosing [Eison and Temple, 1986; Goa and Ward, 1986; Lader, 1988]. It differs behaviorally in that it does not reliably increase punished responding in rat and monkey [Pollard and Howard, 1990; Howard and Pollard, 1990]. Its anxiolytic action was discovered during clinical trials to assess possible antipsychotic action, not by use of an animal test for anxiolysis [Eison, 1984]. Results from several types of preclinical tests have been negative or inconsistent [reviewed briefly in Howard and Pollard, 1990]. Potentiated startle [Kehne et al., 1988] shows some promise, but the only behavioral model of anxiolysis in which buspirone registers a clear, replicated positive effect is punished key-pecking in pigeons [Barrett et al., 1986].

All of the major classes of anxiolytics—benzodiazepines, propanediol carbamates, barbiturates, and buspirone [Baldessarini, 1985; Harvey, 1985]—increase eating in rodents [Bainbridge, 1968; Soubrié et al., 1975; Cooper, 1985; Cooper and Moores, 1985; Berridge and Treit, 1986; Clark and Fletcher, 1986], as does the structurally novel sedative zopiclone [Sanger et al., 1988]. Buspirone, chlordiazepoxide, and meprobamate increased chewing of bedding in a study of conditioned defensive burying [Craft et al., 1988]. It may be that increased eating or chewing in rodents is common to anxiolytics.

To test this hypothesis, we allowed rats daily access to corks until the amount gnawed was stable and then tested drugs of several classes. The results suggest that the test can identify buspirone-like anxiolytics.

MATERIALS AND METHODS

Subjects

Eight male Long-Evans rats from Charles River Breeding Laboratories, Wilmington, MA, served as subjects. They were housed 4 per cage in animal quarters on a regular light/dark cycle (lights on 0600–1800 hr) with free access to food and water except for the period between injection and the end of a test session. Median body weight was 435 g at the initial test, 640 g when baseline gnawing reached asymptote, and 737 g near the end of the 15-month study.

Apparatus

Each subject was assigned a test cage for the duration of the study. The test cages were ordinary single rat housing cages located just above the home cages, in the same rack. Like the home cage, a test cage was made of stainless steel, with a wire mesh bottom and front and a hole in the back which admitted a water spout. (The spout had no intended significance; it was simply part of the standard housing rack.) Test cage dimensions were 17 × 18 × 24 cm (floor area 432 cm^2). The No. 11 cork stoppers (Krackeler Scientific, Durham, NC) weighed 2.00–2.99 g.

Procedure

A session consisted of placing the subject in the test cage with a cork for 30 min. Sessions occurred at mid-morning on 5 consecutive days of the week.

After 30 habituation sessions, with saline being given before session 30 to accustom the subjects to the injection procedure, drug testing began. Mondays and Thursdays (later Thursdays and Sundays) were baseline control days, Tuesdays and Fridays (later Fridays and Mondays) were drug test days.

Meprobamate, valproic acid, zopiclone, alprazolam, diazepam, and oxazepam were suspended in 0.5% methyl cellulose; apomorphine was dissolved in 0.0001 *N* HCl; the other drugs were dissolved in isotonic saline. Injection volume was 1 ml/kg except for meprobamate

TABLE 1. Results of within-subject ANOVA for effects of drugs on amount of cork gnawed away†

Drug	F-value	df	Baseline (g^{-2})	Drug	F-value	df	Baseline (g^{-2})
buspirone[a]	6.570*	7	8	phencyclidine	3.546*	7	3
chlordiazepoxide[a]	2.945*	7	11	chlorpromazine	2.299	7	3
d-amphetamine[a]	7.011*	6	6	alprazolam	1.651	7	4
pentobarbital[a]	2.871*	7	8	zopiclone	5.241*	7	4
imipramine[a]	2.747*	7	8	buspirone	6.983*	7	4
morphine[a]	2.930*	7	10	chlordiazepoxide	5.306*	7	3
meprobamate[a]	2.435	7	6	meprobamate	7.761*	7	2
valproic acid	0.334	7	5	diazepam	3.036*	7	2
				scopolamine	2.811*	7	2
				gepirone	13.711*	7	2
				pentobarbital	0.837	7	2
				d-amphetamine	3.152*	7	3
				apomorphine	*	7	2
				imipramine	1.071	6	3
				morphine	9.343*	6	3
				oxazepam	2.296	6	4

†In chronological order by pairs (the first two drugs were tested in the same period in a counterbalanced design, then the second two, etc.). Exceptions: The control for apomorphine was that of the preceding pair, and oxazepam was tested alone.

[a]Because baselines were high, these drugs were retested later. Results of initial determination, under high baseline, appear in Table 2. Results of second determination, under asymptotic baseline, appear in Figures 1, 2, and 3.

*indicates $P < .05$.

256 mg/kg, which was given as 2 ml/kg. Drugs were injected PO (IP for morphine and phencyclidine, SC for apomorphine) 30 min (60 min for the second test of meprobamate, 0 min for apomorphine) before the session. Food was removed from the home cage at the time of injection.

In general, dose-effect curves were generated in ascending order of dose, beginning with vehicle. Four of the subjects would get one drug while the other 4 got another, then the drug assignments would be reversed to complete the N of 8. Oxazepam was tested alone, with ascending doses in 3 subjects, decending doses in 4 subjects, and 64 mg/kg given to all on the last day. Apomorphine was tested in all subjects on the same day. Drugs were tested in the following order (see Table 1 for a tabulation): buspirone HCl (Bristol-Myers) and chlordiazepoxide HCl (Sigma), *d*-amphetamine SO_4 (SK&F) and Na pentobarbital (Sigma), imipramine (CIBA-GEIGY) and morphine SO_4 (Mallinckrodt), meprobamate (30-min pretreatment, Wallace) and valproic acid (Abbott), phencyclidine (resynthesized by Burroughs Wellcome) and chlorpromazine HCl (SK&F), alprazolam (Upjohn) and zopiclone (Rhône-Poulenc), buspirone (second test) and chlordiazepoxide (second test), diazepam (Hoffman-La Roche) and meprobamate (60-min pretreatment), scopolamine HBr (resynthesized by Burroughs Wellcome) and gepirone HCl (Bristol-Myers), Na pentobarbital (second test) and *d*-amphetamine SO_4 (second test), apomorphine (J.H. Walker), imipramine (second test) and morphine (second test), and oxazepam (resynthesized by Burroughs Wellcome). Second tests were done after it was discovered that baselines for first tests, though stable in the short term, were not asymptotic.

Data Analysis

Each cork was weighed to the nearest 0.01 g before and after the session, and the difference was taken as the basic datum. The mean of all baseline days within a drug test period for a subject was used as a control value (for example, if 5 doses of a drug in addition to vehicle

TABLE 2. Drug-induced changes in cork gnawed (mean grams^{-2} ± SEM): Initial determination, high baseline

Drug	Dose (mg/kg)										
	0	0.25	0.5	1	2	4	8	16	64	128	256
buspirone	−5		1	3	8	13*	17*	14*			
	±2		±2	±3	±4	±5	±4	±4			
chlordiazepoxide	−3		3	6	0	14*	5	3			
	±2		±3	±3	±2	±6	±4	±3			
d-amphetamine	−1	−1	−2	−5*	−5*	−5*					
	±1	±1	±2	±1	±1	±1					
pentobarbital	−3			−3	2	6	4	1			
	±2			±1	±2	±4	±3	±2			
imipramine	−1					−3	−5	−6			
	±2					±2	±2	±1			
morphine	2			−1	−6	−8*					
	±4			±3	±2	±1					
meprobamate	−1								0	7	8
	±2								±1	±4	±4

*indicates $P < .05$, Dunnett's test.

were tested, there were 6 baseline days, and control was the mean of these 6 days). Drug effects for each subject were assessed as the difference between amount gnawed on drug day and mean amount gnawed on baseline days. The values so derived were subjected to analysis of variance and Dunnett's post-hoc test (CRISP package, CRUNCH Software, San Francisco), with $P < .05$. One baseline day outlier for 1 subject early in the study was cast out before the baseline mean was calculated (subject's values for the period were 0.00, 0.00, 0.41, 0.01, 0.00, 0.03, 0.01). In addition, one subject's data for the first test of *d*-amphetamine were cast out as explained in Results below. The higher doses of *d*-amphetamine and morphine produced large decreases in cork gnawing, with many scores of 0; therefore, each value for these 2 drugs was transformed by adding 0.001 and taking the log to the base 10.

RESULTS

Training

In the early sessions, amount gnawed was relatively high and variable within and between subjects. For example, in session 7 the values for the 8 subjects were 0.02, 0.14, 0.45, 0.16, 0.33, 0.20, 0.23, 0.00; the mean was 0.19, roughly 8% of the average cork. In sessions 7–11 the values for a representative subject were 0.23, 0.02, 0.00, 0.46, 0.24. In session 29 the mean for all subjects was 0.07 and the within- and between-subject variability was judged to be sufficiently low to allow drug testing to begin.

Drug Effects on High Baseline (Sessions 31–138)

The left half of Table 1 gives mean baselines chronologically, as well as results of the analyses of variance, for the first 8 drugs tested. Changes from baseline are given in Table 2 (except for valproic acid, the effects of which are considered below).

The structurally novel anxiolytic, buspirone, is the only one of the drugs that produced a large, significant, dose-related increase in cork gnawing with high baseline. The 3 conventional anxiolytics produced increases, but the effect of meprobamate was non-significant, and only 1 dose of chlordiazepoxide had a significant effect.

The stimulant *d*-amphetamine and the opiate morphine significantly and dose-dependently decreased gnawing, as did the tricyclic antidepressant imipramine. *d*-Amphetamine data for 1 subject were anomalous and were excluded from analysis: The values for 2 and 4 mg/kg

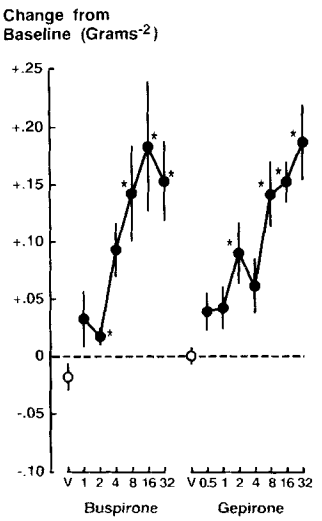

Fig. 1. Effects of buspirone and gepirone on cork gnawing (asymptotic baseline). Each data point represents the mean ± 1 standard error for the 8 subjects. Drugs were given PO 30 min before the 30-min tests. * indicates $P < .05$ by Dunnett's test.

were +0.12 and +0.27, inclusion of which would have changed group means from −0.05 and −0.05 to −0.03 and −0.01. All values for the other seven subjects under 1, 2, and 4 mg/kg of *d*-amphetamine were negative. No individual dose effect of imipramine was significant; however, at 8 mg/kg, 7 of 8 subjects showed decreases (1 was unchanged), and at 16 mg/kg all showed decreases.

Drug Effects on Low Baseline (All Subsequent Tests)

After these 8 drugs had been tested, it became evident that baselines had declined. Therefore, all except valproic acid were retested, and several other drugs were tested to investigate the therapeutic-class specificity of cork gnawing. Results for valproic acid are presented here because the baseline, 0.05 g, was near the asymptotic range of 0.02–0.04, and time for retest was limited.

The right half of Table 1 gives mean baselines chronologically, as well as results of the analyses of variance, for these drugs.

Effects of buspirone and its congener gepirone are shown in Figure 1. These drugs produced large, dose-related increases in gnawing.

Effects of 6 sedative-hypnotic anxiolytics are shown in Figure 2. All 4 benzodiazepines increased gnawing, but the effects were quantitatively different: Chlordiazepoxide's effect was large and orderly; diazepam's effect was significant but marginal, with only 1 dose achieving significance; alprazolam's and oxazepam's effects were orderly but non-significant. Pentobarbital, which had a marginal effect when tested earlier under high baseline, had no effect here. The propanediol carbamate meprobamate produced a large increase at 1 dose. The novel sedative zopiclone's effect was similar to chlordiazepoxide's.

Effects of 8 non-anxiolytics are shown in Figure 3. Two of the drugs increased gnawing: the effect of scopolamine was clear but small; the overall effect of phencyclidine was significant, but not the effect of any individual dose. No dose of *d*-amphetamine produced an increase, and 8 mg/kg abolished cork gnawing. Apomorphine abolished cork gnawing and produced intense sniffing and gnawing of cage mesh during the 30-min test session; during the

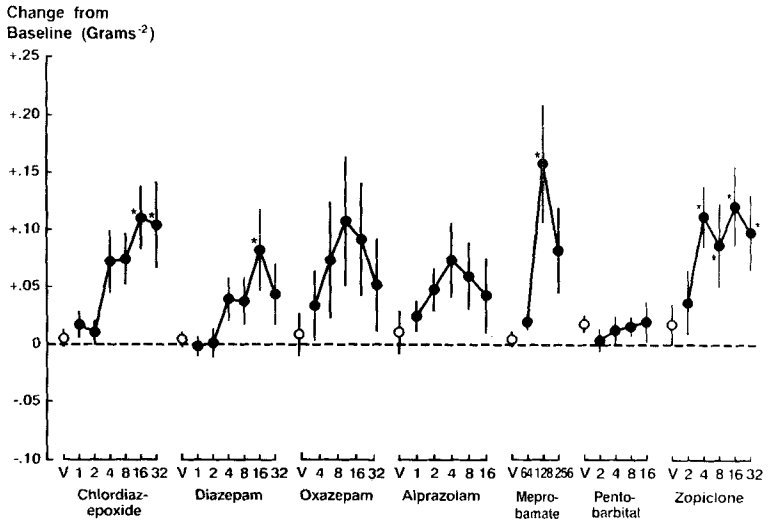

Fig. 2. Effects of sedative-hypnotics on cork gnawing (asymptotic baseline). Each data point represents the mean ± 1 standard error for the 8 subjects (7 for oxazepam). Drugs were given PO 30 min (60 min for meprobamate) before the 30-min test. * indicates $P < .05$ by Dunnett's test.

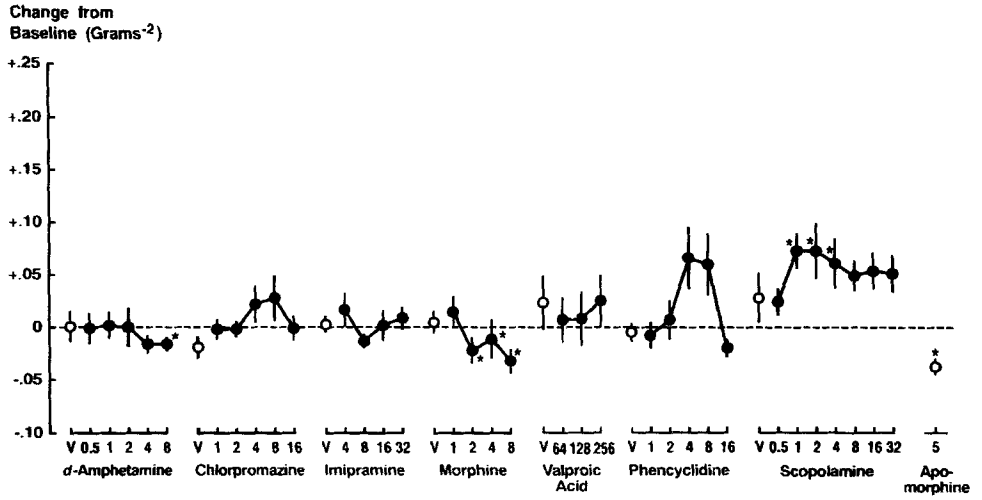

Fig. 3. Effects of non-anxiolytics on cork gnawing (asymptotic baseline). Each data point represents the mean ± 1 standard error for the 8 subjects (7 for imipramine and morphine). Morphine and phencyclidine were given IP, apomorphine SC, other drugs PO, 30 min (0 min for apomorphine) before the 30-min test. * indicates $P < .05$ by Dunnett's test.

following hour the subjects were engaged in intense gnawing of mesh but no gnawing of cork; 1 subject injured its snout in the process and was sacrificed.

When drug testing was finished, 3 subjects were deprived of food except for 10 min post-session for 3 days to determine whether increased appetite might account for drug-induced increases in cork gnawing. On the 3 test days, group mean body weight had declined

by 4.3, 5.3, and 7.0%; mean amounts gnawed were +0.01, +0.01, and 0.00 g. In the same period, group mean values for the four non-deprived subjects were −0.1, −0.1, and −0.1%, and −0.01, −0.02, and 0.00 g.

DISCUSSION

Cork gnawing, like some other spontaneous behaviors such as locomotor activity [Pollard and Howard, 1989], declines with repeated exposure and reaches a steady state that can be used as a baseline upon which to measure drug effects. In these male rats on a regular light/dark cycle, baseline after 30 sessions was relatively high (about 0.10 g per session) and somewhat variable, although it was adequate to register a large, orderly increase by buspirone as well as orderly decreases by non-anxiolytics. Between 30 and about 140 sessions the baseline changed so slowly as to be considered stable within the time frame (3 weeks) required to generate a dose-effect curve for a compound. Once achieved, asymptote (about 0.03 g per session) was maintained for the remaining 11 months of the study. Ongoing research suggests that ovariectomized female rats on a reverse light/dark cycle have a similar pattern of habituation, but their baselines are twice those of the males and intersubject variability is greater.

Inspection of Figures 1 and 2 leads to the conclusion that the effects of the novel anxiolytic buspirone and its congener gepirone are clearly greater than the effects of the barbiturate pentobarbital, the benzodiazepines diazepam and oxazepam, and the triazolobenzodiazepine alprazolam. Peak effects of buspirone and gepirone also appear to be greater than those of the benzodiazepine chlordiazepoxide and the sedative zopiclone. The large peak for meprobamate is probably real, even though only the 1 dose produced a significant effect; the dose-effect curve of meprobamate in the Geller-Seifter conflict test, a standard behavioral assay for anxiolytics, is known to be narrow [Pollard and Howard 1979]. In the ongoing research with ovariectomized female rats on reverse light/dark cycle, buspirone and ipsapirone appear to produce greater increases than chlordiazepoxide; diazepam and phenobarbital produce no increase in the females. Taken together, the data suggest that cork gnawing registers large effects by buspirone-like drugs, moderate to large effects by some sedative-hypnotics, and little or no effect by other sedative-hypnotics. That is, the test identifies buspirone-like drugs but is inconsistent with respect to conventional anxiolytics. Furthermore, the distinctively large effect of drugs that bind to the serotonin 1A receptor—buspirone, gepirone, ipsapirone—suggests that cork gnawing might be a neurochemical tool.

Figure 3 shows that none of the 8 non-anxiolytics increased cork gnawing substantially. The increases produced by phencyclidine and scopolamine were of about the same magnitude as those of diazepam and alprazolam. Phencyclidine could have some anxiolytic properties: It is used recreationally, and it has been shown to increase punished responding to some degree in rat [Cook and Davidson, 1973; Porter et al., 1987] and pigeon [Chait et al., 1981; Wenger, 1980]; scopolamine has long been used as a sedative (Weiner, 1985). Imipramine did not increase gnawing, although, like buspirone, it is an effective anxiolytic after several weeks of dosing. The dopamine agonists *d*-amphetamine and apomorphine, which produce stereotyped gnawing in rats, only decreased cork gnawing; we speculate that this difference arises from the voluntary, manipulative nature of holding and gnawing an object, in contrast to the compulsive nature of repetitive gnawing of cage mesh. Because asymptotic baselines are so low, this preparation may not be sufficiently sensitive to measure general behavioral depression or an increase in behaviors that compete with cork gnawing.

Food deprivation did not increase cork gnawing, which suggests that the positive drug effects could not be accounted for by appetite stimulation. The hyperphagic effect of many anxiolytics appears not to extend to cork gnawing.

A substantial increase in cork gnawing may be a useful index by which to identify buspirone-like anxiolytics. Once a stable baseline is achieved, the preparation can serve as an economical alternative to the only reliable existing behavioral preparation for this purpose,

punished responding in the pigeon. Researchers who wish to set up the cork gnaw assay are invited to contact the authors at Burroughs Wellcome Co.

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