Br. J. Pharmac. (1978), **62**, 19–24

CAN SOCIAL INTERACTION BE USED TO MEASURE ANXIETY?

SANDRA E. FILE & J.R.G. HYDE

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

1 Pairs of male rats were placed in a test box for 10 min and the time they spent in active social interaction was scored. Maximum active interaction was found when the rats were tested under low light in a box with which they were familiar. When the light level was increased or when the box was unfamiliar active social interaction decreased.

2 Exploration (time spent sniffing objects) decreased in the same way in relation to test conditions as did social interaction. As these decreased, defecation, and freezing increased.

3 Anosmic controls showed that the decrease in social interaction across test conditions could not be attributed to olfactory changes in the partner.

4 Chlordiazepoxide (5 mg/kg) given chronically prevented or significantly reduced the decrease in social interaction that occurred in undrugged rats as the light level or the unfamiliarity of the test box was increased. Controls showed that this effect could not be entirely attributed to chlordiazepoxide acting selectively to increase low levels of responding.

5 The effect of chronic chlordiazepoxide contrasts with its action when given acutely; in the latter case it has only sedative effects.

6 Whether this test can be used as an animal model of anxiety is discussed and this test is compared with existing tests of anxiety.

Introduction

When pairs of male rats are placed in a situation in which neither has established territory they engage in social interaction which includes a variety of behaviours (sniff, follow, walk over, crawl under, allogroom), but in which sexual (mount) and aggressive behaviours (kick, bite, box, wrestle) are infrequent (Latane, 1969; File & Pope, 1974; Whatson, Smart & Dobbing, 1976). It is important to distinguish between this active interaction and passive body contact between the rats, i.e. the rats just sitting or lying with their bodies in contact, since the two are controlled by different factors (File & Pope, 1974). The maximum *active* interaction is found when the rats are tested in a box with which they are familiar and which is under a low level of illumination. If the light level is increased or if the test box is unfamiliar active social interaction decreases. This decrease is prevented by one anxiolytic, ethanol (0.4 g/kg) as shown by File & Pool in a communication ('Dutch Courage') to the Experimental Psychology Society in 1976. However, a second anxiolytic, chlordiazepoxide (2.5–7.5 mg/kg) produced a dose-related decrease in active social interaction in all the test conditions (File, Hyde & Pool, 1976).

One purpose of the present experiments was to examine other behavioural changes accompanying the changes in social interaction induced by the different test conditions. A second purpose was to examine the effects of chronically administered chlordiazepoxide since there are reports that the initial sedative effect of benzodiazepines disappears with chronic dosing, whereas the antianxiety effects persist (Warner, 1965; Cook & Sepinwall, 1975a).

Methods

A total of 272 male hooded rats (*Rattus norvegicus*), 200–250 g, were tested. They were housed singly for 5 days before the experimental test, and were allowed food and water *ad libitum*. During this period they were weighed and handled daily and the position of the cages in the rack was changed so that all rats received equal experience of the different levels of illumination.

The rats were randomly assigned to 4 test conditions. Half the rats were allocated to the 'familiar' test conditions. These were placed singly in the test

box for 10 min on 2 consecutive days before the social interaction test. The other half were allocated to the 'unfamiliar' conditions, and were placed in the test room for two 10-min sessions, but remained in their home cages. Half the rats in each of these groups were tested under high light and half under low. The familiarisation sessions took place under the appropriate light level. The high and low light levels were 338 and 23.5 scotopic lux, respectively: scotopic units are appropriate since the rat has a predominantly rod retina.

Each rat was tested for social interaction with an unknown test partner that did not differ by more than 10 g in weight. Both members of a pair had the same prior familiarisation experience and the same drug treatment. Pairs were tested in a random order between 08 h 00 min and 11 h 00 minutes. The test box was 65 × 65 cm with walls 47 cm high. Pairs of rats were placed in this box for 10 min and their behaviour observed on a television monitor in an adjacent room. The time they spent in active social contact was scored by two observers; this gave agreement to within 10 seconds. The following behaviours were scored: sniffing, nipping, grooming, following, mounting, kicking, boxing, wrestling, jumping on, crawling under or over the partner. It must be emphasized that passive contact (sitting or lying with bodies in contact) was not included in this social interaction score. At the end of the session any boluses were removed and the floor and walls of the box wiped with detergent and dried.

Behavioural measures

Six pairs of rats were tested for social interaction in each of the 4 test conditions. Each rat was tested on one occasion only. As well as scoring social interaction, each observer scored the time one rat spent exploring. Because motor activity has been so frequently criticised as a measure of exploration (Sheldon, 1968; Archer, 1973; Robbins & Iversen, 1973; File & Wardill, 1975) it is essential to use a measure of directed exploration. In this part of the experiment, therefore, objects were hung on the walls of the box and exploration was measured by the time spent sniffing and manipulating objects.

In order to see whether changes in social interaction were a direct result of environmental manipulation, or whether they were mediated by olfactory changes (e.g. a rat producing fear pheromone might be a less attractive social object) groups of anosmic rats were tested in three of the test conditions. Six pairs were tested in each condition and none had previously been tested. Anosmia was produced on the day before testing by anaesthetizing the rats with halothane in O_2 and then administering a 5% zinc sulphate solution to the external nares (Alberts & Galef, 1973; Alberts, 1974). The animal was held upside down and zinc sulphate instilled into the nares via a fine plastic cannula until 8 drops emerged from the nostrils; this usually required 0.5 to 0.7 ml of solution. Before we administered the zinc sulphate solution the animals were tested with three odours: female rat urine, fresh rat blood, and 35% ammonia solution. All the rats showed a clear response to the ammonia; and the rat urine and blood evoked a response in about 50% of the animals. The rats were tested again with these odours on the day after the zinc sulphate treatment, immediately before the social interaction test. Any rat still showing a response to any of these odours was excluded from further test.

Chronic chlordiazepoxide treatment

Chlordiazepoxide hydrochloride (CDP, Roche Products Ltd) was dissolved in deionised water to give a concentration of 2.5 mg/ml. On 5 successive days rats received an intraperitoneal injection of CDP (5 mg/kg) or water. One group was replaced in the home cage after the daily injection. A second group was placed for 10 min each day in novel apparatus, 30 min after injection. On the 6th day rats were tested for social interaction 30 min after injection. None of these rats was tested on more than one occasion.

Because it has been suggested (Dews, 1976) that chlordiazepoxide acts not to increase selectively response rates that are depressed by anxiety, but merely to increase low rates of responding *irrespective of how these are caused*, a control experiment examined how CDP varied in its effects as the baseline level of responding was manipulated. Low levels of responding were obtained in 2 ways designed not to alter anxiety: by testing heavier animals (320–350 g) and by testing in the afternoon. These rats were tested in low light, familiar conditions and their scores were compared with those of 3 groups of rats whose levels of social interaction were manipulated by varying anxiety. The latter groups were tested in low light, familiar; low light, unfamiliar; and high light, unfamiliar conditions. There were 6 pairs of rats in each group and all the rats in this experiment were tested twice, once after 5 days pretreatment with CDP and once after 5 days of water injections. Half the pairs received the drug treatment first and half received the water first.

In order to see whether any changes in social interaction could be secondary to changes in motor activity, rats were placed singly in an automated activity box (29 × 29 × 21 cm). Each rat was tested for a 10-min period 30 min after an intraperitoneal injection of CDP or water. Twelve rats were tested after an acute injection (of CDP or water) and 12 were tested after 5 days of chronic treatment (with CDP or water).

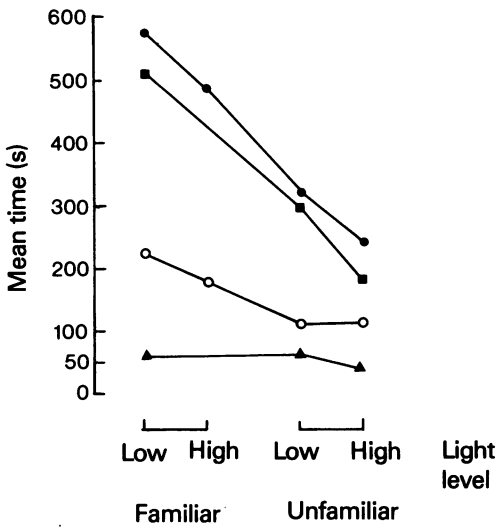

Figure 1 Mean time spent in active social contact (●) and in object exploration (○) by undrugged rats, in each of the 4 test conditions. The social contact scores (■) and exploration scores (▲) for a group of anosmic rats are also shown.

Results

Behavioural measures

In Figure 1 the 4 test conditions (low and high light intensity in familiar conditions (LF and HF) and in unfamiliar conditions (LU and HU)) are arranged in a rank order that represents a monotonic increase in the number of boluses dropped and in the incidence of freezing by rats placed singly in the test box (File & Pool, 'Dutch Courage'). These measures were not useful when the rats were tested in pairs because they occurred too infrequently (cf. Latane & Glass, 1968; Latane, 1969).

Figure 1 shows the mean time spent in exploration and in social interaction in each of the four test conditions. Each point represents the mean from 6 pairs of rats. Pair scores were used since the score from each individual cannot be considered as independent of its partner's score. A pair score was the sum of the time the two individual rats spent exploring or in social interaction and thus the maximum score would be 1200 seconds. From Figure 1 it can be seen that both object exploration and social interaction systematically decreased across the 4 test conditions ($F(3,20) = 7.6$ and 17.7, $P < 0.002$ and 0.001 respectively), and that exploration is the less sensitive measure. Since the data were normally distributed and the s.e. was $<10\%$ of the mean, analysis of variance was used.

A detailed analysis of the individual elements of the social interaction revealed that in all the test conditions most of the interaction was classed as sniffing and following. Table 1 shows the frequency of incidence of grooming the partner, of aggressive behaviours (boxing, wrestling and kicking the partner) and of mounting the partner, in each of the 4 test conditions. In each case the distribution of the behaviour is such that we are able to reject the hypothesis that the particular behaviour occurs equally often in the four test conditions ($\chi^2 = 8.9$, $P < 0.05$; 65.3 $P < 0.001$; 8.0, $P < 0.05$; respectively). These individual behaviours showed the same rank order of decreasing incidence across the test conditions as did sniffing and following.

If the social interaction were decreasing in response to a change in smell of the partner then the group of anosmic rats should have shown a constant level of interaction across the test conditions. As is shown in Figure 1 this was not the case and the anosmic rats showed a systematic decrease in interaction across the test conditions ($F(2,15) = 12.7$, $P < 0.001$). The overall level of social interaction was not reduced by the zinc sulphate treatment, an observation which agrees with an earlier report (Latané, Joy, Meltzer, Lubell & Cappell, 1972); but the level of object exploration was profoundly reduced (see Figure 1).

Chronic chlordiazepoxide treatment

Rats given an acute injection of CDP (5 mg/kg) showed significantly reduced motor activity compared with control rats, means of 200.3 ± 15.3 and 266.2 ± 17.8 respectively ($t(10) = 2.80$, $P < 0.01$). In contrast, rats given chronic CDP injections showed no significant change in motor activity compared with controls, means of 270.5 ± 15.2 and 258.3 ± 38.7 respectively.

The effects of chronic CDP (5 mg/kg) on social interaction are shown in Figure 2. The rats placed in novel apparatus following their daily injection (5

Table 1 Frequency of occurrence of individual behaviours in the four test conditions

	LF	HF	LU	HU
Grooming the partner	11	6	3	2
Aggressive behaviours	61	43	10	9
Mounting	16	12	8	4

LF: low light level, familiar condition; HF: high light level, familiar condition; LU: low light level, unfamiliar condition; HU: high light level, unfamiliar condition.

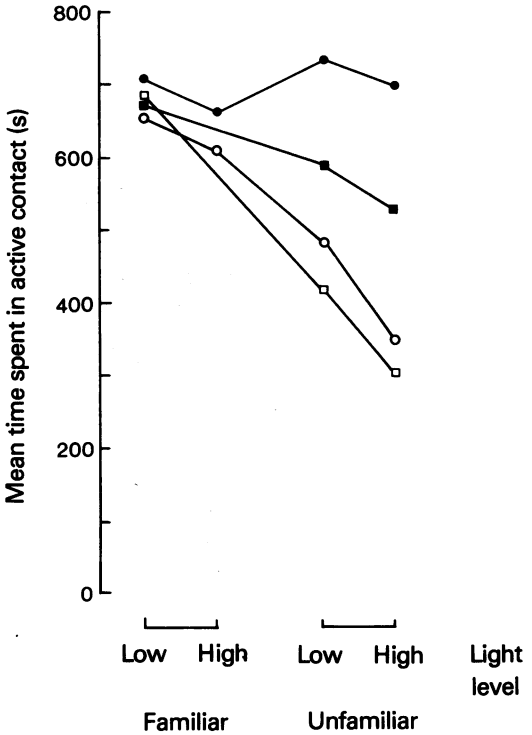

Figure 2 Mean time spent in active social contact for rats tested after chronic injections of chlordiazepoxide (solid symbols) or water (open symbols) in each of the 4 test conditions. Rats were either placed in novel apparatus 30 min after their daily injections (circles) or were replaced in their home cages (squares).

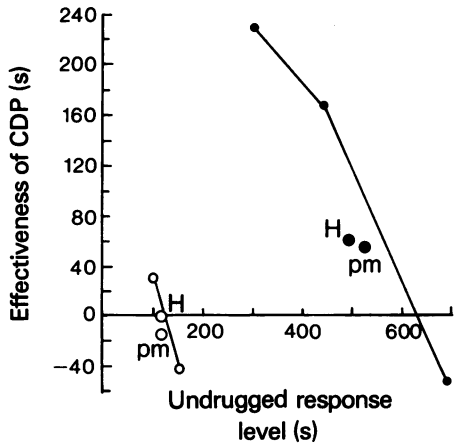

Figure 3 The effectiveness of chlordiazepoxide (CDP) plotted against the undrugged response rate. Scores are shown for rats tested in low light, familiar (LF), low light unfamiliar (LU), and high light unfamiliar (HU) conditions, for social contact (●) and for exploration (○). The scores are also shown for the group of rats tested in the afternoon (pm) and for the group of heavy rats (H).

pairs in each group) showed a significant drug × test condition interaction ($F(3,32) = 6.3$, $P < 0.002$). In other words, whilst the control rats decreased their social interaction with an increase in the light level or unfamiliarity of the box, the CDP-treated rats showed a steady level of interaction across the 4 test conditions. The chronically treated rats that were returned to their home cages after each day's injection were tested in the LF, LU and HU conditions only (6 pairs in each group). These groups also gave a significant drug × test condition interaction ($F(2,30) = 4.0$, $P < 0.03$).

Dews (1976) suggested that the lower the undrugged response level the more effective would CDP be in raising it. A measure of the effectiveness of CDP was obtained from the 3rd group of chronically treated rats by subtracting their undrugged social interaction score from their score when drugged. This effectiveness of CDP score is shown in Figure 3, plotted against the level of interaction when undrugged.

The 3 points joined by the line in Figure 3 were obtained by testing the rats in the LF, LU and HU conditions. This shows that when the light level and unfamiliarity are manipulated the lower the social interaction when undrugged the higher the effectiveness of CDP. It is also clear that CDP is more effective in increasing social contact than it is in increasing exploration. When social interaction is decreased by manipulating factors that do not involve anxiety, does CDP still increase the level of responding? Two other groups had been tested in the LF condition (to minimize anxiety): one had lower social interaction scores because they were tested in the afternoon and the other had lower scores because they were heavy animals. The effectiveness of CDP on these two groups is also shown in Figure 3 where it can be seen that CDP did indeed increase their level of social interaction to some extent, but less than when the unfamiliarity and light level (and hence possibly anxiety) were manipulated.

From this control experiment it seems that some, but not all, of the increased social interaction seen with chronic CDP can be attributed to non-anxiolytic actions of the drug. Further evidence that this is not the whole basis for increased interaction comes from an analysis of the individual behaviours. Relatively infrequent responses, such as sexual and aggressive ones, were increased by chronic CDP, but so also were the most frequent responses of all, sniffing and following.

Discussion

As the illumination or unfamiliarity of the test box was increased so the time spent by pairs of male rats in active social interaction decreased. The same rank order reflects an increase in more traditional measures of emotionality (defaecation and freezing). Exploration decreased in the same way as did social interaction but was less sensitive to the experimental manipulations. The behavioural changes are thus consistent with the suggestion that our experimental manipulations were leading to increased anxiety and that this was reflected in decreased social interaction. However, these measures alone provide insufficient validation since their validity can also be questioned (Archer, 1973).

The results with ethanol (0.4 g/kg) support an interpretation in terms of anxiety, as this drug prevented the decrease in social interaction that normally occurs when the test box is unfamiliar or the illumination is high (File & Pool, 'Dutch Courage'). Further support comes from the similar pattern of results found with chronically administered CDP. The effect of this drug on exploration was much less marked than that on social interaction, suggesting that the latter is more sensitive to the effects of anxiolytics. In this experiment we measured exploration by the time spent sniffing objects hung on the walls of the cage. Most of the exploration that occurred was directed at the objects, very little sniffing of other parts of the box occurred. More exploration of the box might have been expected in the unfamiliar than in the familiar conditions, and this would probably have been found if more than 2 familiarisation sessions had been given.

Two tests have traditionally provided animal models of anxiety: the conditioned emotional response test (Estes & Skinner, 1941) in which anxiety is equated with conditioned fear, and the rat conflict test (Geller & Seifter, 1960). Both tests involve deprivation and electric shock and any experiment involving drugs must incorporate controls for drug-induced changes in motivation or in sensory thresholds. We feel that the test described in this paper provides a useful alternative test for anxiety in that neither deprivation nor electric shock is involved. Moreover, the existing tests of anxiety fail to encompass a potent cause of anxiety, uncertainty. The uncertainty may relate to the nature of the noxious events and/or to the place and time of their arrival. Our test incorporates the element of uncertainty by manipulating the rat's unfamiliarity with the test box. An animal placed in an unfamiliar situation is uncertain about whether a noxious event will occur, what it will be, and where or when it might arrive.

Our results show that an acute dose of chlordiazepoxide produces behavioural sedation, whereas with chronic dosing the sedative effects are diminished and antianxiety effects remain. This is in agreement with data obtained from the Geller-Seifter conflict test (Margules & Stein, 1968; Cook & Sepinwall, 1975a, b) and with clinical results (Warner, 1965). In contrast the conditioned emotional response is reduced only by acute doses of benzodiazepines (Millenson & Leslie, 1974) and not by chronic doses. This discrepancy between the conditioned emotional response and other tests suggests that it might be possible to distinguish pharmacologically between conditioned fear and anxiety (as measured in the rat conflict test and in our social interaction test).

This work was supported by a Science Research Council grant to Dr M.J. Neal. J.R.G.H. holds a Science Research Council Studentship. We are most grateful to Dr D. Palmer for his advice on scotopic light measurements. The chlordiazepoxide was a gift from Roche Products Ltd.

References

ALBERTS, J.R. (1974). Producing and interpreting experimental olfactory deficits. *Physiol. Behav.*, **12**, 657–670.

ALBERTS, J.R. & GALEF, B.G. (1973). Acute anosmia in the rat: A behavioural test of a peripherally-induced olfactory deficit. *Physiol. Behav.*, **6**, 619–621.

ARCHER, J. (1973). Tests for emotionality in rats and mice: A review. *Animal Behav.*, **21**, 205–235.

COOK, L. & SEPINWALL, J. (1975a). Psychopharmacological parameters and methods. In *Emotions—their Parameters and Measurement*. ed. Levi, L. pp. 379–404. New York: Raven Press.

COOK, L. & SEPINWALL, J. (1975b). Behavioural analysis of the effects and mechanisms of action of Benzodiazepines. In *Mechanism of Action of Benzodiazepines*. ed. Costa, E. & Greengard, P. pp. 1–28. New York: Raven Press.

DEWS, P.B. (1976). Effects of drugs on suppressed responding. *Br. J. Pharmac.*, **58**, 451P.

ESTES, W.K. & SKINNER, B.F. (1941). Some quantitative properties of anxiety. *J. exp. Psychol.* **29**, 390–400.

FILE, S.E., HYDE, J. & POOL, M. (1976). Effects of ethanol and chlordiazepoxide on social interaction in rats. *Br. J. Pharmac.*, **58**, 465P.

FILE, S.E. & POPE, J.H. (1974). Social interaction between drugged and undrugged rats. *Animal Learn. Behav.*, **2**, 161–164.

FILE, S.E. & WARDILL, A.G. (1975). Validity of head-dipping as a measure of exploration in a modified hole-board. *Psychopharmacologia*, **44**, 53–59.

GELLER, I. & SEIFTER, J. (1960). The effects of meprobamate, barbiturates, d-amphetamine and promazine on experimentally induced conflict in the rat. *Psychopharmacologia*, **3**, 374–385.

LATANE, B. (1969). Gregariousness and fear in laboratory rats. *J. exp. Social Psychol.* **5**, 61–69.

LATANE, B. & GLASS, D. (1968). Social and nonsocial attraction in rats. *J. Personality Social Psychol.* **9**, 142–146.

LATANE, B., JOY, V., MELTZER, J., LUBELL, B. & CAPPELL, H. (1972). Stimulus determinants of social attraction in rats. *J. comp. Physiol. Psychol.*, **79**, 13–21.

MARGULES, D.L. & STEIN, L. (1968). Increase of "anti-anxiety" activity and tolerance of behavioural suppression during chronic administration of oxazepam. *Psychopharmacologia*, **13**, 74–80.

MILLENSON, J.R. & LESLIE, J. (1974). Conditioned emotional response (CER) as a baseline for the study of anti-anxiety drugs. *Neuropharmacology*, **13**, 1–9.

ROBBINS, T. & IVERSEN, S.D. (1973). A dissociation of the effects of d-amphetamine on locomotor activity and exploration in rats. *Psychopharmacologia*, **28**, 155–164.

SHELDON, M.H. (1968). Exploratory behaviour: the inadequacy of activity measures. *Psychon. Sci.*, **11**, 38.

WARNER, R.S. (1965). Management of the office patient with anxiety & depression. *Psychosomatics*, **6**, 347–351.

WHATSON, T.S., SMART, J.L. & DOBBING, J. (1976). Undernutrition in early life: lasting effects on activity and social behavior of male and female rats. *Dev. Psychobiol.*, **9**, 529–538.

(*Received February* 2, 1977.
Revised September 13, 1977.)