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The effects of hallucinogenic drugs on maze exploration in the rat over a 24 hour period

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Summary

1. The variation with clock-hour in the behaviour of naive rats in a Y-maze has been recorded.

2. The number of entries recorded was highest during the light period while the number of faecal boluses was highest during the dark period. Rearing showed a biphasic periodicity.

3. The responses to LSD, mescaline and amphetamine also showed significant variation with clock-hour.

4. In general, amphetamine, 1·25 mg/kg, increased the number of entries and the number of rears, the effect being greatest at the end of the light period. LSD, 100 μg/kg, and mescaline, 12·5 mg/kg, decreased the number of entries and the number of rears.

5. All three drugs decreased the number of faecal boluses. The possible significance of these responses in terms of an effect on the emotional state of the animals is discussed.

Introduction

Many physiological functions show a daily rhythm (for reviews, see Bruce, 1960 ; Aschoff, 1963). In the rat, a 24 h rhythm has been reported for such functions as motor activity (Richter, 1922 ; Peacock, Hodge & Thomas, 1966), body temperature (Halberg, 1960 ; Friedman & Walker, 1968) and brain concentration of biogenic amine (Scheving, Harrison, Gordon & Pauly, 1968 ; Davies, Ancill & Redfern, 1972).

The biogenic amines noradrenaline, dopamine and 5-hydroxytryptamine are commonly implicated in the mode of action of hallucinogenic drugs (Gaddum 1953 ; Freedman, 1963 ; Dixon, 1968 ; Bebbington & Brimblecombe, 1969), and variations in the concentration of these amines in the brain might be expected to affect the response to hallucinogenic drugs over 24 hours. Such variation has been demonstrated in rectal temperature and locomotor activity (Ancill, Davies & Redfern, 1970).

The experiments described in this paper were undertaken to determine whether the performance of rats in a Y-maze exhibited a 24 h rhythm, and to investigate the effects of lysergic acid diethylamide (LSD), mescaline and amphetamine on this behavioural pattern over a 24 h period.

Methods

Male Sprague-Dawley rats weighing 175–200 g were used. For at least 10 days prior to the experiment the animals were housed in a soundproof room with artificial lighting which was turned on by an automatic switch at 06.00 h and off at 18.00 hours. The temperature of this room was $22 \pm 1°$ C. The animals were fed and watered at irregular intervals during the light phase and were allowed access to the food and water *ad libitum*.

The apparatus consisted of a Y-shaped runway, the walls of which were 305 mm high. Each arm was 380 mm long and 125 mm wide and was constructed of 12·5 mm plywood and sealed with clear polyurethane varnish. A 100 watt electric lamp was placed 1·83 m above the centre of the Y (Steinberg, Rushton & Tinson, 1961).

A trial consisted of placing a rat in the centre of the Y and observing its movements for 3 minutes. The number of entries into the arms of the Y were recorded, as well as the number of rears and the number of faecal boluses voided. An entry into an arm of the runway was recorded when all four feet of the rat crossed the entrance to the arm.

Experiments were carried out every 4 h over a 24 h period starting at 20.00 hours. Since it was only possible to test 10 animals at any one time the experiments were spread over several days, and the administration of the drugs was randomized. At each clock hour a total of 10 animals were used for each drug, as well as 10 saline-injected controls. All animals were injected intraperitoneally 20 min before testing in the Y-maze.

Statistical analysis

A Fortran computer programme was used to provide an analysis of variance between clock hours, drug treatments and experimental days, and to provide correlation coefficients between the different parameters measured.

Evidence of periodicity was normally obtained simply by comparing the combined figures from the three clock hours of the dark phase with the combined figures for the three clock hours of the light phase. As with the comparison between drug treatments at a particular clock hour, the significance of the difference was assessed using Student's t test (for rears and entries) and, because of the low numbers involved, the Mann-Whitney U-test for faecal boluses.

Drugs

The three drugs were administered at the lowest dose levels which had previously been shown to have a significant effect on locomotor activity (Ancill *et al.*, 1970).

(±)-Amphetamine hydrochloride (Sigma), 1·25 mg/kg i.p.; lysergic acid diethylamide (Brocades), 100 μg/kg; mescaline hydrochloride (Sigma), 12·5 mg/kg. All doses expressed as base.

Results

Analysis of variance showed no significant difference between corresponding figures from experiments on different days. The results from all experiments were therefore pooled.

24 Hour rhythms in the number of entries, rears and faecal boluses in saline-treated rats

More entries into the arms of the Y-maze were recorded during the light phase of the illumination cycle than during the dark phase. The difference between the total number of entries during the light phase and the total number of entries during the dark phase was statistically significant ($P<0{\cdot}05$), the peak ($17{\cdot}2 \pm$ S.E.M. $1{\cdot}7$) occurring at 08.00 h with a nadir ($12{\cdot}7 \pm 1{\cdot}8$) at 20.00 hours (Figure 1).

The number of rears showed a biphasic rhythm over 24 h, two peaks occurring one at 04·00 h and one at 20·00 hours (Figure 2). The values recorded at both peaks were significantly different from the two minimum values which immediately preceded them (Student's t test, 04.00 h, $P<0{\cdot}05$; 20.00 h, $P<0{\cdot}01$).

The number of faecal boluses also showed a 24 h variation. The peak and nadir were not pronounced (Fig. 3) but the total number of boluses voided in the dark phase was statistically significantly higher than the number voided in the light phase ($P<0{\cdot}05$).

Amphetamine

As is shown in Fig. 1, amphetamine, 1·25 mg/kg, increased the number of entries into the arms of the maze throughout the 24 h period ($P<0{\cdot}01$).

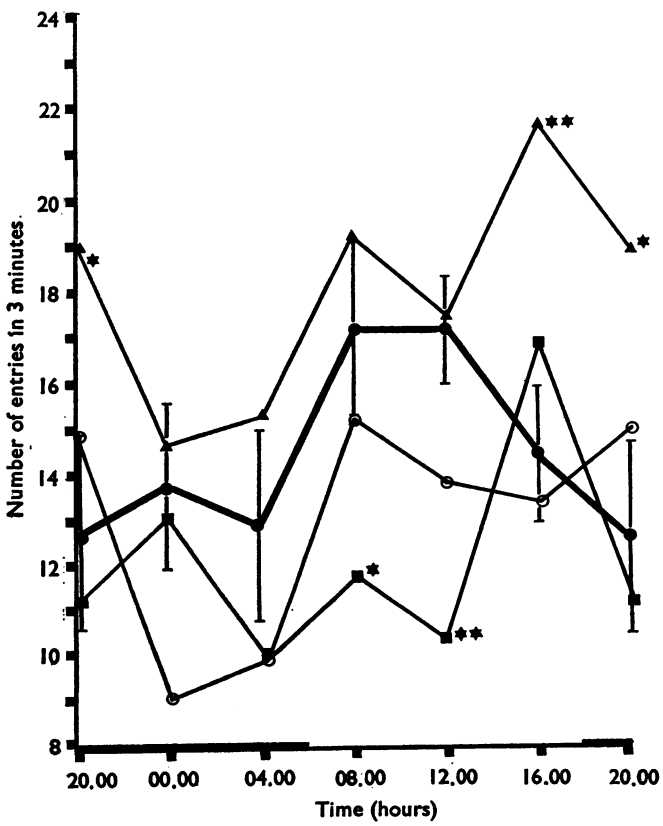

FIG. 1. The number of entries into the arms of a Y-maze over a 24 h period. ●——● saline ; ■——■ mescaline, 12·5 mg/kg i.p. ; ▲——▲ amphetamine, 1·25 mg/kg i.p. ; ○——○ LSD, 100 μg/kg i.p. ; ★—P=0·05 ; ★★—P=0·01 ; S.E.M.S are shown.

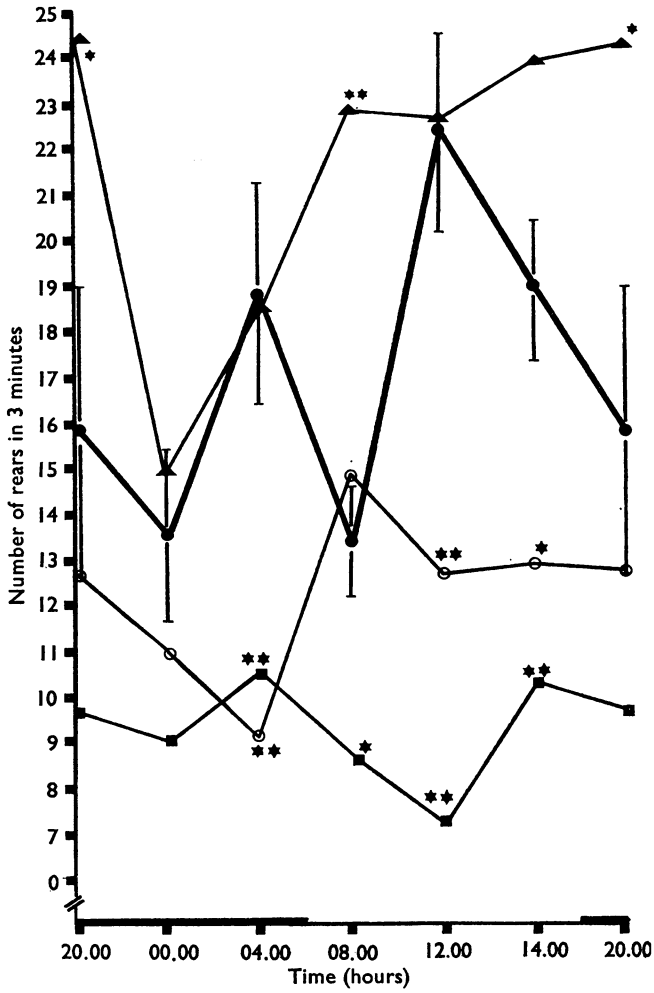

FIG. 2. The number of rears in a Y-maze over a 24 h period. ●—● saline ; ■—■ mescaline, 12·5 mg/kg i.p. ; ▲—▲ amphetamine, 1·25 mg/kg i.p. ; ○—○ LSD, 100 μg/kg i.p. ; ★—P=0·05 ; ★★—P=0·01 ; S.E.M.'s are shown.

However at individual clock-hours, the difference between the response in amphetamine-treated and control animals was statistically significant only at 16.00 h and 20.00 hours. Amphetamine also produced an overall increase in rearing ($P<0·01$) but in contrast to the control rhythm, the rhythm in the number of rears in amphetamine-treated animals (Fig. 2) showed only a single peak. Consequently when the effect at each clock-hour is examined the statistically significant increases occur at 08.00 h and 20.00 hours.

Amphetamine significantly decreased ($P<0·01$) the number of faecal boluses voided during both the light and the dark phases (Figure 3).

LSD

LSD, 100 μg/kg, did not significantly affect the number of entries into the arms of the maze, either overall or at any single clock-hour. There was an overall de-

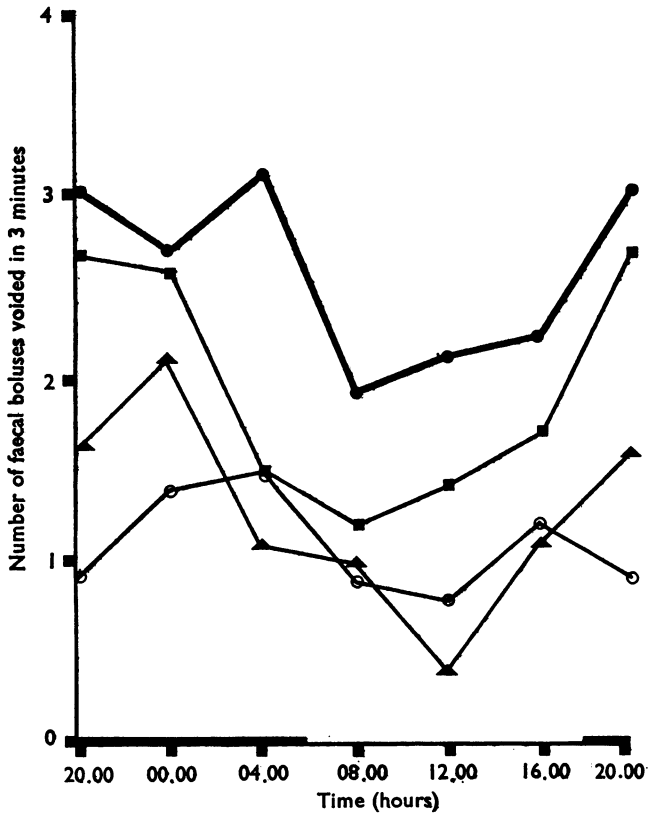

FIG. 3. The number of faecal boluses voided over a 24 h period. ●——● saline ; ■——■ mescaline, 12·5 mg/kg i.p. ; ▲——▲ amphetamine, 1·25 mg/kg i.p. ; ○——○ LSD, 100 μg/kg i.p. For degree of statistical significance, see text.

crease in the number of rears ($P<0·01$) and significant decreases at 04.00, 12.00 and 16.00 hours resuting in the abolition of the 24 h rhythm (Figure 2). LSD also reduced the number of boluses voided throughout the 24 h period ($P<0·01$), during the dark phase ($P<0·01$), and during the light phase ($P<0·05$; Figure 3).

Mescaline

Mescaline, 12·5 mg/kg produced an overall reduction in entries ($P<0·01$) and significantly reduced the number of entries at 08.00 h and 12.00 hours (Figure 1). There was also a marked overall reduction in the number of rears after mescaline ($P<0·01$) and the 24 h rhythm was abolished (Figure 2). The decrease in the number of faecal boluses voided after mescaline was not as great as that produced by either amphetamine or LSD, and did not reach statistical significance (Figure 3).

Discussion

When a rat is placed in an unfamiliar situation, the animal investigates its new surroundings, and it has often been suggested that fear plays an important role in producing this exploratory behaviour. In the situation described in this paper, exploratory behaviour was probably best reflected by the number of entries into

the arms of the maze while defaecation may be said to reflect, at least to some extent, the degree of emotionality and fear present (Broadhurst, 1957). Thus Steinberg *et al.* (1961) showed that the number of faecal boluses voided decreased if rats were given repeated trials in a Y-maze and suggested that these animals may have overcome their fear of the novel environment.

If the amount of defaecation is affected to any significant extent by the stress or fear induced by exposure to an unfamiliar situation, then the results of our experiments do not support Halliday's (1966) hypothesis since over a 24 h period the number of entries was not correlated with the number of faecal boluses ($r = -0{\cdot}77$). There was a significant 24 h variation in both parameters, but whereas the greatest number of entries occurred during the light phase, the number of faecal boluses was highest during the dark phase. It is also clear from our experiments that rearing is not simply another manifestation of exploratory behaviour, since the 24 h variation in the number of rears did not follow the 24 h variation in the number of entries. It may be significant that the rhythm in the number of rears has the same twin-peaked pattern reported for the 24 h variation in noradrenaline concentrations in rat brain (Davies *et al.*, 1972).

A common feature in the drug responses in our experiments is a reduction in the number of faecal boluses. This finding is in agreement with that of Brimblecombe (1963) who showed that in the open-field situation LSD and mescaline did not affect activity but significantly decreased defaecation. It is tempting to suggest that the decrease in defaecation produced by all three drugs in our experiments is centrally mediated and represents a change in the emotional state of the animals. However, it must be remembered that both amphetamine and mescaline have peripheral sympathomimetic activity, while LSD is a potent antagonist of 5-hydroxytryptamine in the gastro-intestinal tract. The fact that amphetamine produced significant increases in the number of rears and the number of entries, whereas LSD and mescaline tended to decrease the scores of these parameters probably reflects the central stimulant properties of amphetamine. These increases were only evident at certain clock-hours, which may possibly help to explain why amphetamine has been reported to have little effect on the number of entries (Rushton & Steinberg, 1963).

Whatever the particular relevance of these changes may be, the significance of these results as a whole would seem to be that the presence of a significant 24 h variation in the parameters measured is clearly reflected in the responses to these drugs ; there is no reason to suppose that the influence of circadian rhythms on the responses to other drugs in this situation would be any less.

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