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Effects of chlorpromazine on exploration and habituation in the rat

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Summary

1. The effects of chlorpromazine, 2 and 4 mg/kg, on exploration were investigated under two independent influences—food deprivation and time of day.

2. Rats had three 3-minute trials in a hole-board apparatus; hole-investigation (head-dips) and general motor activity were recorded.

3. On trial 1 chlorpromazine reduced exploration to half the control level, but on trial 3 it had no effect on motor activity or head-dips.

4. General activity habituated from trial 1 to 3 in all groups, but some of the drug groups did not show significant habituation of head-dips, due to the low level of responding on trial 1.

Introduction

The purpose of this experiment was to investigate the effects of chlorpromazine on exploratory behaviour in the rat, stimulated by two different means. In a previous experiment (File & Day, 1972), exploration in a hole-board (similar to that described by Boissier & Simon, 1962) increased with an increase in food deprivation and also changed with the time of day. Since these two factors did not interact, each independently influences the level of exploration. The level of exploration at 10.00 h and 18.00 h after two hours of food deprivation was equal to that at 14.00 h after six hours of food deprivation. Therefore in this experiment the effect of chlorpromazine on exploration, stimulated primarily by food deprivation (six hours' deprivation at 14.00 h) was compared with its effect on exploration resulting primarily from the time of day (two hours' deprivation at 10.00 h and 18.00 h). The time spent in general motor activity was recorded as well as the number of head-dips made. Chlorpromazine reduces spontaneous activity (Stolerman, 1970, 1971), exploration of a new environment (Marriott & Spencer, 1965; Shillito, 1967; Maxwell, 1968; Kumar, 1971) and reactivity to external stimuli (Delay & Deniker, 1952; Brucke, Hornykiewicz & Sigg, 1970), but it is not known whether this effect applies to all exploratory behaviour, however motivated.

A second interest in this experiment was the effect of chlorpromazine on the habituation of exploratory responses made in the hole-board. The responses of an animal to a new situation, e.g. walking, sniffing, defaecation, and their decline on repeated trials in the situation, can be used as behavioural measures of orienting and habituation (e.g. Squire, 1966; Nadel, 1966; Martinek & Lát,

1969). Furthermore, such measures of habituation have been found to correlate well with other behavioural measures of habituation, e.g. those obtained in a distraction task (File, unpublished observations). It has been found (File, 1973a) that the inter-trial interval has little effect on the rate of habituation, over the range of 1 min to 24 h, although at intervals of <1 min the rate of habituation is slower. A 1 min inter-trial interval was therefore selected for this experiment so that three trials could be given to the animal before the period of rapid drug loss.

Methods

One hundred and thirty-five male hooded rats, 250–300 g, were housed singly in a 12 h light/12 h dark cycle (lights on from 8.00–20.00 h) at a constant temperature of 21° C. They were housed in these conditions with food and water *ad libitum* for three weeks before the start of the experiment. The food was placed in a rectangular trough in the wire lid of the cage.

Apparatus

The apparatus was a wooden box with a floor 660×560 mm in which there were 16 round holes, 38 mm in diameter, 100 mm apart. Each hole was 10 mm deep and the apparatus was placed 20 mm above the floor of the room. The box had sides 470 mm high which prevented the animal from escaping and provided a constant visual environment. Testing took place in the room where the rats were housed, under normal room illumination (80 W strip light 3 m above the floor of the box) and with normal air conditioning, which masked noises from other rooms.

Procedure

Fifteen rats were randomly assigned to each of nine groups. Group A_s, A_2 and A_4 were tested at 10.00 h, groups B_s, B_2 and B_4 were tested at 14.00 h and groups C_s, C_2 and C_4 at 18.00 hours. The suffixes S, 2 and 4 refer to whether the members of the group were tested after an injection of saline, 2 or 4 mg/kg of chlorpromazine respectively. The rats tested at 10.00 h and 18.00 h were food deprived two hours before testing, and those tested at 14.00 h deprived six hours before. All injections were given intraperitoneally one hour before testing. To ensure that the variation from the prescribed time of testing was minimal no more than four rats was tested on any one occasion, which meant that testing took place over 12 days. Within each time of day the order of testing was randomized between the saline and drug groups so that neither day-to-day variations nor order of testing could systemically bias the results.

At the appropriate time of day and deprivation level each rat was placed in the centre of the hole-board and its behaviour recorded by the experimenter for 3 min, from a height of 1·1 m and a distance of 0·84 m from the centre of the board. A head-dip was scored if both eyes disappeared into the hole, and ended when the rat's head was completely clear of the hole. Each head-dip was numbered and entered on a score sheet for the particular hole. This enabled a reconstruction of the sequence of holes explored, as well as revealing any stereotyped

patterns such as repeated dipping at any one hole. This method of scoring gave a reliability of 0·95 between two independent observers. A record was also made of the amount of time spent moving around the box, including the time spent rearing, for all the animals in the drug groups and for five animals in each of the saline groups. This was recorded on an electronic counter, operated by a silent switch.

After the 3 min trial the rat was replaced in its home cage for 1 min, and the floor and walls of the apparatus were wiped to remove traces of the previous path taken. The rat was then replaced in the apparatus and the procedure repeated until each animal had received three trials in the box.

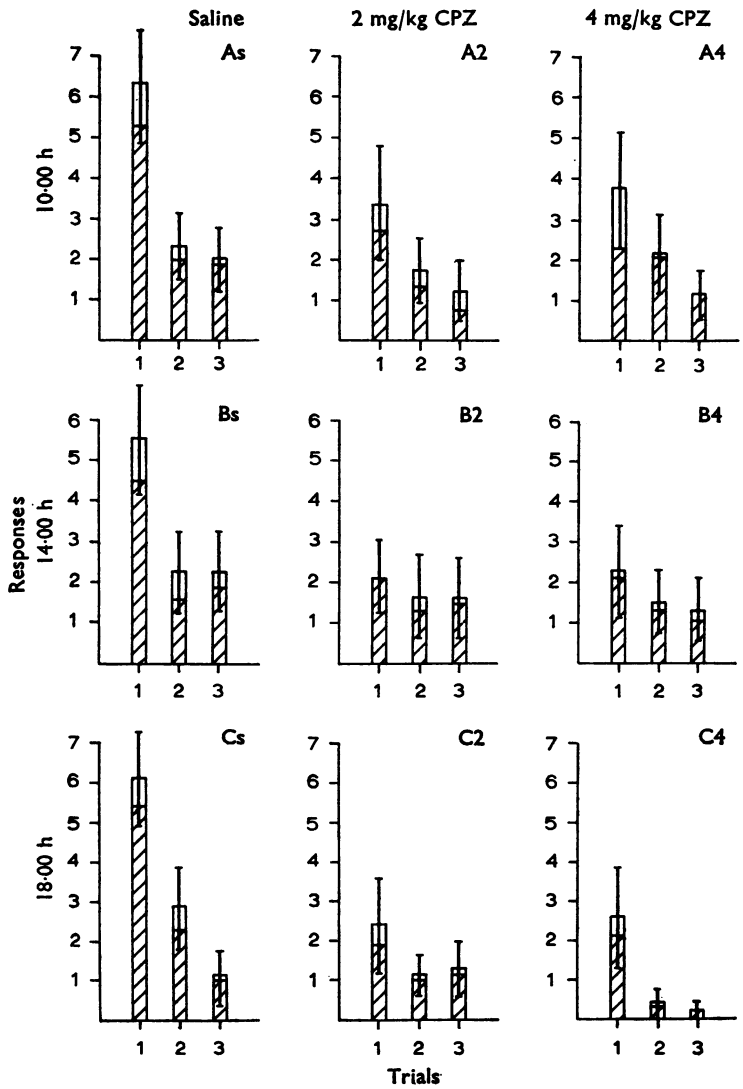

FIG. 1. Exploration over three trials for saline-injected and drug-injected groups tested at three times of day. The full height of each bar represents the mean number of head-dips and the shaded portion the mean number of different holes explored. I indicates the S.D. of the head-dips. CPZ=chlorpromazine.

Results

In Fig. 1 the full height of each bar shows the mean number of head-dips made by each group, and the height of the shaded portion shows the mean number of *different* holes explored, which gives a measure of the variety of holes investigated. These measures are shown separately for each of the three trials in the apparatus.

Consider first, the number of head-dips made on the first trial. This gave a measure of the rat's exploration of the novel situation and was a mean of about six head-dips for all three of the saline groups. This confirms an earlier finding (File & Day, 1972) that the level of exploration at 10.00 h and 18.00 h after two hours of food deprivation was equal to that at 14.00 h after six hours of deprivation. The effect of chlorpromazine was to halve this initial exploration, under all the testing conditions, but the effect of 4 mg/kg was no greater than that of 2 mg/kg. A 3×3 analysis of variance was conducted on the head-dip scores for trial 1 and confirmed that the drug effect on exploration was significant at $P<0{\cdot}001$ level ($F=17{\cdot}79$, d.f.$=2,126$) and that the motivational conditions (i.e. food deprivation and time of day) had no significant effect nor did they interact with the drug effect ($F=1{\cdot}68$, d.f.$=2,126$ and $F=0{\cdot}11$, d.f.$=4,126$ respectively, $P>0{\cdot}05$).

From Fig. 1 it can be seen that very much the same pattern of results is reflected in the number of holes explored. Thus chlorpromazine not only reduced the total amount of exploration but it similarly reduced the variety of exploration. There was no evidence of stereotypy, i.e. repeated head-dipping at the same hole. On the second trial there was a mean of 2 different holes investigated by the rats. Sometimes these were holes not explored on the previous trial and sometimes they represented a return to holes previously explored. There seemed to be no difference between drugged and saline animals in this respect, but the small number of holes investigated by the drugged animals precluded statistical comparisons. There was no systematic preference for any particular set of holes, e.g. those at the edge of the board, and there was no difference between the drugged and saline animals in whether they explored holes at the centre of the board or at the edge.

The time spent moving around the hole-board gives a measure of the animal's activity which is not influenced by any drug-induced changes in speed of locomotion. However, it was noticed that drug-injected animals moved around slowly, whereas the saline-injected animals moved rapidly from place to place. The degree of ataxia after chlorpromazine was not marked, even in the 4 mg/kg group, and did not prevent the rats from investigating the holes and other features of the apparatus. Figure 2 shows the mean time (in seconds) spent moving around the hole-board for each of the nine groups, for all three trials in the apparatus. Again the effect of chlorpromazine was to reduce the time spent moving, from the saline level of about 24 s to about half that level, but again the effect of 4 mg/kg was no greater than that of 2 mg/kg. The effect of chlorpromazine on amount of activity was significant ($F=8{\cdot}09$, d.f.$=2,96$, $P<0{\cdot}001$) but neither the motivational conditions nor their interaction with the drug had a significant effect ($F=0{\cdot}59$, d.f.$=2,96$ and $F=0{\cdot}71$, d.f.$=4,96$ respectively, $P>0{\cdot}05$).

Returning to the head-dip score, in all cases there was a reduction in the number of head-dips made from the first to the third trial, reflecting habituation to the

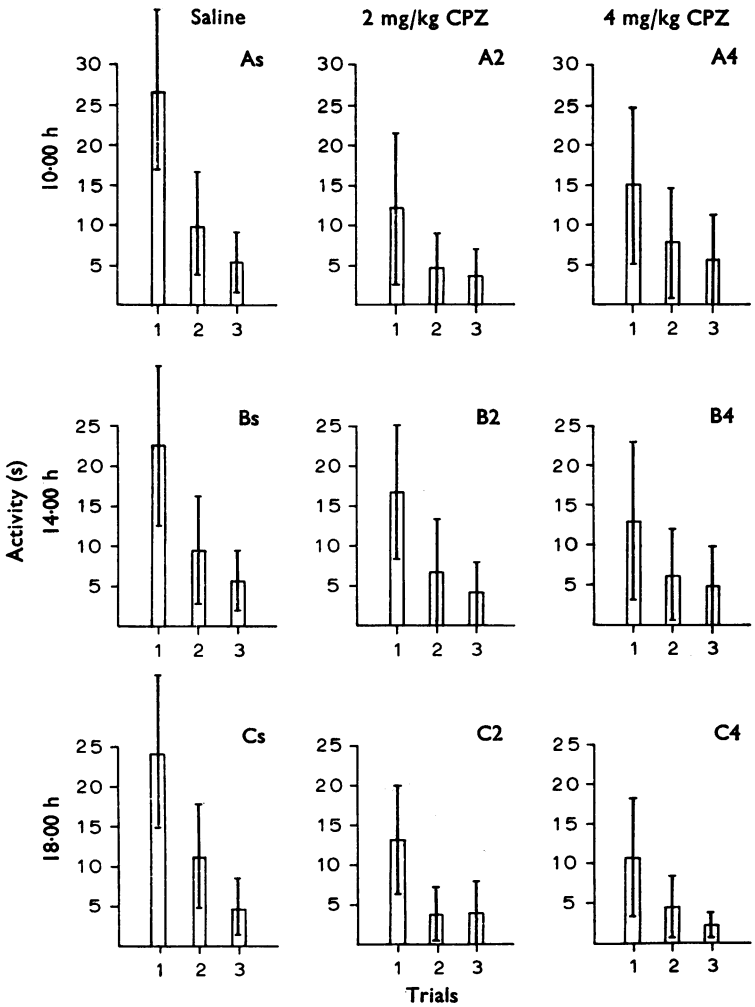

FIG. 2. Mean activity over three trials for saline-injected and drug-injected groups tested at three times of day. Activity=time(s) spent moving. I indicates the S.D. of the scores. CPZ=chlorpromazine.

situation. This habituation was significant for all the saline groups, but was only significant for the 2 mg/kg group tested at 10.00 h and the 4 mg/kg groups tested at 10.00 h and 18.00 h. The groups B_2, B_4, and C_2 failed to show a level of habituation significant at the 5% level. Details of the statistical tests are given in Table 1. It is clear that the drug did not abolish habituation completely, and it is possible that the failure to habituate in some cases was due to the low level of responding on trial 1.

Some confirmation for this interpretation comes from the activity scores, which also showed a reduction from trial 1 to trial 3. Since activity was only measured in five rats for each of the saline groups the results for these three groups were combined, and these showed a significant habituation from trial 1 to trial 3 and, more importantly, there was now a significant habituation of activity in all the drug groups. Once again the details of the statistical tests can be seen in Table 1.

TABLE 1. *Wilcoxon T tests of the difference between responses on trial* 1 *and trial* 3

A. Head-dips (one-tailed tests)

Group	T	N	*P*	
AS	6	15	<0·005	
BS	13·5	15	<0·005	
CS	0	15	<0·005	
A_2	10	14	<0·005	
A_4	0	9	<0·005	
C_4	0	10	<0·005	
B_2	23	11	>0·05	N.S.
B_4	12·5	10	>0·05	N.S.
C_2	21·5	11	>0·05	N.S.

B. Activity (one-tailed tests)

Group	T	N	*P*
saline	0	15	<0·005
A_2	9·5	14	<0·005
A_4	5·5	15	<0·005
B_2	4	15	<0·005
B_4	7	14	<0·005
C_2	11	15	<0·005
C_4	1	14	<0·005

Finally, considering performance on trial 3, by which time the apparatus had become more familiar to the animal and the measures no longer reflect exploration of a novel environment. At this stage there was no difference between the number of head-dips made by the saline-treated and drug-treated groups ($F=2·37$, d.f.=2,126, $P>0·05$); nor did the motivational conditions have a significant effect ($F=2·13$, d.f.=2,126, $P>0·05$); the interaction between these two factors was not significant ($F=0·45$, d.f.=4,126, $P>0·05$).

A similar pattern of results is reflected in the amount of time spent moving. This was not significantly affected by the drug ($F=0·47$, d.f.=2,96, $P>0·05$), by the motivational conditions ($F=1·13$, d.f.=2,96, $P>0·05$), or by the interaction between these factors ($F=0·68$, d.f.=4,96, $P>0·05$).

Discussion

Two measures of directed exploration were taken—the total number of head-dips made and the number of different holes investigated—which would reflect any drug-induced changes in overall level or variety of exploration. In addition a measure of motor activity was taken which was free of any drug-induced changes in speed of locomotion. It must, however, be remembered that changes in motor activity may be related to factors other than exploration (Kumar, Stolerman & Steinberg, 1970; Hughes, 1972), especially if the situation is a fearful one, such as the open field (Denenberg, 1969). However, fear is less important in maze exploration (Halliday, 1968) and in this respect the hole-board resembles a maze since there is no occurrence of 'freezing' and very little defaecation or urination. All three of the measures taken on trial 1 were reduced by 2 and 4 mg/kg chlorpromazine, which is in agreement with previous findings (e.g. Marriott & Spencer, 1965); in some situations, the lower dose does not affect exploration (Heimstra, 1962; File, 1973c). The responses were not sensitive to the drug dose level, but this is similar to results with mice (Shillito, 1970) where both 2 and 4 mg/kg chlorpromazine halved the level of exploration on trial 1. In this case a higher dose, 8 mg/kg, did lead to a greater reduction in exploration, but at this point the mice were so sedated that there was virtually no activity in the

test situation. In the experiment reported in this paper the lack of interaction between the drug factor and the motivational conditions indicated that exploratory activity at the three times of day and deprivation levels was as well matched for the drug-injected animals as it was for the saline-injected controls. The results from the controls replicated previous findings (File & Day, 1972), indicating the reliability of the head-dip situation.

The decrease in level of responses from trial 1 to 3 can be attributed to habituation, due to familiarity with the situation, for even 9 min of activity is far below the level which would be fatiguing for an adult rat. All the saline-injected and drug-injected group showed significant habituation of their motor activity, and all the saline-injected groups also showed significant habituation of the head-dip response. Not all the drug-injected groups showed significant habituation of this parameter, but in view of the results from the activity scores it is most likely that this failure was primarily due to the low level of response on trial 1. A very similar result has been obtained on the effects of atropine on habituation (Lowe, 1971), where once again the extremely low level of responding (bar-pressing) made conclusions about subsequent habituation impossible. The brainstem reticular formation is an area constantly implicated in models of orienting and habituation (e.g. Hernandez-Peon, 1960; Sokolov, 1960; Groves & Lynch, 1972) and is a major site of action of chlorpromazine (e.g. Martin, de Maar & Unna, 1958; Bradley & Hance, 1957; Tokizane, Kawakami & Gellhorn, 1957). The results from this experiment suggest that chlorpromazine certainly affects the mechanisms involved in orienting and exploration, but probably does not affect those involved in habituation.

By the third trial in the apparatus, the situation had become familiar to the rats and therefore their behaviour no longer reflected exploration of a novel environment. In this situation chlorpromazine no longer affected head-dips or activity, in agreement with a previous study on mice (Shillito, 1970). Shillito concluded that a mouse had only to move a little in trial 1 to obtain sufficient information for its behaviour to match that of saline-treated control mice in trial 2. However, in a situation where the experimenter has no control over the stimulus input to the animal it cannot be assumed that the same level of exploration, as measured by the number of tunnel entries or head-dips, necessarily means that both the drug-injected and saline-injected animals had learned the same amount about their environment. To investigate this problem it would be necessary to use a situation where the stimulus input could be controlled and parametrically manipulated, as in the distraction task described by File & Russell (1972). Other experiments (e.g. Shillito, 1967) which have involved a second trial in the apparatus, but in an undrugged state, are not directly comparable with this study and involve issues of state-dependent learning (Overton, 1964), not investigated in this study.

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