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Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice

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Abstract. A single prior undrugged exposure to the elevated plus-maze has been reported to reduce open arm activity on retest and to attenuate/abolish the anxiolytic response to benzodiazepines at retest intervals ranging from 48 h to 14 days. The present study was designed to examine the generality of these findings by comparing the effects of prior maze experience on baseline behaviour and response to diazepam in two murine models of anxiety. Parallel experiments were conducted in which DBA/2 mice were exposed/not exposed to the plus-maze, treated daily with saline or diazepam (2–4 mg/kg daily for 8 days) and then tested on either the elevated plus-maze or in the light/dark test of exploration. Results show that, in both tests, diazepam reduced behavioural indices of anxiety in maze-naive mice only. However, interpretation of this apparent loss of diazepam efficacy is at least partially confounded by the observation that maze experience per se altered baseline behaviour in both procedures, *reducing* open arm activity in the plus-maze and *increasing* light compartment activity in the light/dark test. The apparent elimination of an anxiolytic response to diazepam in two animal models of anxiety by prior plus-maze experience is discussed in relation to experience-related baseline shifts in behaviour.

Key words: Elevated plus-maze – Prior experience – Diazepam response – Light/dark box – Anxiety – Retest – Mice

The elevated plus-maze is currently one of the most widely used animal models of anxiety. The procedure is based upon the natural aversion of rodents to heights and open spaces (Montgomery 1955), does not involve extensive training or the use of noxious stimuli, and therefore has a high degree of ecological validity (Lister 1990). The plus-maze has been validated for both rats (Pellow et al. 1985) and mice (Lister 1987), and has been shown to be bidirectionally sensitive to pharmacological manipulations designed to influence anxiety (e.g. Pellow and File 1986; Benjamin et al. 1900; Moser et al. 1990; Critchley et al. 1992; Rodgers et al. 1992a).

An intriguing feature of the plus-maze model is the marked attenuation or even abolition of the anxiolytic effect of chlordiazepoxide (Lister 1987; File 1990; File et al. 1990) and diazepam (Rodgers et al. 1992b) by a single previous undrugged experience of the maze. This phenomenon has been called "one trial tolerance", a description consistent with the observation that its effect is equivalent to 21 days pretreatment with chlordiazepoxide (File 1990). It has been reported to occur with inter-test intervals ranging from 24 h to 2 weeks (Lister 1987; File 1990; File et al. 1990; Rodgers et al. 1992b) and appears to depend critically upon initial experience of an open arm but is independent of drug condition on initial exposure and the material from which the maze is constructed (Lister 1987; File 1990; File et al. 1990; Rodgers et al. 1992b). Furthermore, as diazepam retains its anxiolytic activity in successive trials in the punished drinking test and anxiogenic stimuli (FG 7142 and cat odour) retain their efficacy over two successive plus-maze trials (File and Zangrossi 1993), one trial tolerance to the anxiolytic effects of benzodiazepines appears to be a highly specific phenomenon.

Several reports suggest that control scores remain stable across successive plus-maze tests (e.g. Pellow et al. 1985; Lister 1987; File et al. 1990) and hence it would appear that the reduction/loss of benzodiazepine efficacy through prior maze experience cannot be explained by baseline changes in behaviour. In direct contrast, however, it has recently been reported that prior undrugged maze experience can result in a significant reduction in percent time spent by rats (Shepherd 1992; Almeida et al. 1993; Treit et al. 1993) and mice (Lee and Rodgers 1990; Rodgers et al. 1992b) on the open arms of the maze. Furthermore, as this retest profile is itself unaltered by diazepam pretreatment (Rodgers et al. 1992b; Treit et al. 1993), we have suggested that prior experience of the

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plus-maze may qualitatively alter subsequent responsivity to this procedure.

Prior exposure to the plus-maze could influence future responses to potentially dangerous environments in at least two ways. Thus, the shift in behavioural baseline and reduced response to diazepam may be test specific in that they occur only when animals are retested on the elevated plus-maze; on initial exposure, animals are acquiring very specific information about the maze which then modifies both basal and diazepam-induced responses to this test. Alternatively, prior experience might exert a more general influence on emotional reactivity and hence response to benzodiazepines; on initial exposure, animals simply experience environmental novelty which subsequently influences basal and diazepam-induced responses to other potentially dangerous situations. One way to test these possibilities would be to examine the effects of prior maze experience on baseline behaviour and response to diazepam in a different animal model of anxiety.

In the present study, we have addressed this issue using the elevated plus-maze and light/dark exploration (Crawley and Goodwin 1981) tests of anxiety. In the former test, an anxiolytic profile is indicated by an increase in percent open arm entries and percent time spent on open arms (Pellow et al. 1985; Lister 1981; Rodgers et al. 1992a), while in the latter, such a profile is indicated by an increase in percent activity/time spent in the aversive light compartment (Costall et al. 1989; Onaivi and Martin 1989). The treatment regimen was based on that reported by Rodgers et al. (1992b) in which male mice received diazepam daily for 8 days with the aim of inducing tolerance to the sedative effects of the drug. Twenty-four hours prior to the first injection, subjects were exposed/not exposed to the plus maze and, 30 min following the final injection, were tested either in the plus-maze or the light/dark paradigm.

Materials and methods

Animals. One hundred and twenty 8-to-10-week-old male DBA/2 mice (Biomedical Services, University of Leeds), weighing 25–33 g, were used. Animals were housed in groups of ten per cage (cage size: 45 × 28 × 13 cm) and maintained under a reversed light-dark cycle (lights on: 1900 hours) in a temperature-controlled room (21 ± 1° C). Food and water were freely available.

Drugs. Diazepam (Roche Products Ltd, UK) was ultrasonically dispersed in 0.9% saline to which Tween 80 (2 drops per 10 ml) had been added; a corresponding saline/Tween mixture served as vehicle control. All injections were performed intraperitoneally (side alternated daily to reduce peritoneal irritation) in a volume of 10 ml/kg. Treatments were coded, with codes broken only after complete data analysis.

Apparatus. The elevated plus-maze was a modification of the apparatus validated for NIH mice by Lister (1987), and comprised two open arms (30 × 5 cm) and two closed arms (30 × 5 × 15 cm) extending from a common central platform (5 × 5 cm). The apparatus was elevated to a height of 45 cm above floor-level. The central platform and maze floor were constructed from black Plexiglas while the side walls of the closed arms were made of clear Plexiglas. As previously reported (Lee and Rodgers 1990; Rodgers et al. 1992a, b), grip on the open arms was provided by inclusion of a slight raised edge (0.25 cm) and open arm activity was further encouraged by testing under dim red light (2 × 60 W).

The light/dark box was based on that described by Crawley and Goodwin (1981) and subsequently validated by Costall et al (1989). It comprised an open-topped arena (45 × 27 × 27 cm), one third painted flat black and two-thirds flat white. A partition (height 27 cm) with a small opening (7.5 × 7.5 cm) divided the compartments and the floor was marked with 9 cm squares. The light compartment was brightly illuminated (direct 1 × 60 W) and the dark compartment dimly illuminated (indirect 1 × 60 W red).

Procedure. All testing was conducted under dim red background illumination during the dark phase of the light cycle. Two parallel experiments were preformed, in each of which equal numbers of animals ($n = 30$) were initially allocated either to plus-maze exposure or non-exposure conditions. On day 1 of each study, mice in the exposure condition were individually placed onto the centre platform of the maze facing an open arm, and removed 5 min later. Mice in the non-exposure group were transported to the laboratory but remained in their home cages. Exposed and non-exposed mice were then randomly allocated to one of three treatment conditions ($n = 10$) and, on days 2–9, received daily injections of vehicle, 2.0 or 4.0 mg/kg diazepam. Thirty minutes after the final injection, mice were placed either on the central platform of the elevated plus-maze (experiment 1) or in the centre of the light compartment of the light-dark box (experiment 2). A 5-min test duration was employed on day 9 and, to reduce any lingering olfactory cues, both sets of apparatus were wiped with a clean damp cloth between successive tests. All test sessions were recorded by a vertically mounted videocamera linked to a monitor and VCR in an adjacent laboratory.

Behavioural analysis. All videotapes were scored by an observer blind to treatment condition. For the elevated plus-maze, behaviours scored were number of rears, number of open and closed arm entries (plus total entries) and time spent on the various sections of the maze (open, closed and centre platform; Lee and Rodgers 1990; Rodgers et al. 1992b). Arm entries were defined as entry of all four paws into the arm. Distribution of behaviour (arm entries and time spent) on the maze was additionally calculated as "percent total" both for frequency and duration measures. For the light/dark test, behaviours scored off videotape were number of line crosses and rears in the light and dark compartments (plus total line crosses and rears), number of transitions (i.e. whole body movements) between compartments and the time spent in the two compartments. To facilitate comparisons with data derived from the plus-maze, the distribution of behaviour (line crosses, rears and time) in the light/dark test was additionally calculated as "percent total" (light compartment/total × 100) for both frequency and duration measures.

Statistics. All data were initially subjected to two-factor (maze experience; drug condition) independent analyses of variance (ANOVA). In instances of significant experience × drug interactions, F-values for main effects are not reported. Follow-up comparisons were performed using the appropriate error variance terms from the ANOVAs.

Results

Experiment 1: effects of prior plus-maze experience on subsequent anxiolytic response to diazepam in the elevated plus-maze

Data are summarized in Table 1 and Fig. 1. ANOVA on total arm entries failed to reveal a significant interaction between prior maze experience and drug treatment ($F_{2,54} = 1.57$, NS), nor were significant main effects observed (maze experience: $F_{1,54} = 0.1$, NS; diazepam: $F_{2,54} = 0.8$, NS). However, highly significant experience × drug interactions were observed for rearing

Table 1. Effects of chronic diazepam (2–4 mg/kg, IP, 8 days) on plus-maze behaviours in mice with or without prior maze experience. Data are expressed as mean values ± SEM. See also Fig. 1

Behaviour	Plus-maze naive diazepam (mg/kg)			Plus-maze experience (diazepam (mg/kg)		
	0	2	4	0	2	4
Total entries	16.6 ± 0.83	22.7 ± 2.25	22.1 ± 2.13	21.6 ± 1.95	20.5 ± 2.29	20.8 ± 3.20
Total rears	18.6 ± 1.12	17.3 ± 1.12	19.4 ± 1.38	24.3 ± 1.28	13.9 ± 2.03	12.4 ± 1.23
% time closed	65.7 ± 1.69	43.4 ± 2.27	41.0 ± 2.26	74.3 ± 2.01	72.1 ± 2.59	76.2 ± 4.55
% time centre	21.0 ± 1.09	20.2 ± 1.96	21.0 ± 1.40	18.5 ± 1.78	17.3 ± 1.45	15.3 ± 1.66

$^{\bullet}P < 0.025$ vs plus-maze naive
$^{\bullet\bullet}P < 0.005$
$^{***}P < 0.005$ vs vehicle

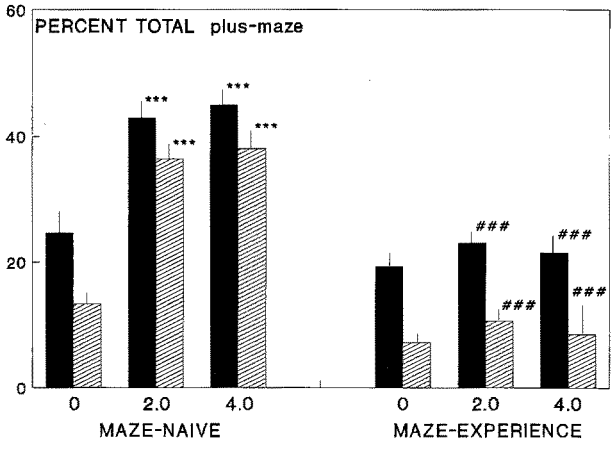

Fig. 1. The effect of chronic diazepam treatment 2–4 mg/kg, IP, daily for 8 days) on percent open arm entries (*black bars*) and percent open arm time (*hatched bars*) in maze-naive and maze-experienced male mice. Data are presented as mean values ± SEM for percent total entries and percent total time on the maze. For further details, see text and Table 1. $^{***}P < 0.005$ vs vehicle control; $\#\#\#P < 0.005$ vs corresponding maze-naive groups

($F_{2,54} = 11.1$, $P < 0.01$), percent open arm entries ($F_{2,54} = 8.7$, $P < 0.01$) and percent open arm time ($F_{2,54} = 11.5$, $P < 0.01$).

Further analyses indicated that rearing was enhanced by prior maze exposure (vehicle naive versus vehicle experienced, $P < 0.025$) and that diazepam 2–4 mg/kg suppressed this behaviour only in the maze-experienced group ($P < 0.005$ versus corresponding vehicle control); see Table 1. Percent open entries were increased by diazepam 2–4 mg/kg, an effect observed in maze-naive mice only ($P < 0.005$ versus corresponding vehicle and $P < 0.005$ versus maze-experienced mice). Similarly, although diazepam (2–4 mg/kg) pretreatment increased percent open arm time, this effect was again restricted to maze-naive mice ($P < 0.005$ versus corresponding vehicle control and $P < 0.005$ versus maze-experienced mice); see Fig. 1. Table 1 shows that the diazepam-induced increase in percent open arm time in maze-naive mice was accompanied by a reciprocal reduction in closed arm time ($F_{2,54} = 13.0$, $P < 0.01$) with no detectable change in time spent on the central platform ($F_{2,54} = 1.68$, NS).

The apparent inhibitory effect of prior maze exposure per se on percent open entries and percent open time (vehicle controls comparisons, Fig. 1) approached, but failed to reach, statistical significance. However, video-analyzed data were also available for the first exposure of the "experienced"group, thereby allowing test-retest comparisons (day 1 versus day 9 scores) for control mice in the maze experienced condition. This within-groups analysis (related *t*-tests) revealed that, on retest, control mice had significantly lower scores for both percent open entries (day 9: 19.3 ± 1.9 versus day 1: 30.7 ± 1.6, $P < 0.01$) and percent open time (day 9: 7.2 ± 1.2 versus day 1: 15.8 ± 1.1, $P < 0.01$) measures.

Experiment 2: effects of prior plus-maze experience on subsequent anxiolytic response to diazepam in the light/dark exploration test

Data are summarized in Table 2 and Fig. 2. Table 2 shows that prior plus-maze experience did not influence the total number of line crosses in the light/dark test ($F_{1,54} = 0.5$, NS). However, line crosses were significantly altered by diazepam treatment ($F_{2,54} = 17.2$, $P < 0.01$) while the maze-experience × drug interaction approached significance ($F_{2,54} = 2.6$, $F_{\text{crit}0.05} = 3.15$). Further analysis indicated that both diazepam doses increased line crossings in maze-naive animals ($P < 0.005$) while, in plus-maze-experienced mice, such an effect was seen only with 2 mg/kg diazepam ($P < 0.01$). For total rearing, ANOVA indicated a significant overall effect of prior maze experience ($F_{1,54} = 5{,}8$, $P < 0.01$), with experienced groups generally showing a lower level of rearing than maze-naive mice. However, no significant effects were observed for diazepam ($F_{2,54} = 2.1$, NS) or the experience × drug interaction ($F_{2,54} = 0.6$, NS) on this measure. Prior experience of the plus-maze also significantly influenced transitions ($F_{1,54} = 3.8$, $P < 0.05$), with exposed vehicle-treated mice showing a higher level of transitions compared to their maze-naive counterparts ($P < 0.05$). Although diazepam tended to elevate transitions, neither this effect ($F_{2,54} = 2.5$, NS) nor the experience × drug interaction ($F_{2,54} = 1.7$, NS) proved statistically reliable.

For percent line crosses in the light compartment (Fig. 2), ANOVA revealed a significant effect for diazepam treatment ($F_{2,54} = 4.1$, $P < 0.025$) and an effect for prior

Table 2. Effects of chronic diazepam (2–4 mg/kg, IP, 8 days) on light/dark box behaviours in mice with or without prior maze experience of the elevated plus-maze. Data are expressed as mean values ± SEM. See also Fig. 1

Behaviour	Plus-maze navie diazepam (mg/kg)			Plus-maze experience diazepam (mg/kg)		
	0	2	4	0	2	4
Total line crosses	135.9 ± 8.47	204.9 ± 8.53***	210.0 ± 15.60***	153.4 ± 7.94	199.2 ± 12.90**	179.8 ± 8.27
Total rears	33.9 ± 3.96	38.9 ± 2.40	30.5 ± 2.63	30.1 ± 2.83	29.8 ± 3.02	26.6 ± 1.98
Transitions	8.9 ± 1.10	12.3 ± 1.21	12.3 ± 1.20	12.8 ± 1.41	15.2 ± 1.65•	11.7 ± 1.21

•$P < 0.05$ vs plus-maze naive
**$P < 0.01$,
***$P < 0.005$ vs vehicle

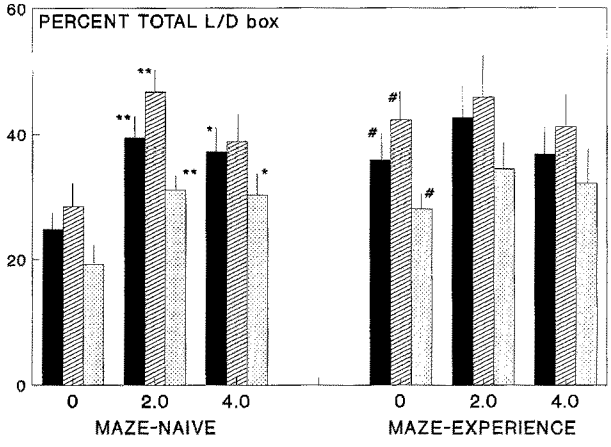

Fig. 2. The effect of chronic diazepam treatment (2–4 mg/kg, IP, daily for 8 days) on the behaviour of plus-maze-naive and plus-maze-experienced male mice tested in the light/dark paradigm. Data are presented as mean values ± SEM for percent line crosses (*black bars*), percent rears (*hatched bars*) and percent time (*stippled bars*) in the aversive light compartment (i.e. light compartment/total × 100). For further details, see text and Table 2. *$P < 0.05$, **$P < 0.025$–0.01 vs vehicle control, #$P < 0.05$ vs corresponding maze-naive groups

maze experience that approached statistical significance ($F_{1,54} = 2.2$, NS); the experience × drug interaction was not significant ($F_{2,54} = 1.2$). Follow-up tests on vehicle-treated mice indicated that prior exposure to the plus-maze increased percent line crossings in the light compartment ($P < 0.05$). Furthermore, diazepam treatment increased percent line crossings in maze-naive groups only (2 mg/kg, $P < 0.025$; 4 mg/kg, $P < 0.05$). Importantly, the effect of prior plus-maze exposure per se was of an equivalent magnitude (45–50% increase) to that produced by diazepam 2–4 mg/kg in maze-naive mice.

Very similar patterns of effect were obtained for percent rearing and percent time spent in the light compartment. For rearing, although neither maze experience per se ($F_{1,54} = 2.0$, NS) nor the interaction term ($F_{2,54} = 1.5$, NS) was reliable, the main effect for diazepam closely approached significance ($F_{2,54} = 3.0$, $F_{crit} = 3.15$). Follow-up tests on control groups indicated that plus-maze exposure per se increased ($P < 0.05$) percent rearing in the light compartment, an effect similar in magnitude to that seen in maze-naive mice treated with 2 mg/kg diazepam ($P < 0.01$). ANOVA on percent time in the light compartment revealed significant main effects for maze experience ($F_{1,54} = 3.15$, $P < 0.05$) and diazepam ($F_{2,54} = 4.8$, $P < 0.025$) but no evidence of an interaction ($F_{2,54} = 0.7$, NS). Further analysis showed that maze experience per se increased percent time spent in the light compartment (controls: $P < 0.05$) and that this effect was again similar to that induced by diazepam in maze-naive mice (diazepam 2 mg/kg, $P < 0.025$, 4 mg/kg, $P < 0.05$).

Discussion

The present study addresses a number of questions concerning the influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze paradigm: (i) are the effects of prior plus-maze experience on the anxiolytic efficacy of diazepam limited to retesting on the maze? (ii) does prior plus-maze experience per se modify behavioural reactivity to environments other than the plus-maze? (iii) do shifts in behavioural baseline contribute to the apparent loss of benzodiazepine efficacy in animals previously exposed to the plus-maze? The approach adopted involved parallel studies in which the effects of prior maze experience were examined in two murine models of anxiety, the elevated plus-maze and light/dark exploration tests.

Our results confirm that prior experience of the plus-maze eliminates the anxiolytic response to diazepam (Lister 1987; File 1990; File et al. 1990; Rodgers et al. 1992b). In the absence of any change in total entry scores, daily treatment with 2–4 mg/kg diazepam for 8 days increased percent open entries and percent open time in maze-naive, but not maze-experienced, animals. Intriguingly, a similar pattern emerged in the parallel experiment in which mice were treated identically to those in the first study with the exception that they are ultimately tested in the light/dark procedure. Prior exposure to the plus-maze again *appeared* to result in a complete abolition of the anxiolytic response to diazepam. Without producing signs of behavioural impairment, pretreatment with diazepam 2–4 mg/kg significantly increased percent line crosses, percent rears and percent time spent in the aversive light compartment in maze-naive mice only.

Together, these data would appear to suggest that the phenomenon of "one-trial tolerance" is not limited to retesting on the elevated plus-maze, perhaps indicating a more general influence of maze experience on the anxiolytic efficacy of benzodiazepines. However, this conclusion must be qualified by the effects of prior maze experience on baseline (vehicle control) behaviour in both paradigms.

Prior maze experience per se had no effect on total arm entries on maze retest, confirming earlier findings (Lister 1987; Lee and Rodgers 1990; Rodgers et al. 1992b), but did enhance rearing in the present study. Furthermore, as previously reported (Lee and Rodgers 1990; Rodgers et al. 1992b; Shepherd 1992; Almeida et al. 1993; Treit et al. 1993), prior maze experience also reduced percent open entries and percent time spent on the open arms; this effect, while apparent in the between-groups comparison, was most clearly seen in the within-group (day 1 versus day 9) analysis. Although animals were repeatedly handled and injected during the study, previous work from this laboratory has clearly shown that the retest reduction in open arm measures can be attributed to prior maze experience and not to other aspects of the test procedure (Rodgers et al. 1992b). In the light/dark test, previous exposure to the elevated plus-maze altered neither total line crossings nor rearing but, unexpectedly, actually *reduced* behavioural indices of anxiety. Thus, significant increases in inter-compartment transitions (Crawley and Goodwin 1981) and percent total line crosses/rears/time spent in the light compartment (Costall et al. 1989) were observed in maze-experienced mice. Furthermore, the degree of behavioural change in these parameters closely mirrored that produced by diazepam pretreatment in maze-naive mice. While this effect of prior maze experience is very different to the influence of repeated exposure to the light/dark test, where either stable performance (Onaivi and Martin 1989) or reduced time in light (Barry et al. 1987) have been reported, it is reminiscent of the increase in open arm behaviour in the plus-maze following prior exposure to the holeboard test (Lister 1987). In view of these apparently dissimilar effects of prior maze experience on basal reactivity to familiar and unfamiliar environments, its influence on the anxiolytic efficacy of diazepam should be considered separately for the two tests.

In the plus-maze retest paradigm, behavioural baselines (control responses) for percent open entries and percent open time shifted downwards, making it more (rather than less) likely that a positive response to diazepam would be observed. That exactly the opposite result was obtained supports the view that prior maze experience profoundly alters the nature of reactions to this environment (Rodgers et al. 1992b). Undoubtedly, this effect is due to retention of information from trial 1. Such learning may relate to the brevity of the test and/or the absence of harmful sequelae, with the loss of diazepam efficacy on trial 2 reflecting a relative absence of an approach/avoid conflict. Although the plus-maze retest profile of enhanced avoidance of open arms would appear inconsistent with this view, it is possible that prior knowledge of the maze (e.g. escape is not possible via open arms) reduces the tendency to explore these areas, thereby reducing conflict and eliminating a positive response to diazepam.

Alternatively, trial 1 learning may represent the acquisition of a phobic-like response to the open arms, with the absence of diazepam anxiolysis related to the insensitivity of phobias to benzodiazepines (e.g. Nutt 1990). This argument would be consistent with Itoh et al. (1990), who have reported that prior forced exposure of mice to the open arms of a plus-maze reduces open arm escape latencies and increases closed arm time upon retest, and have specifically developed this paradigm for the study of drug effects on learning and memory. More recently, Shepherd (1992) has found both within- and between-session reductions in the time spent by rats on the open arms of a maze, i.e. within-session reductions from 50% open time in minute 1 of the test to 5% in minute 5. Furthermore, File et al. (1990) have published data which suggest that "one trial tolerance" is crucially dependent upon initial experience of the open arms and that this experience is associated with some form of learning that is ultimately expressed in an insensitivity to the anxiolytic effects of benzodiazepines. Although this view would be compatible with present findings, it is important to emphasize that maze-experienced mice are not completely insensitive to the effects of diazepam, with a suppression of rearing evident in animals retested in the plus-maze (see also Rodgers et al. 1992b) and a stimulation of locomotor activity in those tested in the light/dark paradigm. It is therefore interesting to note that File and Zangrossi (1993) have recently suggested that, rather than inducing tolerance to the anxiolytic effects of benzodiazepines, prior maze experience induces a phobic-like state against which benzodiazepines are ineffective.

In the light/dark test, plus-maze experience per se induced a behavioural profile that was indistinguishable from that induced by diazepam in maze-naive mice (see Fig. 2). As such, the most parsimonious explanation for the absence of diazepam anxiolysis is that maze-experienced mice were already showing the maximal response possible, i.e. a "ceiling effect". This interpretation would imply that the effects observed in the light/dark test are nothing other than an artifact of the experientially induced shift in behavioural baseline. However, since the combination of diazepam and maze experience did not produce a greater "anxiolytic" effect than maze experience alone, these two manipulations may share a common substrate. This proposal would be consistent with the view that exposure to the elevated plus-maze initiates adaptive changes in benzodiazepine receptor mechanisms (File and Hitchcott 1990), but further implies that such changes may generally alter behavioural responsivity to *any* potentially dangerous environment. Nevertheless, the fact remains that prior maze experience appears to enhance anxiety-related behaviour in the plus-maze but to reduce such behaviour in the light/dark test. Both of these actions interfere with the anxiolytic efficacy of diazepam and further studies will be required to fully clarify the mechanisms involved.

In this context, two very recent studies add further intrigue to the influence of prior experience on plus-maze performance. Da Cunha et al. (1992) have reported that single or repeat 30-s exposure of rats to a non-functional passive avoidance box (i.e. novel arena) produces an anxiogenic profile in the elevated plus-maze; the greater

the number of exposures, the stronger the anxiogenic effect. This finding agrees well with the "anxiogenic-like" effects of repeated plus-maze exposure in rats and mice (Lee and Rodgers 1990; Rodgers et al., 1992b; Shepherd 1992; Almeida et al. 1993; Treit et al. 1993; Experiment 1, present study) but is at variance with other cross-test paradigms in which "anxiolytic-like" effects of prior novelty have been reported in mice, e.g. the influence of prior holeboard testing on plus-maze profiles (Lister 1987) and the influence of prior maze experience on light/dark test performance (experiment 2, present study). A very recent study from our own laboratory (Rodgers and Cole 1993) further emphasizes the complexities involved in that immediate prior exposure (5 min) to a novel arena was found to reduce open arm activity (anxiogenic) in DBA/2 mice but enhance open arm activity (anxiolytic) in T1 mice. These findings combine to suggest that the species/strain employed, the tests used, and the order of test exposure may critically determine the effects of prior experience on baseline behaviour and pharmacological response in animal models of anxiety. They also clearly indicate that any generalizations concerning such effects should be made with extreme caution.

In conclusion, present results show that prior maze experience alters subsequent behavioural and pharmacological reactions of male mice to the elevated plus-maze and light/dark tests of anxiety. Although further work is required to elucidate the underlying mechanisms, File and colleagues (1992) have recently reported that prior maze experience is associated with a modification of chlordiazepoxide's effects on the release of GABA in cortex and 5-HT in hippocampus. While prior maze experience also decreased the *basal* release of 5-HT (but not GABA) from cortex and hippocampus, the relevance of this neurochemical change to current findings is uncertain; it was measured in rats (versus mice), associated with stable test-retest behavioural profiles (versus reduced retest open arm activity) and only observed in unhandled animals (versus daily handling and injection).

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