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Some critical determinants of the behaviour of rats in the elevated plus-maze

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Abstract

The effects of daytime testing periods, repeated test exposures, and level of illumination were tested on the behaviour of rats in an elevated plus-maze consisting of two open and two enclosed arms. Rats made significantly more entries into the open arms and spent significantly more time in the open arms when testing was carried out between 8 h and 12 h than when performed between 14 h and 17 h. Repeated exposure to the test apparatus tended to reduce time spent by rats in the open arms, number of entries into the open arms and total locomotor activity. Finally, it was found that an increase of the level of illuminance was followed by a decrease of all behavioural parameters. Since conflicting results have been reported for drug treatments evaluated in the elevated plus-maze (e.g., for compounds acting at the 5-HT_{1A} receptor), the present results, based on the experimental conditions used, provide one possible explanation for these discrepancies.

Key words: Animal model of anxiety; Elevated plus-maze; Rat

Introduction

A variety of animal models of anxiety have been developed for testing antianxiety drugs. For example, a number of studies have shown that anxiolytic drugs tend to increase the locomotor activities of rodents in an unfamiliar environment (for review see File, 1985; Crawley, 1985; Treit, 1985, 1991; Lister, 1990). Pellow et al. (1985) validated in rats the so-called elevated plus-maze, developed from the work of Montgomery (1955) and of

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Handley and Mithani (1984), in which rats are exposed to a maze consisting of two open and two closed elevated arms. This situation can be considered as an approach-avoidance unconditioned conflict test since rats on their first exposure to the maze normally avoid the open arms, restricting most of their activity to the closed arms (Montgomery, 1955). An antianxiety effect is indicated by an increase in the proportion of activity in the open arms of the maze (i.e. an increase in the percentage of time spent in the open arms or an increase in the percentage of entries into the open arms) (Pellow, 1986). In such a situation a variety of anxiolytic agents, such as chlordiazepoxide, diazepam, alprazolam, adinazolam and phenobarbital were found to increase the time spent by rats in the open arms as well as the number of entries (File and Pellow, 1985; Pellow et al., 1985; Pellow and File, 1986; File and Aranko, 1988; Critchley and Handley, 1987; Johnston and File, 1988; Wilks and File, 1988; File and Johnston, 1989; Moser, 1989; Dunn et al., 1989; Söderpalm et al., 1989; Guimaraes et al., 1990; Kshama et al., 1990; Moser et al., 1990; Wada and Fukuda, 1991). However, more recently conflicting results on drug effects in the elevated plus-maze have been reported for compounds acting at the 5-HT_{1A} receptors. Although there are some reports that 5-HT_{1A} agonists or partial agonists (i. e. 8-OH-DPAT or buspirone) can have clear anxiolytic-like effects in the elevated plus-maze, the effects of such compounds in this test are often equivocal. For example, some studies (Critchley and Handley, 1987; Fellow et al. 1987; Moser, 1989; Moser et al. 1990) showed that buspirone or 8-OH-DPAT decreased open-arm activity, suggesting an anxiogenic-like effect. In contrast, Dunn et al. (1989) and Kostowski et al. (1989) reported anxiolytic-like effects with these compounds while others found no evidence for an anxiolytic- or anxiogenic-like effect of buspirone or 8-OH-DPAT in the elevated plus-maze (Pellow and File, 1986; Kshama et al., 1990; Klint, 1991; Wada and Fukuda, 1991). In view of these data, certain authors concluded that the plus-maze is either insensitive to certain classes of anxiolytics or is measuring a process in rodents that is unrelated to human anxiety (Moser et al., 1990). Others suggested that dose may be a significant factor in determining the anxiolytic or anxiogenic profile of 5-HT_{1A} receptor ligands in the elevated plus-maze (Söderpalm et al., 1989). Treit (1991) indicated that the anxiogenic-like effect of these compounds in some studies (Critchley and Handley, 1987; Moser et al., 1990) might be attributed to a concomitant reduction in total arm activity (i.e. behavioural sedation) after administration of high doses. Pellow et al. (1987), however, showed that buspirone decreased open-arm activity without significantly suppressing total arm activity. An alternative explanation of these discrepant results could be related to the different strains of rats used (Soderpalm et al., 1989). Finally, these possible sources of inter-laboratory variability can be related to the different experimental conditions used such as level of illumination or daytime observation periods, and only few studies provide clear information on these factors. The aim of the present study was to examine several important methodological factors (i.e. illumination level, diurnal time observation and repeated test exposures) which may contribute to variability in rats' behaviour in the elevated plus-maze.

Materials and Methods

Animals

Animals were male Wistar rats (Ibm : RoRo; Biological Research Laboratories, 4414, Füllinsdorf, Switzerland) weighing 150–230 g, housed in groups of four and maintained in

a room with free access to food and water, in temperature- (22 ± 2°C) and humidity- (55% RH) controlled animal quarters on a 12 h light-dark cycle with light onset at 6 h. An adaptation period to the animal maintenance facilities of at least 7 days was allowed prior to the start of the experiments.

Apparatus

All parts of the apparatus were made of grey PVC plastic. The maze was elevated to a height of 50 cm with two open (50 × 10 cm) and two enclosed arms (50 × 10 × 50 cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10 × 10 cm). The two enclosed arms could be darkened by two covers (50 × 10 cm) (see experiment 1).

Procedure

The rats were placed individually in the centre of the maze and observed for 5 min via a closed circuit TV camera by an observer located in an adjacent room. The time spent in open arms, the number of open-arm entries, and the number of closed-arm entries (defined as entry of all four limbs into an arm of the maze) during the observation period was recorded and the maze was thoroughly cleaned after removal of each rat. The results were expressed as mean ratio of open arm to total arm entries, mean ratio of time spent in open arms to total time for individual rats, and mean total number of entries made by the rats.

Experiment 1: Effects of level of illumination

Three groups of rats (A: $n = 23$; B: $n = 12$ and C: $n = 20$) were used and the measures described above were scored under three conditions. In the first condition (A), a desk lamp (75 W) provided the only room illumination. It was placed 130 cm above the open central area of the maze, so that the levels of illumination on the central platform, on each open arm and on each closed arm were 100, 80 and 90 lux respectively (this condition was used for all further experiments). In the second condition (B), the illumination level in the experimental room was increased by a scattered light source which consisted of two neon tubes fixed on the ceiling (240 cm above the apparatus). Under this condition, light intensities were as follows: 230 lux on the centre of the platform; 220 on each open arm and 100 lux on the closed arms. Finally, the third condition (C) only differed from the second one in that the two enclosed arms were darkened with black covers. In this condition the level of illumination on the closed arms was 2 lux. Testing was carried out between 8 h and 12 h.

Experiment 2: Effect of time of day at which testing occurs

Two groups of rats (AM: $n = 23$ and PM: $n = 18$) were used. In the first group, all behavioural testing was carried out between 8 h and 12 h and in the second group between 14 h and 17 h. Illumination and other conditions were as described for condition A in experiment 1.

Experiment 2: Determination of optimal time interval for repeating exposures

Five groups of rats (+2 h: $n = 8$; +1 day: $n = 16$; +2 days: $n = 16$; +7 days: $n = 8$ and +8 days: $n = 8$) were used. Each group was tested twice with different intervals between the sessions: 2 h, 1 day, 2 days, 7 days or 8 days after the first exposure. Testing was performed between 8 h and 12 h. Illumination and other conditions were as described for condition A in experiment 1.

Experiment 4: Effects of multiple exposures

On 4 consecutive days, the same 8 rats were tested five times. The second exposure was carried out 2 h after the first session, the third after 24 h, the fourth after 48 h and the last after 72 h. Testing was performed between 8 h and 12 h. Illumination and other conditions were as described for condition A in experiment 1.

Statistics

In experiments 1 and 2 differences between groups were statistically evaluated by the Mann-Whitney test. In experiment 3 data were analysed by the Wilcoxon signed-rank test. In experiment 4 statistical significance of differences between groups was ascertained by a combined repeated measures ANOVA and a posteriori paired *t*-test. All statistical analyses were performed on the percentage of open arm entries, the percentage of time spent in the open arms, and the total number of arm entries.

Results

Experiment 1: Effects of level of illumination

Analysis with the Mann-Whitney test shows that conditions B and C (both high illumination levels), in comparison with condition A (low illumination level), significantly reduced percentage of time spent in open arms, percentage of entries into open arms and total activity. There was no significant change between conditions B and C for any of the behavioural parameters (Fig. 1).

Experiment 2: Effects of time of day at which testing occurs

Analysis with the Mann-Whitney two sample test showed that afternoon testing markedly reduced percentage of entries into open arms and percentage of time in open arms, as compared with morning testing (Fig. 2).

Experiment 3: Determination of optimal time interval for repeating exposures

Analysis with the Wilcoxon signed-rank test revealed significant differences between groups for percentage of time spent in open arms and for percentage of entries into open arms 1 day and 2 days after the first exposure and for total activity 2 h after the first

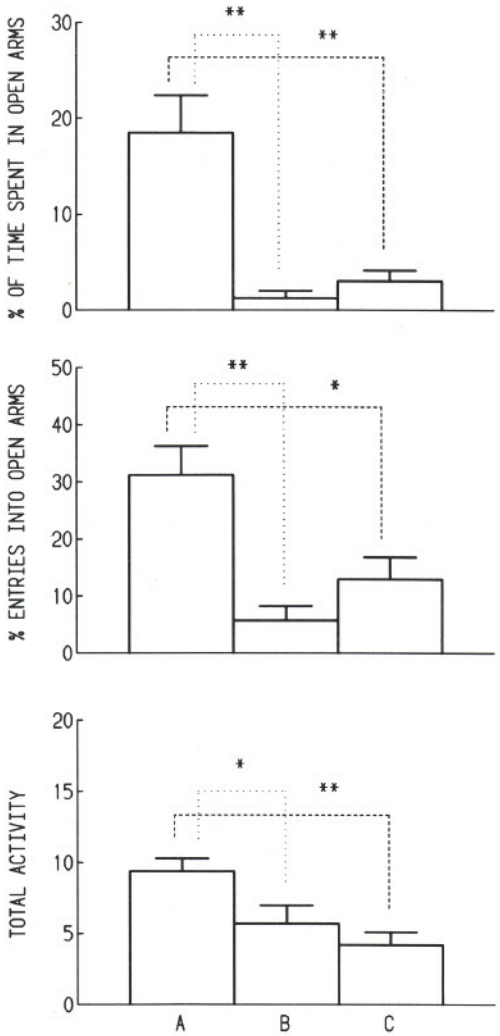

Fig. 1. Mean (±SEM) percentage of time spent in the open arms (top), percentage of entries made into the open arms (middle), and total number of arm entries (bottom) in rats in the elevated plus-maze with different illumination level. A: low illumination level above both open and closed arms; B: high light level above both open and closed arms; C: high illumination level above open arms only. * $P < 0.05$ and ** $P < 0.01$ (Mann-Whitney test).

exposure and on day 2 but not on day 1. A second exposure 2 h after the first one and on days 1 and 2 markedly reduced all behavioural parameters. By days 7 and 8 the animals reached the same level of performance as seen on first exposure (Fig. 3).

Experiment 4: Effects of multiple exposures

Repeated measures ANOVA showed that there were significant differences in all behavioural parameters over the 3-day testing period. The second exposure, 2 h after the

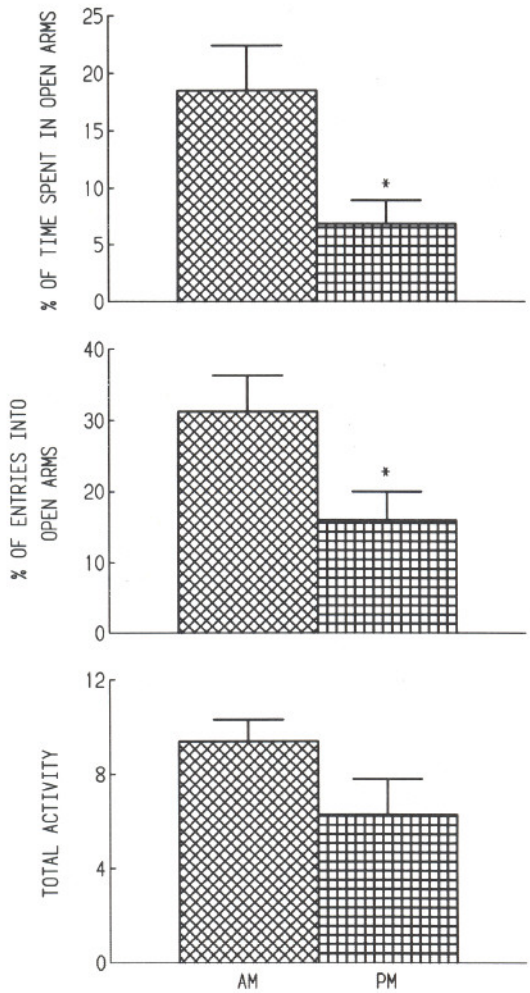

Fig. 2. Mean (±SEM) percentage of time spent in the open arms (top), percentage of entries made into the open arms (middle), and total number of arm entries (bottom) of rats in the elevated plus-maze on different daytime periods. AM: testing was performed between 8 h and 12 h; PM: testing was performed between 14 h and 17 h. * $P < 0.05$ (Mann-Whitney test).

first one, significantly reduced percentage of entries into open arms. In comparison with the first exposure, third, fourth and fifth exposures dramatically decreased all parameters, so that performance reached levels near to zero (Fig. 4).

Discussion

The behavioural assessment of anxiety in animals is problematic because anxiety essentially concerns certain aspects of human behaviour. It is therefore important to consider what the elevated plus-maze situation actually measures and to control as much as possible some crucial determinants which could influence the spontaneous behaviour of

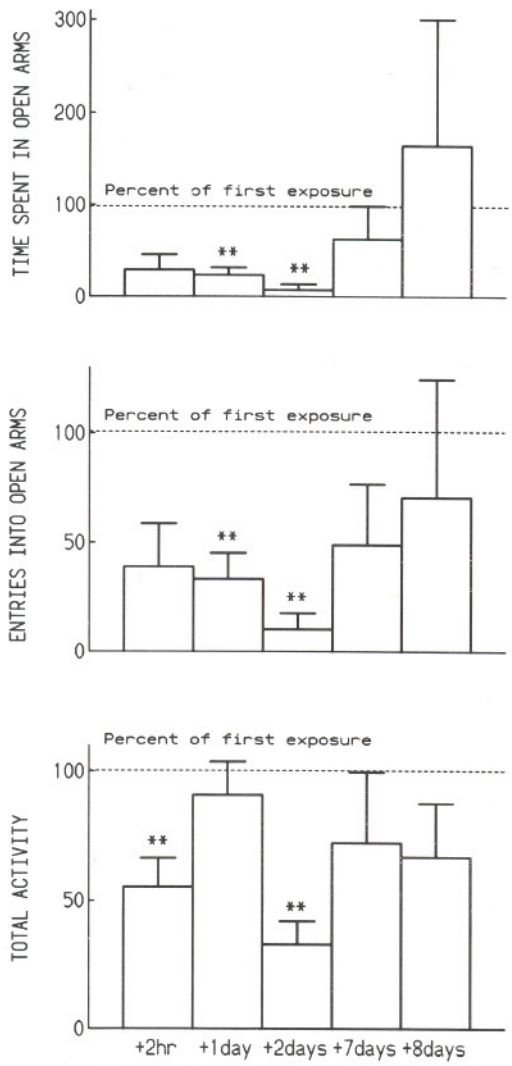

Fig. 3. Effects of two exposures performed at various time intervals (2 h; 1, 2, 7 or 8 days) on the performance of rats in the elevated plus-maze. Results are expressed as percent of baseline at first exposure (mean ± SEM). * $P < 0.05$ and ** $P < 0.01$ (Wilcoxon signed-rank test).

animals in such a situation. Our results showed that the level of illumination of the experimental area is a critical determinant of the number of entries of the rats into open arms. In a highly illuminated room, rats showed a greater avoidance of the open arms than in a less intensely illuminated one. These results confirm earlier studies which showed that brightly lit test areas are aversive to many strains of rats and mice (Crawley and Goodwin, 1980; File, 1980; Crawley, 1985; Morato and Castrechini, 1989; Benjamin et al., 1990). Furthermore, McBlane et al. (1992) indicated that alteration of light intensity is able to influence the response to 5-HT_{1A} agonists in the elevated plus-maze test. They showed that 8-OH-DPAT significantly reduced the mean open/total entries ratio in the lower (172 lux), but not in the higher (211 lux) illumination condition. In contrast, the present results are

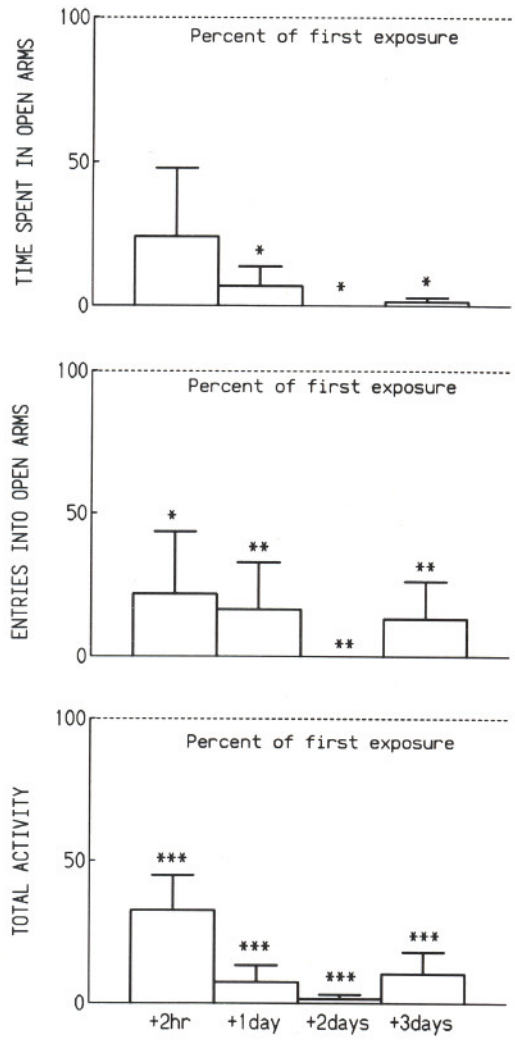

Fig. 4. Effects of multiple exposures on the performance of rats in the elevated plus-maze. Results are expressed as percent of baseline at first exposure (mean ± SEM). * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ (Bonferroni's t-test).

not in agreement with those obtained by Falter et al. (1992) which claimed that the baseline of rats' activity in the elevated plus-maze was not responsive to exogenous influences, including light intensity or stressful experience prior to testing. These latter results are surprising, in view of the relative ease with which rats' performance in the elevated-plus maze can be affected by pharmacological agents. However, this resistance to modification of the baseline pattern of performance in control rats might be partly due to the fact that the authors did not use the same experimental conditions as described by Pellow et al. (1985), such as size of the maze and observation time. Indeed, the length of each arm of their apparatus as well as the walls of the closed arms were shortened (respectively from 50 to 45 cm and from 50 to 15 cm) and the width lengthened (from 10 to 15 cm). Furthermore, these authors used an observation time of 4 min instead of the

usual 5 min testing time. In addition, it can be speculated that the lack of effect of the illumination might be due merely to the fact that the baseline means of time spent by rats in open arms were too low to be further decreased. Finally, our results confirm published data from Pellow et al. (1985) insofar as the preference of rats for the closed arms was not related to the level of illuminance within.

The second factor evaluated here, the time of day at which testing was done, also played an important role in the performance of rats in the maze, insofar as animals tested in the morning (2 h after the onset of their light cycle) showed greater activity in open arms than those observed in the afternoon at the end of the light cycle. Repeated exposures also clearly affected level of performance in rats. Our experiments showed that already on the second exposure to the maze there was a reduction both of the time spent in the open arms and total activity. These results resemble those obtained in mice which showed that re-exposure to the maze decreased time spent by animals in the open arms (Lister, 1987; Lee and Rodgers, 1990; Rodgers et al., 1992). These authors hypothesized that re-exposure results in an anxiogenic-like behavioural profile suggesting a possible anticipatory anxiety reaction. This shift in behavioural baseline would be consistent with test "sensitization" which, in turn, may reflect adaptative changes ("one-trial tolerance") induced by initial maze exposure (File, 1990; Rodgers et al., 1992). Furthermore, this effect had disappeared 7 days later, suggesting that the test "sensitization" may diminish as the interval between tests increases. Finally, the effect of multiple testing was to produce an initial dramatic decrease of the behavioural parameters then to completely suppress rats' activity in open arms. However, these data are not in accordance with those of Pellow et al. (1985) who reported that avoidance of the open arms was unchanged after 3 days of repeated testing.

In summary, when using the elevated plus-maze it seems to be necessary to identify potential drug effects under strictly defined experimental conditions. Optimal test conditions are difficult to describe because inter-laboratory differences in procedure can never be totally ruled out and they would be specific to each laboratory. However, the present variables should be taken into account by each laboratory when attempting to identify the optimal conditions for their investigation.

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