Psychopharmacology (1994) 113:543–546

Psychopharmacology

Raised corticosterone in the rat after exposure to the elevated plus-maze

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Received March 9, 1993 / Final version April 30, 1993

Abstract. Rats given one or two 5-min trials in the elevated plus-maze had plasma corticosterone concentrations significantly higher than the home cage control group and there was no sign of habituation in the group given two trials. In rats given two plus-maze trials the corticosterone responses were significantly higher in the group given 10-min rather than 5-min trials. A previous experience of cat odour (1 week earlier) has no effect on the plasma corticosterone response, but did have an anxiogenic effect that could be detected by a decrease in the percentage of time spent on the open arms of the plus-maze. The results are discussed with reference to the nature of anxiety generated by trials 1 and 2 and by the trial duration in the plus-maze, and with respect to dissociation between behavioural and endocrinological measures.

Key words: Corticosterone – Plus-maze – Anxiety – Cat odour – Habituation

The elevated plus-maze is a widely used animal test of anxiety, which is based on the relative avoidance of the open elevated arms of the maze, as opposed to the arms enclosed with high walls. There has been growing evidence that repeated exposure to the elevated plus-maze does not simply lead to habituation of the behavioural responses. For example, the scores of undrugged rats may stay constant over several trials (Pellow et al. 1985) and although benzodiazepines are extremely effective when rats or mice are naive to the plus-maze, they are ineffective in those with 5 min previous plus-maze experience (Lister 1987; File 1990). It has therefore been suggested (Rodgers et al. 1992; File and Zangrossi 1993; File et al. 1993a) that a single experience of the plus-maze changes the nature of the anxiety that is generated and that by trial 2 the animals have acquired a specific fear or phobia of heights. A single 5-min exposure to the elevated plus-maze significantly elevates plasma corticosterone concentrations above the levels of rats left in their home cages (File et al. 1988) and exposure to the open arms produces greater increases than exposure to the closed arms (Pellow et al. 1985). The purpose of experiment 1 was to compare the elevation in plasma corticosterone concentration caused by the first 5-min trial in the plus-maze with the elevation occurring after the second 5-min trial. If trial 2 generates a distinct form of anxiety then it would be expected that the corticosterone response would remain high, rather than habituate over trials. In other animal tests of anxiety, the plasma corticosterone response habituates with repeated exposure. Thus, in the social interaction test exposure to the low light, unfamiliar test condition leads to a significant elevation above the levels of the home cage control group (File et al. 1988) and on repeated exposure to both low and high light conditions there is habituation of the corticosterone response (File and Peet 1980). In a test of phobic anxiety, exposure to the odour of predator, there is habituation of the corticosterone response after repeated exposures (File et al. 1993b).

There is also evidence that the duration of exposure to the elevated plus-maze is an important factor in determining the nature of the anxiety generated on trial 2. Thus, when both trials are 10 min in duration, diazepam retains its anxiolytic efficacy (Critchley and Handley 1987; Almeida et al. 1991; File et al. 1993a), whereas when they are 5 min in duration diazepam is no longer effective on trial 2 (Lister 1987; File 1990; File et al. 1990; Rodgers et al. 1992; File et al. 1993a). The purpose of experiment 2 was therefore to compare the plasma corticosterone responses of rats after their second 5- or 10-min trial in the plus-maze. The responses of rats otherwise experimentally naive were compared with a group that had previously received exposure to cat odour. Exposure of rats to cat odour produces marked changes during the odour exposure and anxiogenic effects can also be detected for several hours (Blanchard et al. 1990; Zangrossi and File 1992).

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Materials and methods

Animals

Male hooded Lister rats (Olac Ltd, Bicester, UK) weighing approximately 220 g were housed with food and water freely available in a room maintained at 22 °C with lights on from 0700 to 1900 hours. The rats were housed in groups of five until 5 days before testing, when they were singly housed. During this period all rats received daily handling.

Plus-maze

The plus-maze was made of wood and had two open arms (50 × 10 cm) and two enclosed arms of the same size with walls 40 cm high; it was elevated 50 cm above the ground. Each rat was placed in the central square (10 × 10 cm) and allowed 5 or 10 min to freely explore the maze. A video camera was mounted vertically over the plus-maze and an observer scored the rats from a monitor in an adjacent room.

Chemicals

[1,2,6,7-^{3}H] Corticosterone (222 mCi/mmol) was obtained from Amersham International. Corticosterone (4-pregnene-11β, 21-diol-3, 20-dione) was obtained from Sigma, USA. Phosphate buffer (pH = 7.4, room temperature) of the following composition was used (g/l): Na_2HPO_4 11.7, NaH_2PO_4 3.1 and NaCl 9. Charcoal suspension (in phosphate buffer pH = 7.4) of the following composition was also used (g/l): activated charcoal 2.5, dextran 0.25 and gelatine 1. CBG (corticosteroid-binding globulin) was obtained from charcoal-stripped plasma from a full-term pregnant woman and was used in the reaction solution at a proportion of 1.75% of the final volume of phosphate buffer. [^{3}H]corticosterone was added to the reaction solution to give a radioactive count of 20–30 × 10^3 cpm/per tube.

Behavioural tests

Experiment 1. Twenty-three rats were randomly allocated to home-cage (n = 8), one plus-maze exposure (n = 7) and two exposures to the plus-maze (n = 8). Rats were given a 5-min exposure to the plus-maze between 0930 and 1130 hours. Those exposed to the plus-maze twice were given their second trial 3 days after the first.

Experiment 2. Thirty-two rats were randomly allocated among the four test conditions (n = 8 in each group): 2 × 5 min plus-maze and 2 × 10 min plus-maze (naive and previously cat odour-exposed rats). All cat odour exposures had taken place 7 days earlier in a small, dimly lit room quite separate from the room in which plus-maze testing occurred. The rats had been exposed to two trials of cat odour exposure on successive days, totalling 20 (n = 8) or 65 (n = 8) min, and rats from each cat odour exposed group were randomly allocated so that n = 4 of each group received the 5- and 10-min plus-maze exposures.

All rats received their plus-maze trials between 0930 and 1130 hours and 3 days separated the two trials. An observer scored the times spent on open and closed arms, an arm entry being defined as all four feet in the arm.

Corticosterone assay

Immediately after the plus-maze exposure each animal was returned to its home-cage in the animal house and 30 min later it was sacrificed in a separate room. Rats in the home-cage group in experiment 1 were left undisturbed in their cages in the animal house until sacrifice. Rats were guillotined and trunk blood was collected in heparinized tubes. Sample collection was carried out between 1000 and 1200 hours. The tubes were centrifuged (3000 rpm for 15 min at room temperature) and the plasma extracted and frozen at − 25 °C for later analysis, using the method of Murphy (1967), and as outlined below.

The assay was performed in triplicate and chloroform (1.5 ml) was added to the sample tubes (200 µl of sample + 200 µl of phosphate buffer) and to the standard tubes (0– 100 ng cold corticosterone in 200 µl charcoal stripped horse-serum + 200 µl phosphate buffer) and left overnight at room temperature in a shaking bath to extract the corticosterone. The water-soluble fraction in each tube was removed by aspiration and discarded. The chloroform in the remaining fraction was dried down by a constant nitrogen flow. CBG--[^{3}H] corticosterone reaction solution (200 µl) was added to each tube and left in a shaking water both at 40 °C for 30 min, then immediately moved to an ice-cold bath and left for 40 min. Ice-cold charcoal suspension (1 ml) was added to each tube and left for an additional 15 min. Immediately afterwards, all tubes were centrifuged for 5 min (3000 rpm at 4 °C) and the supernatant, containing the corticosterone, decanted off. Scintillation fluid (Ultima Gold; Packard) was added to all tubes and the radioactivity measured by liquid scintillation counting. The amount of corticosterone in each sample was expressed as ng/ml plasma.

Statistics

The results from experiment 1 were analysed by one-way analyses of variance, followed by Duncan's tests for significances between individual groups. It is the significance of these that are shown in Fig. 1. The results for experiment 2 were analysed by two-way analyses of variance with the duration of the plus-maze trial as one factor and previous exposure to cat odour as the second.

Results

Exposure of rats to the elevated plus-maze resulted in significant increases in plasma corticosterone concentrations [$F(2,20) = 4.6$, $P < 0.05$] after both one and two exposures, see Fig. 1. There was no evidence of habituation of the corticosterone response after two 5-min trials in the plus-maze.

For rats given two trials in the plus-maze those receiving 10-min trials had plasma corticosterone concentrations significantly higher than those receiving 5-min trials [$F(1,25) = 7.3$, $P = 0.01$], but the previous experience of cat odour had no significant effect [$F(1,25) = 1.1$]; see

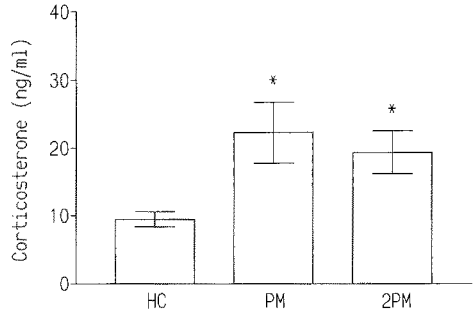

Fig. 1. Mean (± SEM) plasma corticosterone concentration (ng/ml) for rats left undisturbed in their home-cages (*HC*) or exposed for 5 min to the plus-maze once (*PM*) or twice (*2PM*). *$P < 0.05$ compared with home-cage control group

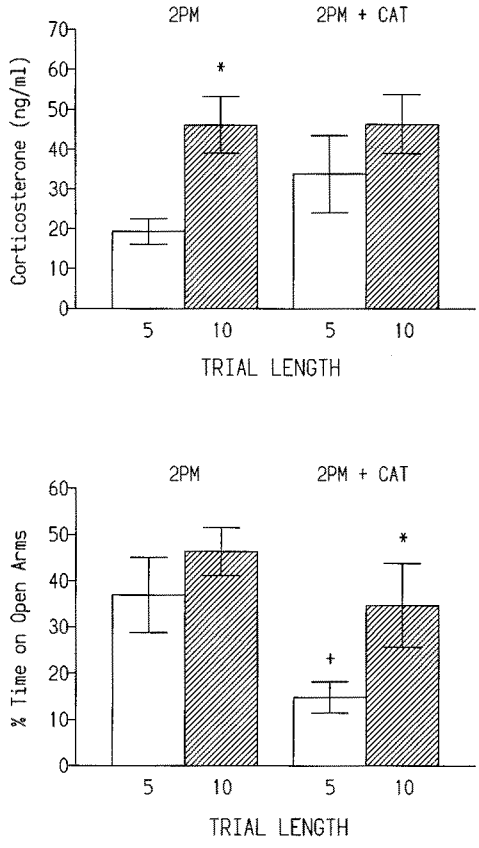

Fig. 2. *Top panel*: mean (± SEM) plasma corticosterone concentrations for rats given two 5-min or 10-min trials, with no previous experimental experience (*2PM*) or with a previous exposure to cat odour (*2PM + CAT*). *Bottom panel*: mean (± SEM) % of time (s) spent on the open arms of the plus-maze for these groups. $^{*}P < 0.05$ compared with respective 5-min group. $^{+}P < 0.05$ compared with 5-min 2PM group

Fig. 2 top panel. In contrast, it can be seen from the bottom panel of Fig. 2 that the duration of the plus-maze trials had little effect on the behaviour in the plus-maze of the previously naive rats. However, the prior experience of cat odour 1 week earlier did significantly reduce the percent of time spent on the open arms of the plus-maze [$F(1,28) = 6.1, P < 0.05$], without a change in the number of closed arm entries [mean (± SEM) for no cat = 13.6 ± 1.4 and for cat 11.4 ± 1.3]. In the cat odour groups the percent time spent on the open arms of the plus-maze scores was significantly lower for those given 5-min rather than 10-min trials, but there was no difference in the number of closed arm entries (11.2 ± 0.9 for 5-min trials, 13.8 ± 1.7 for 10-min trials).

Discussion

The results show that rats exposed to the elevated plus-maze showed significantly raised plasma corticosterone concentrations after both the first and second 5-min exposure. This contrasts with the habituation found after repeated exposure to the social interaction test (File and Peet 1980), the holeboard, startle stimuli (File 1982) or the odour of a cat (File et al. 1993b). The results are therefore compatible with the suggestions that on trial 2 in the plus-maze a new type of fear/anxiety is replacing that experienced on trial 1. The plasma corticosterone response to the plus-maze and the nature of the anxiety generated on subsequent trials in the plus-maze remains to be investigated.

The plasma corticosterone response remained high after two trials in the plus-maze, whether these were 5 or 10 min in duration, and in terms of this response the 10-min trials were clearly more stressful than the 5-min ones. Whilst there is clear evidence that with 5-min trials, trials 1 and 2 in the plus-maze are generating different types of anxiety (File and Zangrossi 1993; File et al. 1993a), the nature of the anxiety generated with 10-min trials is unknown. Since benzodiazepines are effective on trial 2 when both trials are 10 min (Critchley and Handley 1987; Almeida et al. 1991; File et al. 1993a) it is clear that a different form of fear/anxiety exists when the rat has experienced two 10-min trials rather than two 5-min trials. Further evidence for this difference comes from the additive effects of cat odour in the latter, but not the former, case.

The lack of additive effects of cat odour and two 10-min trials in the plus-maze on plasma corticosterone concentrations could have been due to a ceiling level of response being approached. However, this cannot account for the lack of a significant additive effect of cat odour and two 5-min trials in the plus-maze. At the very least it seems that the behavioural measures were more sensitive than the corticosterone response to the long-term effects of cat odour. A more marked dissociation between behavioural and endocrinological responses was found after five repeated exposures to cat odour, in which the plasma corticosterone response habituated, but not the behavioural response of avoidance (File et al. 1993b). Mineka (1985) distinguished two components of the response to phobic stimuli – disturbance and avoidance. If the elevation in plasma corticosterone reflects the disturbance caused by exposure to cat odour then it seems that measures of disturbance habituate more rapidly than those of avoidance. This would make excellent sense ethologically when the phobic stimulus is the odour of a predator. The results of the present experiment suggest that the long-term effects of exposure to cat odour were more marked on the behavioural avoidance of the open arms than on the endocrinological response to the plus-maze.

Acknowledgements. We are grateful to Dr. Brian Gillham, UMDS Department of Biochemistry, for the gift of CBG and for his advice and assistance with the corticosterone assay. These experiments were supported by grants from the Wellcome Trust and Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, Brasil.

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