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A Test of Anxiety that Distinguishes Between the Actions of Benzodiazepines and Those of Other Minor Tranquilisers and of Stimulants

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FILE, S. E. AND J. R. G. HYDE. *A test of anxiety that distinguishes between the actions of benzodiazepines and those of other minor tranquilisers and of stimulants.* PHARMAC. BIOCHEM. BEHAV. 11(1) 65–69, 1979.—The effects of minor tranquilisers and of stimulant drugs were studied in the Social Interaction test of anxiety in which the illuminance and unfamiliarity of the test arena are manipulated. Acute administration of sodium phenobarbitone (25 mg/kg) was without effect. Acute administration of sodium phenobarbitone (35 mg/kg) and of meprobamate (60 mg/kg) produced sedation: both locomotor activity and social interaction were reduced. On the other hand, amphetamine sulphate (2 mg/kg) and caffeine citrate (20 mg/kg) reduced social interaction, but increased locomotor activity. Chronic administration dissociated the pattern of results produced by sodium phenobarbitone (35 mg/kg) from that produced by flurazepam (0.5 mg/kg). With chronic treatment (5 days) neither drug reduced motor activity, but whereas phenobarbitone increased social interaction regardless of the test illuminance and unfamiliarity, the increase produced by flurazepam was limited to the more stressful test conditions, i.e., when the arena was unfamiliar or brightly lit.

Flurazepam Phenobarbitone Meprobamate Amphetamine Caffeine Social interaction
Locomotor activity Rats Acute and chronic administration

WE have recently described [4] a test of anxiety that is based on the social interaction between pairs of male rats. Social interaction is greatest when the rats are tested in an arena that is under low illumination and with which they are familiar; there is significantly less social interaction when the test arena is unfamiliar to the rats, or when it is brightly lit. We found that repeated administration of chlordiazepoxide (5 mg/kg for 5 days) prevented or significantly reduced the decrease in active social interaction that normally occurs in unfamiliar or brightly lit environments. This effect is in contrast to that found after a single dose [3]. Acute administration of chlordiazepoxide produced a dose-related decrease in active social interaction in all the test conditions, a decrease in motor activity, and an increase in passive contact (i.e., the rats sitting or lying with their bodies in contact, but without showing any active interaction). Thus is seemed that our test distinguished between sedation, produced by acute chlordiazepoxide, and the behavioural profile produced by 5 days of treatment. These differences between acute and chronic treatment have also been found for diazepam and desmethyl diazepam in the mouse [1] and in man [13].

The purpose of the present study was to extend our attempts to validate the Social interaction test, by determining whether the behavioural profile seen with benzodiazepines is indeed specific to the benzodiazepines, or whether it is also shown by other minor tranquilisers, e.g., meprobamate and phenobarbitone. Since the chronic administration of benzodiazepines increases social interaction in certain test conditions, we also examined the effects of two stimulants, amphetamine and caffeine, in order to see if the effects of these drugs could be distinguished from those of benzodiazepines.

Since the pattern of results produced by the benzodiazepines is different for acute and chronic (5 days) administration, the effects of phenobarbitone were also examined both acutely and chronically. The effects of meprobamate were examined only after acute administration because the solvent (propylene glycol) has been found to produce significant behavioural changes with chronic treatment (unpublished results). The doses of sodium phenobarbitone (25 and 35 mg/kg) and the dose of meprobamate (60 mg/kg) were chosen on the basis of the results from the Geller-Seifter conflict test [7] and were those that produced similar results in that test to those seen with 5 mg/kg chlordiazepoxide, or equivalent doses of other benzodiazepines. For the chronic studies the dose of sodium phenobarbitone was selected on the basis of the results with acute administration: and the dose of flurazepam (0.5 mg/kg) was chosen on the basis of results from pilot experiments where it was found to be approximately ten times more potent than chlordiazepoxide.

METHOD

Animals

A total of 432 male hooded rats (from Olac Ltd. Bicester) was tested. Each was housed singly prior to the social interaction test. Food and water were available ad lib. The light schedule was 11 hr on, 13 hr off, with lights on at 0700 hr. The rats ranged from 200 g to 350 g at the time of testing, but

within each experiment (a drug group+appropriate controls) the weight range of the rats did not exceed 50 g.

Drugs

Flurazepam (Roche Products Ltd) was dissolved in deionised water, meprobamate (Wyeth) in propylene glycol, and sodium phenobarbitone (May and Baker Ltd), amphetamine sulphate (B.D.H.) and caffeine citrate (B.D.H.) in saline. All the drugs were administered intraperitoneally in a volume of 2 ml/kg, and the control rats received equal-volume injections of the appropriate solvent.

Apparatus

The test arena had a wooden floor 65×65 cm and walls 47 cm high. The low and high light levels were 13 and 333 scotopic lux, respectively. (It is appropriate to use scotopic units since the rat has a predominantly rod retina.) A camera was mounted immediately above the test arena and the rats were observed on a video monitor in an adjacent room. Infrared photocells along the walls of the arena were connected to counters and provided an automated measure of motor activity.

Procedure

Within each experiment rats were randomly allocated among the drug and control groups: they were then randomly assigned to the test conditions, such that six pairs in each drug group were allocated to each test condition. No pair of rats was tested more than once. Three test conditions were used: low light, familiar: low light, unfamiliar: high light, unfamiliar. Rats were allocated to test partners on the basis of weight, so that they did not differ from each other by more than 10 g.

During the five days of single housing, the rats were weighed and handled daily. On the two days before the social interaction test the rats in the familiar test conditions were placed singly in the test arena, under the appropriate light level, for 10 min. The rats in the unfamiliar test conditions were placed in the test room for 10 min, under the appropriate light level, but remained in their home cages.

On the day of social interaction testing the rats received their appropriate IP injections 30 min before the test, except for meprobamate and phenobarbitone where the intervals were 60 min and 15 min, respectively. Each pair of rats was placed in the centre of the arena for a 10-min trial and their behaviour was scored by two observers (who each scored one rat) from a video monitor in an adjacent room. Both members of a rat pair had the same prior familiarisation experience and the same drug treatment. Since the behaviour of one rat cannot be considered as independent of its partner's behaviour, pair scores were always used, thus there would be a maximum score of 1200 sec.

The following behaviours were scored as active social interaction: sniffing, nipping, grooming, following, mounting, kicking, boxing, wrestling, jumping on, crawling under or over the partner. The majority of the interactions were investigatory in nature (sniffing and following the partner) and sexual and aggressive behaviours were rare. Passive contact, when the rats were sitting or lying with their bodies in contact, but without interacting with each other, was not included in the active interaction score.

The rats were tested in a randomised order between 0800 and 1200 hr. After each pair of rats was removed the test arena was carefully wiped and dried, to remove any trace of odour trails left by the previous pair.

Statistics

Since the data were normally distributed, there was homogeneity of variance and the standard error was approximately 10% of the mean, parametric statistics were used. The data were subjected to two-way analyses of variance with the drug treatment as one factor and the test conditions as the other. An anxiolytic profile is revealed by a significant drug×test condition interaction, i.e., the drug significantly modifies the decrease in social interaction that is normally shown across the test conditions. Since the interpretation of a drug×test condition interaction is difficult if the drug also changes the overall level of interaction, a further restriction is placed on the definition of an anxiolytic profile: that the drug×test condition interaction should be produced without significant change in the baseline level of response.

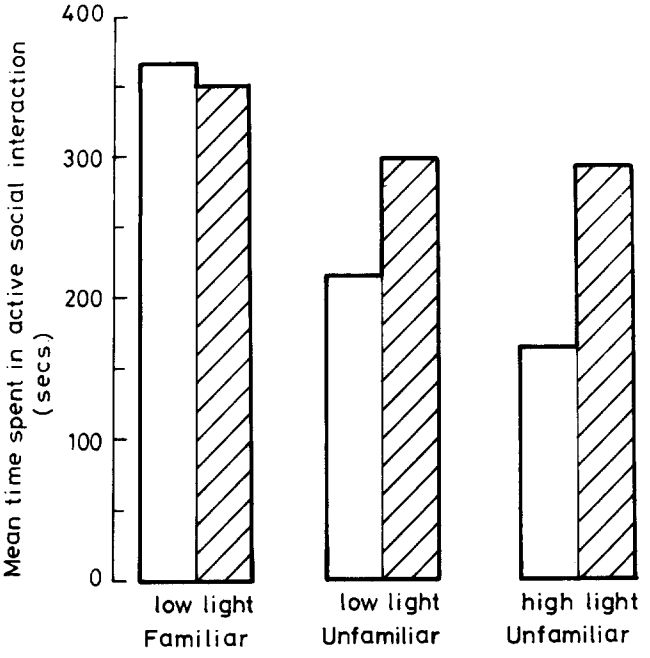

FIG. 1. The mean time spent in active social interaction for rats tested in 3 conditions, after 5 days of flurazepam administration (0.5 mg/kg ▨) and after 5 days of water injection (□).

RESULTS

Figure 1 shows that flurazepam (given chronically) produces a pattern of results similar to that previously seen with chronic administration of chlordiazepoxide: the flurazepam-treated rats showed significantly less change in social interaction across the 3 test conditions, compared with the controls, i.e., a significant drug×test condition interaction, $F(2,30)=4.5$, $p<0.02$. It should be emphasised that the definition of an anxiolytic profile is this test condition×drug interaction, i.e., the failure of the drug treated rats to respond in the normal way to the manipulations of familiarity and light level. With chronic administration flurazepam had no significant effect on motor activity (see Table 1).

TABLE 1

LOCOMOTOR ACTIVITY DURING THE SOCIAL INTERACTION TEST. THE SCORES ARE THE MEANS FROM 18 PAIRS OF RATS TESTED IN EACH DRUG GROUP

Chronic (5 days) administration			
water control	Flurazepam 0.5mg/kg for 5 days	saline control	Sodium Phenobarbitone 35mg/kg for 5 days
685	670	680	726*

Acute Administration				
Propylene Glycol control	Meprobamate 60mg/kg	saline control	Sodium Phenobarbitone 25mg/kg	35mg/kg
715	563*	665	645	526*

Saline control	Amphetamine Sulphate 2mg/kg	caffeine citrate 20mg/kg
697	754*	774*

*Significantly different from controls on analysis of variance, see text for details.

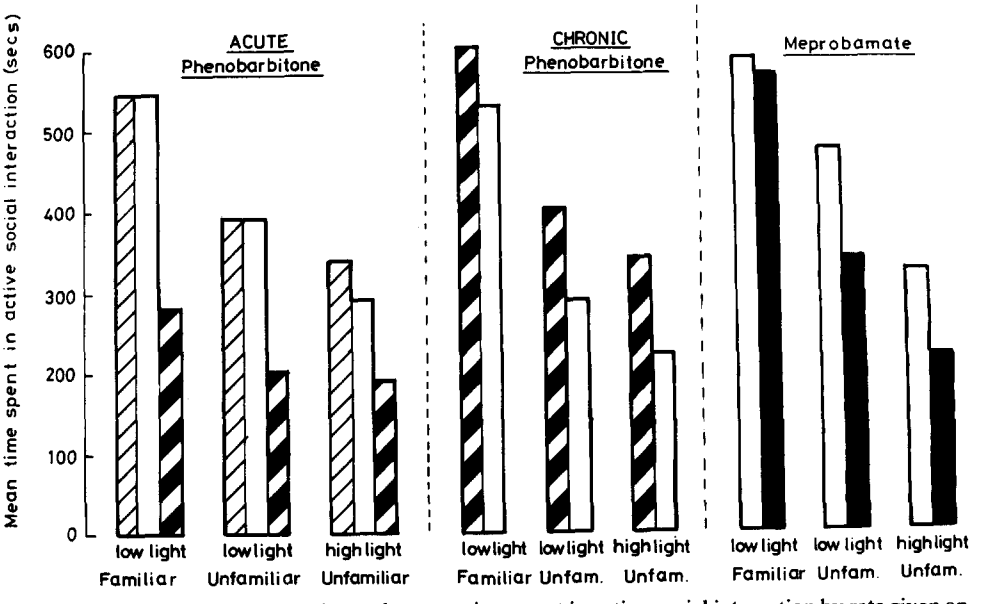

FIG. 2. The left-hand histogram shows the mean time spent in active social interaction by rats given an acute dose of sodium phenobarbitone (25 mg/kg ▨ an 35 mg/kg ◪) and by a group of saline-injected control rats □). Each point is the mean score from six pairs of rats, and independent groups of rats were tested in the 3 different test conditions. The central panel shows the results for rats treated with sodium phenobarbitone (35 mg/kg ◪) for 5 days and for rats given 5 days of saline injections (□). The right hand histogram shows the results for rats given an acute dose of meprobamate (60 mg/kg ■) and for control rats given propylene glycol injections (□).

The results with meprobamate contrast with those seen with flurazepam. It had a sedative effect, which was shown by a significant reduction in both motor activity, $F(1,30)=15.5, p<0.001$ (Table 1) and in active social interaction, $F(1,30)=5.6, p<0.05$ (see Fig. 2).

Given acutely, phenobarbitone sodium (25 mg/kg) was without effect on either motor activity or on social interaction. However, the higher dose (35 mg/kg) on acute administration produced significant reductions in both motor activity (see Table 1) and in social interaction (see Fig. 2), $Fs(1,30)=26.2$ and 36.2, respectively, $p<0.001$. In addition to its marked sedative effect phenobarbitone (35 mg/kg) produced a significant drug×test condition interaction, $F(2,30)=3.9, p<0.05$.

When phenobarbitone (35 mg/kg) was administered chronically, there was tolerance to its sedative effects, and in contrast it caused an overall increase in active social interaction, $F(1,30)=14.1, p<0.001$: see Fig. 2, as well as an in-

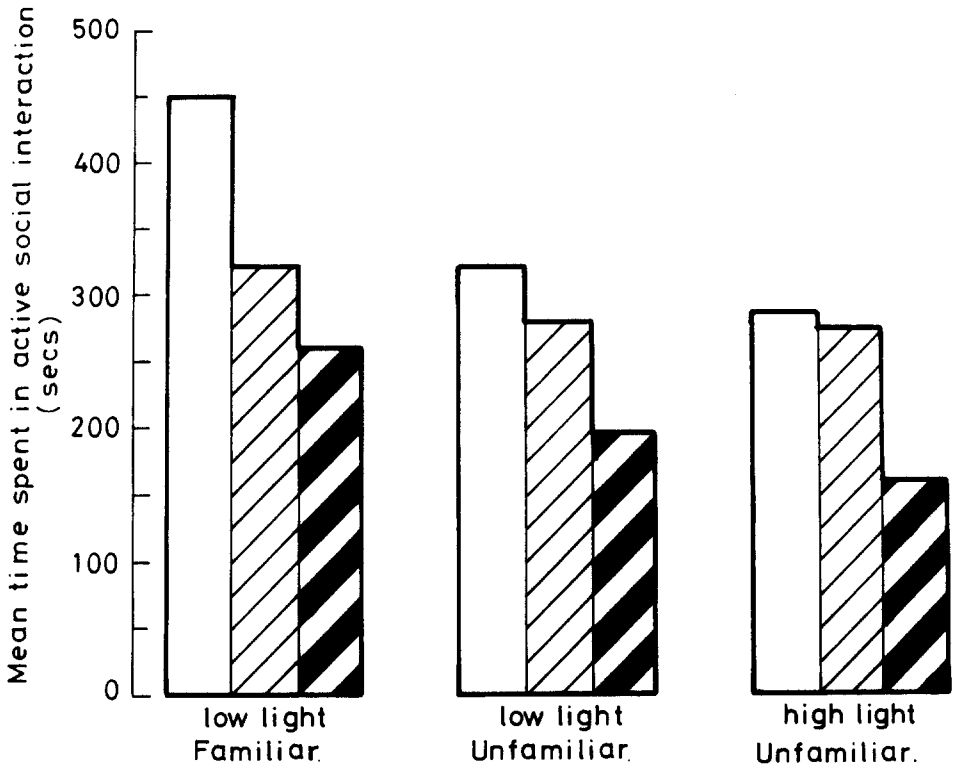

FIG. 3. The mean time spent in active social interaction, in 3 test conditions, for rats injected with caffeine citrate (20 mg/kg ▩),amphetamine (2mg/kg ◪) and for saline injection controls (□).

crease in motor activity, $F(1,30)=4.2$, $p<0.05$. The pattern of results seen with chronic phenobarbitone can be distinguished from that found with flurazepam. Phenobarbitone increased social interaction in all 3 of the test conditions, but the rats treated with this drug still showed a significant decrease in social interaction across the test conditions, $F(2,30)=26.8$, $p<0.001$, and there was no drug×test condition interaction, $F(2,30)=0.5$. In contrast, the flurazepam-treated rats showed no significant change in the level of social interaction when the test conditions were manipulated, thus producing a significant drug×test condition interaction. In other words, flurazepam increased social interaction only when the test conditions were stressful, i.e., brightly lit or unfamiliar.

Figure 3 shows the results from the two stimulant drugs, amphetamine and caffeine. Both drugs significantly reduced the level of social interaction, $F(1,30)=21.1$ and 6.1, $p<0.001$ and 0.02, respectively, whilst significantly increasing the level of motor activity, $F(1,30)=4.9$ and 8.2, $p<0.05$ and 0.01, respectively.

DISCUSSION

The absolute scores of the various control groups differed for several reasons. First, these experiments spanned more than 2 years and the rats from the animal suppliers may have changed in many ways: baseline levels of social interaction have been found to differ markedly in rats from different sources [5]. Secondly, although within each experiment the rats were very similar in weight, between experiments there was a considerable range: and the weight of the rats has a significant effect on the level of social interaction [4]. Thirdly, the control groups received different vehicles. The data shown in Fig. 2 all came from experiments conducted at a similar time, and in which the rats did not exceed 250 g. The data from Fig. 1 came from an experiment conducted 28 months later in which the rats were 100 g heavier. The day-to-day variation was not high, and the standard errors of the mean scores for each group were around 10% of the means.

The behavioural profile in the Social Interaction test [4] that was originally found with chronic (5 days) administration of chlordiazepoxide has now also been found with chronic administration of flurazepam in the rat and diazepam and desmethyldiazepam in the mouse [1]. The chronic effects of benzodiazepines contrast with the sedative effects found with acute administration [1,3], and none of the benzodiazepines produced significant motor sedation when given chronically. These results are similar to those reported by Malick [9] where, with chronic administration of diazepam, mice developed tolerance to the CNS depressant effects, but the antagonism of isolation-induced fighting was not diminished.

Given acutely, both meprobamate (60 mg/kg) and sodium phenobarbitone (35 mg/kg) produced marked sedation, and thus their effects resembled those of acutely administered benzodiazepines. The doses of meprobamate and sodium phenobarbitone causing sedation are not high compared with those used in other animal tests, and the dose of meprobamate is less than the lowest dose used in the Geller-Seifter test and considered not to be sedative [7]. With chronic administration of phenobarbitone there was not only tolerance to the sedative effects, but an increased level of both social interaction and motor activity. However, the phenobarbitone-treated rats still showed a significant change

in social interaction when the test conditions were manipulated. This contrasts with the effects seen with chronic benzodiazepine treatment where the rats were insensitive to manipulations of test conditions. Thus in order to distinguish between the profile produced by chronic phenobarbitone and that produced by chronic administration of benzodiazepines it is necessary to test the rats in more than one test condition. If only one test condition had been used, e.g., high light, unfamiliar, the 2 drugs would have appeared to have had the same effect of increasing social interaction. It is only when it can also be seen that phenobarbitone, but not flurazepam, increases social interaction in the low light, familiar test condition, and the results of at least two test conditions are considered together that the distinction can be made.

The stimulants, like acutely administered meprobamate, phenobarbitone and benzodiazepines, reduced social interaction. The results with amphetamine are similar to those reported by Syme and Syme [12]. The stimulants can, however, be distinguished from the other drugs by the concomitant increased level of motor activity that they produce.

Whilst it is easy to distinguish behaviourally between the effects of stimulants and of minor tranquilisers, within the limitations of the doses used in this experiment, the social interaction test appears to be the first one to distinguish between the actions of meprobamate and phenobarbitone on the one hand, and the benzodiazepines on the other. One of the most widely used animal tests of anxiety is the Geller-Seifter conflict test, but in this test meprobamate, barbiturates and benzodiazepines all produce similar profiles [6,7]. The conditioned emotional response test [2] has also been used as an animal test of anxiety. However, this test gives very variable effects with benzodiazepines [8] and, in general, they produce significant effects when given acutely, but not when given chronically [11]. This is the opposite way round from that found clinically [13], in the Geller-Seifter test [10] and in the social interaction test [1,3]. The social interaction test would appear to have the further advantage of being able to distinguish between the acute and chronic effects of both barbiturates and of benzodiazepines.

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