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A Sensitive Open Field Measure of Anxiolytic Drug Activity

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BRITTON, D. R. AND K. T. BRITTON. *A sensitive open field measure of anxiolytic drug activity*. PHARMAC. BIOCHEM. BEHAV. **15**(4) 577–582, 1981.—Anxiolytic drugs including diazepam (DZP), chlordiazepoxide (CDX), pentabarbitol (PB) and ethanol (EtOH) produce specific alterations in the behavior of fasted rats given access to a single food pellet secured in the center of a novel open field environment. These drugs increase the total amount of food eaten in a 15 min test and the mean amount eaten per approach to the food pedestal. This latter effect appears to be the more sensitive index of anxiolytic drug action and occurs at doses which have no effect on rearing or grooming. DZP was effective following either acute or chronic (15 day) treatment at doses which had no effect on the food consumption by fasted rats tested in their home cages. The effects of the sedative benzodiazepine, flurazepam, were similar to those of DZP but were not statistically significant. Behavioral effects similar to those of DZP were seen in animals receiving additional handling prior to testing or in animals habituated to the open field. Neither the anti-psychotic haloperidol nor morphine mimicked the actions of DZP.

Anxiolytic drugs Diazepam Chlordiazepoxide Pentabarbitol Ethanol Open field test Handling Eating

THE ability of benzodiazepines (BDZ's) and other anxiolytic agents to attenuate the effects of punishment or novelty on animal behavior has served a basis for a variety of conflict paradigms to assess drug action. The anti-conflict potencies of various drugs have been shown to be positively correlated with their clinical anxiolytic potencies [2].

Conflict paradigms rely upon eliciting some predictable response by an animal, inhibiting that response by introducing a response contingent aversive component and overcoming the behavioral effects of the aversive component by drug treatment. It has been important to show that anxiolytics do not alter an animal's sensation of aversion (e.g., they have little or no analgesic action which would make animals insensitive to pain and the analgesic morphine is without anti-conflict action). It is equally important to demonstrate that the drug effect is not to enhance the reward value of the goal towards which the behavior is directed. That is, the drug should not alter responses in the absence of the aversive component. These criteria have been met by the operant paradigm developed by Geller *et al.* [8,9] in which BDZ's restore bar pressing for food when the response rate has been suppressed by contingent shock. In other tests for anticonflict activity, BDZ's have been shown to release drinking by thirsty animals when the drinking is otherwise suppressed by contingent shock [16], to overcome the effects of novel taste on rats given access to sweetened milk [12], and to increase locomotor responses to environmental novelty [4]. Soubrie and colleagues demonstrated the ability of minor tranquilizers to increase food [15] and water [14] intake by deprived animals in both novel and familiar environments. Additionally, BDZ's have been shown to release foot-shock suppressed ambulation in mice [1] and, when administered chronically, to increase social interaction by pairs of rats in an open field [6].

We were interested in studying anxiolytic drug effects in a setting which would not require extensive pretraining of animals and would not introduce physical stress such as electric shock. In order to do this, we have exploited the tendency of environmental novelty to elicit behavior which is incompatible with the act of eating. Therefore, fasted animals could be presumed to be motivated by hunger to approach the food and, by novelty, to avoid prolonged exposure to the center of the open field where the food was available.

METHOD

All animals were Sprague Dawley albino male rats weighing between 250 and 350 g at the time of testing. Approximately one week following arrival at the vivarium rats were placed in individual cages. Except as otherwise noted,

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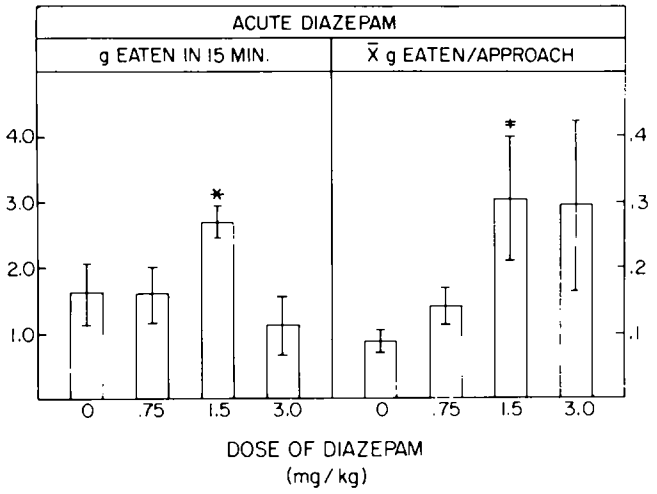

FIG. 1. Effects of acute diazepam (single injection) on the total amount of food eaten and the mean g eaten per approach to the food pedestal. Animals were treated with carrier only, 0.75, 1.5 or 3.0 mg/kg DZP (n=8 per group). *Significantly different from control ($p<0.05$), ‡significantly different from control ($p<0.005$).

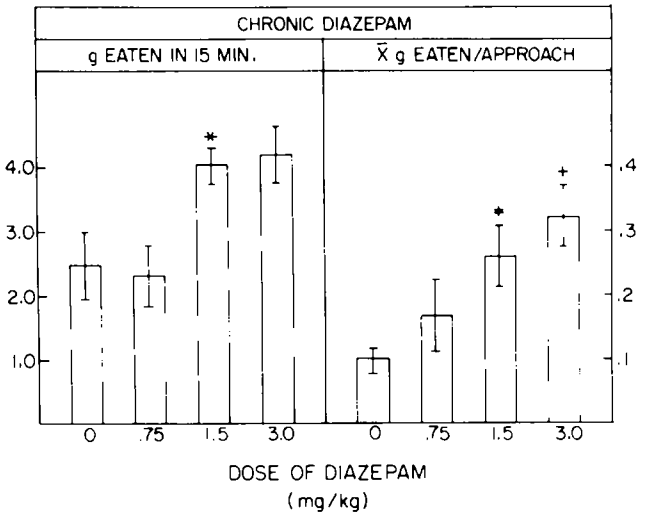

FIG. 2. Effects of chronic diazepam (15 daily injections) on the total amount of food eaten and the mean g eaten per approach to the food pedestal (mean g/App). Significant differences from control: *$p<0.05$, †$p<0.01$, ‡$p<0.005$. Control (n=10), 0.75 mg/kg (n=8), 1.5 mg/kg (n=10), 3.0 mg/kg (n=3).

animals were briefly handled on each of the 3 days immediately prior to testing. Lighting was on an 0800–1700 hr schedule and animals were given ad lib food (Purina Lab Chow) and water except as otherwise noted. Food was removed 24 hrs prior to testing.

Injections were given 30 min prior to testing. Drugs were dissolved in 0.9% saline or a mixture of propylene glycol : ethanol : and 0.9% NaCl (50:10:40). Injections were given subcutaneously in a volume of 1.0 ml/kg body weight, except ethanol which was injected IP as a 10% solution in 0.9% NaCl. Chronically treated animals received 15 daily injections of DZP or carrier and were tested 30 min after the last injection.

Thirty min following injection animals were tested for 15 min, either in their home cages or in an open field. The open field was 40 cm in diameter and highly illuminated. A single food pellet weighing 5.5–6.5 g was secured on a pedestal in the center of the field. During the 15 min observation period records were kept of the total amount of food eaten, the number of approaches to the food pedestal, the latency to the first bite, amount of rearing and grooming and the incidence of urination. Other animals were tested in their home cages by being given access to a preweighed food pellet for 15 min. At the end of the 15 min test period the remaining food and crumbs beneath the cage were collected and weighed.

Animals used to study habituation to the open field were maintained on a restricted diet of ~10 g per day of Purina Lab Chow beginning 4 days prior to the first exposure to the open field. They were then tested for 7 consecutive days without drug and injected with either carrier or DZP (1.5 mg/kg) on the 8th day of exposure.

Data was analyzed by the Kruskal-Wallis analysis of variance followed by individual comparisons using the Mann-Whitney U test [13]. Data of the incidence of urination was analyzed by the Fisher Exact Probability Test.

RESULTS

The effects of a single injection of diazepam (DZP) at doses of 0.75, 1.5 or 3.0 mg/kg, are shown in Fig. 1. In the open field, only the group receiving the dose of 1.5 mg/kg showed an increase in the amount of food eaten during the 15 min test. The measure of the mean g of food eaten per approach to the food pedestal (mean g/App) was increased by

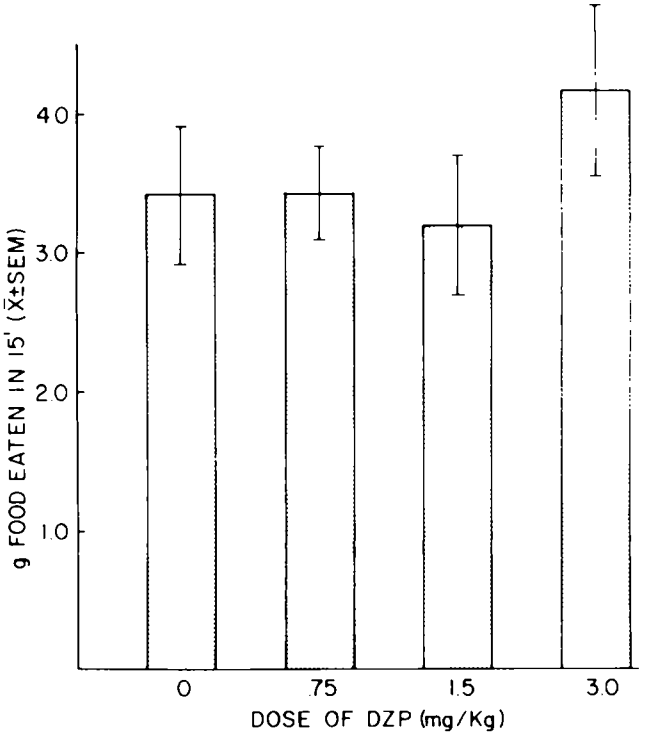

FIG. 3. Effects of diazepam of 15 min food consumption by fasted rats in their home cages. Animals were tested 30 min after the last of 17 daily injections of drug or carrier (n per group same as in Fig. 2).

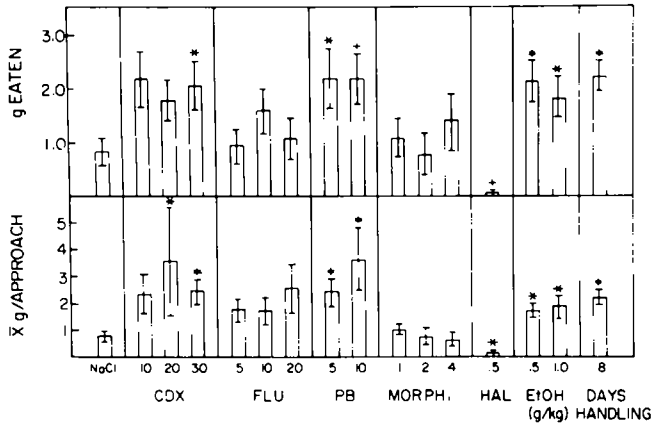

FIG. 4. Effects of chlordiazepoxide (CDX), flurazepam (FLU), pentabarbitol sodium (PB), morphine (MORPH), haloperidol (HAL), Ethanol (EtOH) and an extra 5 days of handling (8 days total) on total g food eaten and the mean g eaten per approach to the food pedestal (mean g/App). Values represent the mean±SEM. The number of animals per group is shown in Table 1. Significant differences from control (C): $*p<0.05$, $\dagger p<0.01$, $\ddagger p<0.005$.

all three doses, reaching significance at the 1.5 mg/kg dose. If the animals were treated with daily injections for 15 days prior to testing, DZP produced a dose related increase in the mean g/App which was significant at both of the higher doses (Fig. 2) and an increase in the total amount of food eaten at the 1.5 and 3.0 mg/kg doses with only the former being significant. The lack of significance in the 3.0 mg/kg group reflects an n of only 3 animals.

Since DZP has been shown to have hyperphagic actions [13,14] which could interfere with the anticonflict test, the chronically treated animals were retested two days after the open field testing for food consumption in their home cages. Figure 3 shows that DZP, at these doses, does not alter the amount of food consumed by fasted animals during a 15 min session in their home cages.

A variety of other compounds were tested for their ability to alter the amount and pattern of food consumption by fasted animals in the open field (see Fig. 4). Chlordiazepoxide, a benzodiazepine with anxiolytic properties, acted like diazepam in increasing the amount of food eaten and the mean g/App. Similar results were obtained for pentabarbitol sodium and for ethanol. The benzodiazepine, flurazepam (FLU) which is prescribed primarily as a seda-

TABLE 1

EFFECTS OF DRUGS AND HANDLING ON OPEN FIELD BEHAVIOR

Treatment Dose	(n)		Frequency of Grooming (Mean ± SEM)	Frequency of Rearing (Mean ± SEM)	Incidence of Urination	Approaches to Food (Mean ± SEM)
Saline		(14)	4.7 ± 1.2	47.8 ± 7.3	7/14	8.4 ± 2.0
Diazepam						
0.75	mg/kg	(8)	—	47.6 ± 8.0	—	12.0 ± 4.1
1.5	"	(8)	—	45.4 ±	—	14.1 ± 3.1
3.0	"	(8)	—	‡17.0 ± 3.4	—	3.4 ± 1.3
Chlorodiazepoxide						
10.0	mg/kg	(11)	3.6 ± 0.7	34.4 ± 8.7	*3/14	9.0 ± 2.5
20.0	"	(9)	2.9 ± 0.5	43.3 ± 9.7	3/ 9	7.9 ± 1.3
30.0	"	(9)	2.1 ± 0.7	42.0 ± 6.6	4/ 9	8.3 ± 1.6
Flurazepam						
5.0	mg/kg	(8)	3.5 ± 0.9	36.7 ± 5.1	6/ 8	4.4 ± 1.0
10.0	"	(8)	2.4 ± 0.7	41.0 ± 8.9	7/ 8	8.4 ± 2.3
20.0	"	(8)	*1.7 ± 0.7	‡17.7 ± 3.0	4/ 8	3.0 ± 0.9
Pentobarbitol						
5.0	mg/kg	(8)	2.8 ± 0.6	41.7 ± 7.8	6/ 8	7.8 ± 0.7
10.0	"	(9)	*1.2 ± 0.3	*27.0 ± 6.8	4/ 9	8.9 ± 1.3
Ethanol						
0.5	mg/kg	(10)	2.4 ± 0.5	34.7 ± 3.0	3/10	11.8 ± 1.4
1.0	"	(9)	4.0 ± 0.9	*24.6 ± 4.0	5/ 9	9.8 ± 1.9
Morphine Sulfate						
1.0	mg/kg	(7)	5.6 ± 2.3	†83.1 ± 12.2	2/ 7	9.6 ± 2.6
2.0	"	(7)	4.6 ± 1.2	69.9 ± 16.0	1/ 7	6.7 ± 2.8
4.0	"	(8)	*1.6 ± 0.4	60.1 ± 8.1	*0/ 8	14.7 ± 6.0
Haloperidol						
0.25	mg/kg	(4)	*0.0 ± 0.0	‡ 0.0 ± 0.0	0/ 4	* 0.0 ± 0.0
0.5	"	(6)	*0.0 ± 0.0	‡ 0.7 ± 0.4	*0/ 6	* 0.3 ± 0.3
8 Days Handling		(12)	4.7 ± 0.8	‡78.5 ± 4.6	8/12	11.4 ± 2.0

— Insufficient observations on these parameters.

Significantly different from saline group: $*p<0.05$, $\dagger p<0.01$, $\ddagger p<0.005$ by Rank Sum Test (Texas Instruments Program).

TABLE 2

EFFECTS OF REPEATED EXPOSURE TO THE OPEN FIELD

Day of Exposure	Frequency of Grooming (Mean ± SEM)	Frequency of Rearing (Mean ± SEM)	Incidence of Urination	No. of Approaches to Food (Mean ± SEM)
1	3.14 ± 0.86	56.4 ± 3.9	4/7	18.4 ± 2.7
2	3.14 ± 1.19	*37.6 ± 8.5	2/7	12.4 ± 3.2
3	3.14 ± 0.40	†30.9 ± 7.0	1/7	14.4 ± 2.7
4	2.43 ± 0.57	‡26.3 ± 3.8	1/7	13.6 ± 2.4
5	2.67 ± 0.61	‡22.0 ± 3.0	*0/7	12.3 ± 2.1
6	1.00 ± 0.22	‡24.0 ± 5.4	*0/7	*11.9 ± 1.3
7	0.83 ± 0.31	‡11.8 ± 2.0	*0/7	* 8.3 ± 0.9

Significantly different from day 1: *$p<0.05$, †$p<0.01$, ‡$p<0.005$.

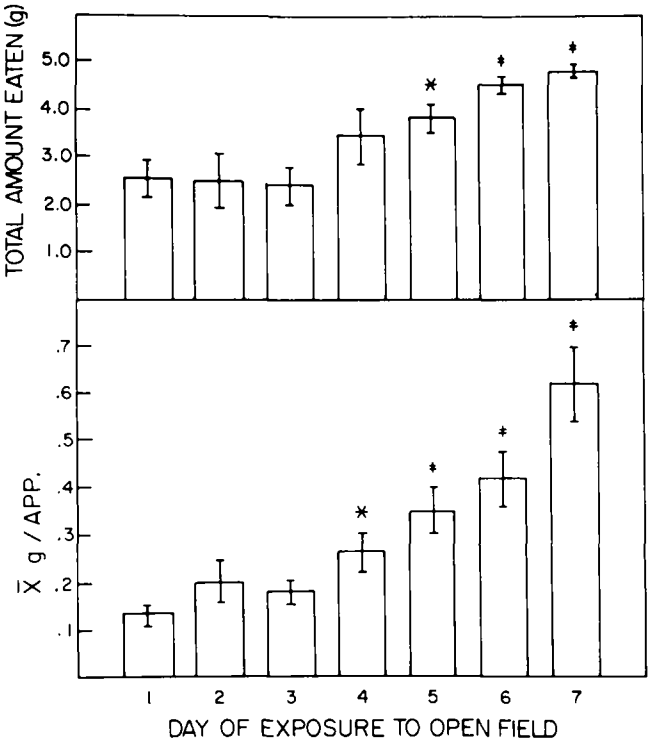

FIG. 5. Effects of repeated exposure to the open field on the total amount eaten during the 15 min session (upper figure) and the mean amount eaten per approach to the food pedestal (mean g/App.) (lower figure). Significantly different from day 1: *$p<0.05$, ‡$p<0.005$.

tive rather than an anxiolytic also increased these measures but the increase was not statistically significant. At the highest dose of FLU (20.0 mg/kg) there was, however, a significant reduction in both rearing and grooming (Table 1). The failure of this compound to produce anticonflict effects was apparently due to its sedative effects which caused some animals in the 20.0 mg/kg dose group significant ataxia with a consequent failure to approach the food pedestal. Morphine caused an increase in rearing significant in the 1.0 mg/kg group and a significant decrease in grooming at the dose of 4.0 mg/kg. There was no significant effect of morphine on total food consumption or on the mean g/App. Haloperidol at a dose of 0.5 mg/kg, significantly decreased the total food consumed, the number of approaches, the mean g/App, amount of rearing and the amount of grooming. A smaller sample of 4 animals tested at a dose of 0.25 mg/kg haloperidol showed similar behavior.

Two non-pharmacologic approaches were taken to alter the animals' behavior in this test. In the first, animals were handled daily for 8 days instead of the usual 3 days prior to testing. The results of this gentling procedure, as shown in Fig. 4 and Table 1, were to increase both the amount of food eaten and the mean g/App. In the second procedure, 8 animals were maintained on a restricted diet (10 g per day) for a total of 12 days. Starting on the fifth day, animals were tested daily in the open field as previously described. As shown in Table 2 and Fig. 5, this process of habituation resulted in changes similar to those seen with anxiolytics. By the second day there was a significant reduction in rearing. By day 4 there was a significant increase in the mean g/App and by day 5, an increase in the amount of food eaten and a decrease in urination. On the 8th day four animals received DZP (1.5 mg/kg). DZP failed to alter any of the observed parameters following the habituation (data not shown).

DISCUSSION

The drugs which have previously been reported to have anti-conflict properties in other paradigms (DZP, CDX, PB and EtOH) produced similar response profiles. The most sensitive measures to be significantly affected were the amount of food eaten and the mean g eaten per approach to the food pedestal. Of these two measures, the latter appears to be the more sensitive and occurs at doses which have no significant effect on the number of approaches to the pedestal, the amount of rearing or the amount of grooming. At higher doses this group of drugs produces sedative-like effects evident as a decrease in rearing and a greater incidence of animals which do not approach the food but remain relatively immobile throughout the session. At these higher doses there is also a significant decrease in grooming in animals treated with DZP, CDX and PB.

The hyperactivity produced by morphine was manifest as an increase in rearing. Morphine had no significant effect on any of the food related parameters at any of the three doses tested. The effect of haloperidol was to decrease activity in a general fashion including the amount eaten, the number of approaches to the food, the amount of rearing and the amount of grooming.

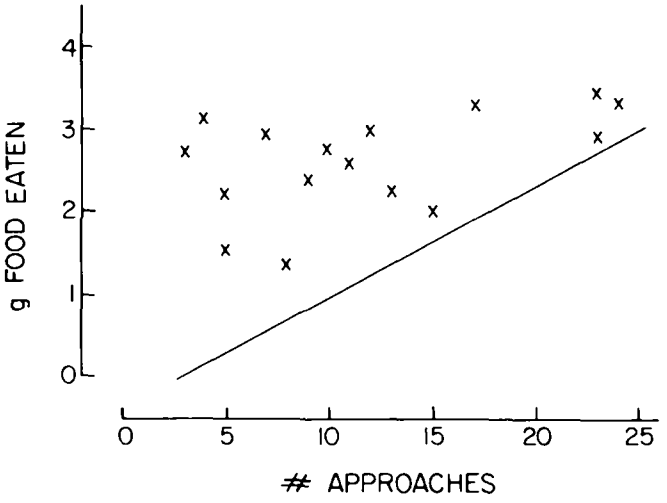

FIG. 6. Correlation of total g food eaten during a 15 min test of the number of approaches to the food pedestal. The solid line represents saline treated animals (n=30), r=.884 ($p<0.0001$); x's represent animals treated acutely with diazepam (1.5 mg/kg) (n=16, r=.481, $p<0.05$). Data combined from this and a previous study. (Britton *et al.*, submitted for publication).

The results of this study show that benzodiazepines and other anxiolytic drugs, such as pentabarbitol and ethanol, alter behavior of fasted rats in the open field. In a setting where eating conflicts with the animal's tendency to respond to the novel environment (e.g., by avoiding the center of the field) anxiolytics facilitate the "resolution" of that apparent conflict by increasing the amount of food eaten per approach to the food pedestal.

An analysis of the results suggests that the mean g eaten per approach reflects a pattern of behavior associated with the degree of the animal's conflicting response tendencies. The measure of mean g eaten per approach could potentially be altered by drug effects on any system(s) involved with either the number of approaches an animal makes to the food or the amount of food eaten during the 15 min session. For a constant amount of food consumption, treatments which reduce approaches to the food pedestal would increase the measure of mean g/App and could be seen as having anticonflict properties. Thus, one might expect any drug with sedative properties to decrease food approaches and thereby, artifactually to show a positive response in this test. In the case of the anxiolytics, there are arguments against this interpretation. Firstly, this class of drugs shows significant increases in the mean g/App at doses which have no effect on the number of approaches to the food pedestal. Secondly, since tolerance develops rapidly to the sedative actions of BDZ's [11], one would expect the indices of sedation to be much reduced in animals chronically treated with DZP. However, animals show no tolerance to the measure of the mean g/App for up to 15 days of treatment.

Since BDZ's increase eating in sated animals [10,17], testing conditions which allowed the expression of this effect risk increasing the mean g/App by mechanisms more directly related to food consumption *per se* than to processes involved in elaborating responses to a novel environment. Since DZP did not affect food consumption by fasted animals tested for 15 min in their home cages, it appears that fasting produces a ceiling effect on appetite which is not overcome by DZP treatment during the 15 min duration of the tests. Comparing the amount of food eaten and the mean g/App by naive animals to that by animals habituated to the open field, it is apparent that the exposure of animals to the novel environment has a suppressive effect on the amount and pattern of food consumption. It is this suppressive effect of novelty which is attenuated by anxiolytics. Soubrie *et al.* [15] have reported a benzodiazepine-induced increase in food consumption by fasted rats and mice in both novel and familiar environments suggesting a hyperphagic action of the drugs unrelated to any anxiolytic effect. The habituation results (Fig. 5) show that, while the total amount eaten is increased by about 90% on day 7 compared to day 1, the mean g eaten per approach is increased approximately 400%. Also, this latter measure increases significantly over day 1 before there is a significant change in the total amount eaten. It, therefore, appears that the mean g per approach is a more sensitive measure of the animal's response to the novel environment as well as a more sensitive measure of anxiolytic drug effects. Figure 6 shows the correlations of the amount of food eaten during the 15 min test with the number of approaches made to the food pedestal for animals treated with carrier or DZP (1.5 mg/kg). This figure combines data presented here and that from another study (Britton *et al.*, submitted for publication). As this figure indicates, the DZP treated animals tended to eat more per approach regardless of the number of approaches. This relationship also held for groups receiving CDX, PB, EtOH or excess handling prior to testing. This tendency is consistent with the observations of Cooper and Francis [3] that BDZ treated mice showed increased duration of eating in the open field.

The fact that morphine failed to have demonstrable anticonflict effects in this test is consistent with the lack of effects of morphine in the Geller operant paradigm [7]. While it has been argued that morphine does have anxiolytic actions in man and that these effects can be demonstrated in the startle response paradigm used by Davis [5], it would seem likely that in the present procedure, any anxiolytic/anticonflict properties of morphine are obscured by more prominent actions of the drug, including its ability to induce hyperactivity.

This procedure offers a sensitive measure of anxiolytic drug action with some advantages over previously reported conflict procedures: (1) No pre-training of animals is required, (2) The test is of short duration allowing testing of large groups of animals, (3) The equipment required is extremely simple and inexpensive, (4) The response is not confounded by treatments which would alter operant responding *per se*, and (5) The animal experiences no actual physical stress such as electric shock.

Possible artifacts in using this procedure to screen for anxiolytics could arise from agents which have direct effects on appetite, thus altering the reward value of approaching the food pedestal. That this is not a factor for diazepam is indicated by the observation that this drug has no effect on food consumption by fasted animals tested in home cages under otherwise similar conditions. Other agents which may have anxiolytic properties but also have a strong sedative action may not show positive results in this test due to the ataxia associated with sedatives. The procedure, therefore, appears most sensitive to relatively pure anxiolytics.

ACKNOWLEDGEMENTS

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