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Pharmacology, Biochemistry and Behavior 72 (2002) 449–455

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

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Prior maze experience required to alter midazolam effects in rats submitted to the elevated plus-maze

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Received 25 September 2001; received in revised form 29 November 2001; accepted 7 December 2001

Abstract

In rodents, prior maze experience increases open arm avoidance (OAA) and compromises the anxiolytic effects of benzodiazepines in a subsequent exposure to the elevated plus-maze (EPM), a phenomenon referred to as "one trial tolerance" (OTT). Nevertheless, a possible correlation between these intriguing events remains unclear. Using maze-naive and maze-experienced (free exploration of the EPM for 5 min) rats, Experiment 1 confirmed the anxiolytic effects of midazolam (MDZ; 0.125–1.0 mg/kg) in maze-naive rats, while both increased OAA and OTT to the MDZ anxiolytic effects were observed in maze-experienced rats. However, our results from Experiment 2, designed to assess whether open, enclosed or both arms experience is involved in increased OAA and OTT, showed that MDZ retained its efficacy in rats confined either to an open or enclosed arm, where no significant changes in open arm exploration were observed when compared to the maze-naive group, therefore suggesting that prior experience in the whole apparatus may be involved in the loss of the anxiolytic MDZ effects. Results are discussed in terms of a possible correlation between increased OAA and the OTT phenomenon elicited in a subsequent exposure to the EPM.

Keywords: Anxiety; Elevated plus-maze; Open arm avoidance; One trial tolerance; Midazolam; rat

1. Introduction

The elevated plus-maze (EPM) test is the most popular of all currently available animal models to study experimental anxiety (File, 1992; Hogg, 1996; Rodgers, 1997; Rodgers and Cole, 1994) and to screen new anxiolytic drugs (Dawson and Tricklebank, 1995; Handley and McBlane, 1993; Hogg, 1996; Lister, 1987). This model is largely based on the natural aversion of rats and mice (rodents) to open spaces (Fernandes and File, 1996; Treit et al., 1993) and has been extensively validated using pharmacological and behavioral criteria (Lister, 1990; Pellow et al., 1985). Benzodiazepines (BZs) and other anxiolytic compounds selectively increase (De-Souza et al., 1998; Griebel et al., 2000; Holmes and Rodgers, 1999; Menard and Treit, 1999; Pellow et al., 1985; Rodgers and Cole, 1994; Rosa et al., 2000; Teixeira and Carobrez, 1999), while anxiogenic compounds decrease (De-Souza et al., 1998; Menard and Treit, 1999; Pellow et al., 1985; Rodgers and Cole, 1994; Teixeira and Carobrez, 1999) both the percentage of entries and time spent on the open arms of the maze. Confinement to the open arms produces significantly more anxiety-related behavior (freezing, defecation, elevated plasma corticosterone concentrations) than confinement to the enclosed arms of the EPM (Pellow et al., 1985).

There is mounting evidence that prior maze experience not only alters behavioral but also pharmacological responses in the EPM (File and Zangrossi, 1993; Holmes and Rodgers, 1998; Rodgers and Shepherd, 1993). Indeed, although early results regarding the influence of prior maze experience showed that repeated testing did not modify baseline open arm exploration (File et al., 1990; Lister, 1987; Pellow et al., 1985), nowadays, there is general agreement that the retest of rodents increases open arm avoidance (OAA) in the EPM (Bertoglio and Carobrez, 2000, in press; Dawson et al., 1994; Fernandes and File, 1996; Gonzalez and File, 1997; Griebel et al., 1993; Holmes and Rodgers, 1998, 1999; Rodgers and Shepherd, 1993; Rodgers et al., 1996; Treit et al., 1993). In fact, progressive OAA starts around the second minute of Trial 1 (Bertoglio and Carobrez, in press; Holmes and Rodgers, 1998; Rodgers et al., 1996) and it seems to be independent of the circadian phase and illumination conditions (Berto-

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0091-3057/02/$ – see front matter
PII: S0091-3057(02)00710-4

glio and Carobrez, in press) as well as of extra-maze cues (Rodgers et al., 1997). In addition to these observations, prior maze experience also appears to alter the nature of the behavioral responses elicited in a subsequent exposure to the maze (File and Zangrossi, 1993; Rodgers and Shepherd, 1993), while the anxiolytic effects of BZs are reduced (or even abolished) in maze-experienced rodents (File et al., 1990; Holmes and Rodgers, 1999; Lister, 1987; Rodgers and Shepherd, 1993). This phenomenon, referred to as "one trial tolerance" (OTT) (File et al., 1990), was first described by Lister (1987) and has been found to be independent of the drug state on Trial 1, of intertrial interval, and of the material from which the maze is constructed (File, 1993). It is also interesting to point out that this phenomenon has been observed in other animal models of anxiolytic activity, such as the mouse four-plate (Hascoet et al., 1997), cat odor avoidance (McGregor and Dielenberg, 1999), and light/dark tests (Holmes et al., 2001), but not in the Geller–Seifter conflict, Vogel conflict, and social interaction tests (File and Zangrossi, 1993; Howard and Pollard, 1991).

A number of hypotheses have been suggested to explain this loss of BZs effectiveness in the EPM, including locomotor habituation (Dawson et al., 1994), sensitization of fear of the open arms (Rodgers and Shepherd, 1993), and a qualitative shift in the emotional state elicited on the subsequent exposure to the maze (Holmes and Rodgers, 1998, 1999; Rodgers and Shepherd, 1993), against which BZs are ineffective (File and Zangrossi, 1993; File et al., 1993). On the other hand, several studies using rats have argued that OTT, at least to the chlordiazepoxide effects, might be prevented by lidocaine-reversible bilateral lesions of the basolateral amygdala immediately after Trial 1 (File et al., 1998) or of the dorsomedial hypothalamus immediately before Trial 2 (File et al., 1999), by the introduction of a motivational conflict situation (light and hot air blow) (Pereira et al., 1999), as well as by increasing the time duration of the EPM trials in both rats and mice (File et al., 1993; Holmes and Rodgers, 1999).

An intriguing and still unclear issue concerning the EPM model is the precise source of aversion to the open arms (Hogg, 1996). Treit et al. (1993) suggested that, rather than the height, the lack of thigmotaxis in the open arms is the main avoidance-promoting feature. Moreover, File et al. (1990) proposed that the experience on the open arms is the crucial factor, while Bertoglio and Carobrez (2000) suggested that, rather than the ratio of open/enclosed arms, the existence of at least two environments with different levels of aversion (open and closed arms) was the key feature in the Trial 1 avoidance learning process.

Taking into account these different findings, the aim of the present study was to evaluate how prior maze experience (Trial 1) alters behavioral and pharmacological responses elicited in a subsequent exposure to the EPM in Trial 2. Experiment 1 evaluated the effects of midazolam (MDZ) in maze-naive and maze-experienced rats, while Experiment 2 assessed whether open, enclosed, or both arms experience is involved in OAA and/or the OTT phenomenon.

2. Methods

2.1. Subjects

The subjects were male Wistar rats weighing 250–300 g, aged 13–15 weeks at the time of testing, housed in groups of five to six per cage (50 × 30 × 15 cm), under a standard light cycle (12-h light/dark phase; lights on at 06:00 h), in a temperature-controlled environment (23 ± 1 °C) and with free access to food and water. The period of adaptation to laboratory conditions was at least 48 h before testing. The experimental sessions were conducted during the diurnal phase (between 12:00 and 16:00 h).

2.2. Drugs

MDZ (Dormonid, Roche, Brazil) was dissolved in saline (0.9%) that, alone, served as a vehicle control. Solutions were administered intraperitoneally (1.0 ml/kg).

2.3. Apparatus

The EPM was made of wood and consisted of two opposite open arms, 50 × 10 cm (surrounded by a 1-cm-high Plexiglas ledge), and two enclosed arms, 50 × 10 × 40 cm, elevated to a height of 50 cm above the floor. The junction area of the four arms (central platform) measured 10 × 10 cm. The floor of the maze was painted with impermeable dark epoxy resin, in order to avoid urine impregnation.

Table 1
Control profiles (mean ± S.E.M.) of maze-naive (EPM-1) and maze-experienced (EPM-2) rats, as well as of rats confined either to one open (OAC) or enclosed (EAC) arm, showing ANOVA results, followed by Newman–Keuls test

Group parameter	EPM-1	EPM-2	OAC	EAC	ANOVA results (*df* = 3,39)
Percentage open entries	45.5 ± 3.8	33.0 ± 2.2	34.1 ± 3.9	40.2 ± 4.4	$F = 2.7$; $P = .058$
Percentage open time	28.2 ± 3.4	14.4 ± 1.8*	19.3 ± 2.6	22.9 ± 2.8	$F = 5.2$; $P < .005$
Risk assessment	2.73 ± 0.25	2.88 ± 0.36	3.13 ± 0.55	2.26 ± 0.25	NS
Enclosed entries	8.6 ± 0.5	9.3 ± 0.5	9.8 ± 0.7	9.4 ± 0.6	NS

df = Degrees of freedom; NS = not significant.
* $P < .05$ compared to EPM-1 group.

2.4. Procedures

The experiments were carried out in a low-illumination (44-lux) conditions room. Behavior was recorded by video-camera. A monitor and a video-recording system were installed in an adjacent room. A trained observer scored the parameters from the videotape. After each trial, the maze was cleaned with ethanol solution (10% v/v).

2.5. Experiment 1—Effects of MDZ in maze-naive and maze-experienced rats

Among the 108 rats used, 50 were maze-naive while 58 had been pre-exposed, without drug treatment, to the EPM (maze-experienced group) 48 h earlier for 5 min. Within each group, the animals were randomly allocated to different treatment conditions (saline; 0.125; 0.25; 0.5 or 1.0 mg/kg MDZ; $n = 10-13$) and submitted to the EPM for 5 min. The injection–test interval employed was 30 min.

2.6. Experiment 2–Effects of prior maze experience on MDZ efficacy

One hundred and fifty-five naive rats were randomly assigned to three experimental groups. In Trial 1, one of these groups was submitted to the EPM for 5 min, while the others were confined either to an open or enclosed arm for the same time. The open (OAC) or enclosed arm confinement (EAC) groups were obtained after the isolation of the rat in a confinement maze with barriers installed in the exits from one open or enclosed arm, respectively, in the EPM.

Forty-eight hours after Trial 1, within each group, the subjects were randomly allocated to different treatment conditions (saline; 0.125; 0.25; 0.5 or 1.0 mg/kg MDZ; $n = 9-13$) and submitted to the EPM (Trial 2) for 5 min. The injection–test interval employed was 30 min.

2.7. Behavioral analysis

Behavioral parameters analyzed in rats submitted to the EPM were the frequency of open and enclosed arm entries and the amount of time spent on the central platform, open, and enclosed arms (four paws inside the arm). These data were used to calculate percentage open entries [%OE; open entries/(open + enclosed entries) × 100] and percentage time spent in open arms (%OT; open arm time/300 × 100). In addition, aborted attempts to reach the open arms, performed by rats from the central platform or enclosed arms, were counted as frequency of tries. Risk assessment (RA) behavior was interpreted according to the formula: RA={[fre-

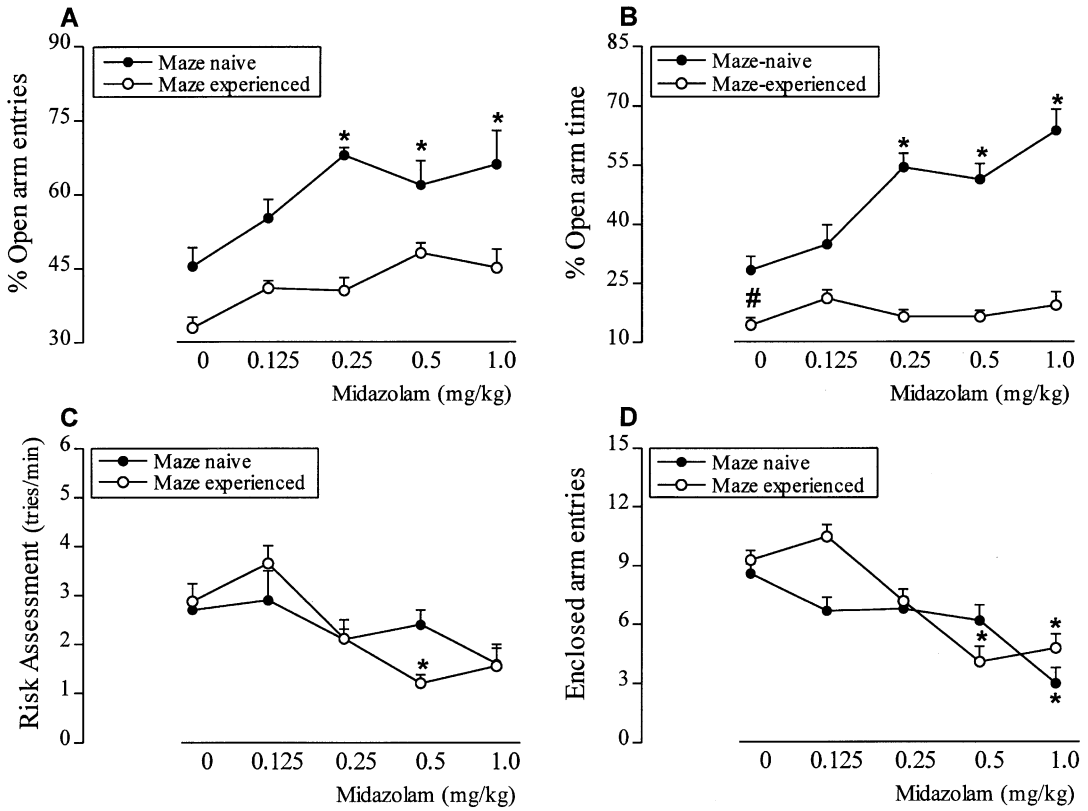

Fig. 1. Midazolam effects on the percentage of entries (A) and of time spent (B) in open arms, on risk assessment behavior (C), as well as on the enclosed arm entries (D), in both maze-naive and maze-experienced rats submitted to the EPM, revealed by two-factor (maze experience vs. drug treatment) ANOVA followed by post hoc Newman–Keuls test ($P < .05$). Data are presented as mean ± S.E.M. * = Statistical difference from respective control group; # = statistical difference from control maze-naive rats.

quency of tries/(300 − time spent in open arms)] × 60}. Thus, it was possible to estimate the frequency of tries per minute performed by rats from protected areas of the maze.

2.8. Statistics

Data obtained from both experiments were analyzed by two-factor (for Experiment 1, maze experience vs. drug treatment and for Experiment 2, Trial-1-experience vs. drug treatment) analyses of variance (ANOVA). Control behavioral profiles of rats from both experiments were analyzed by one factor (group). Further comparisons were performed using Newman–Keuls tests. The level of statistical significance adopted was $P < .05$.

2.9. Ethics

All procedures were approved by our Institutional Ethics Committee and are in accordance with NIH Animal Care Guidelines.

3. Results

Table 1 illustrates the ANOVA results, followed by Newman–Keuls test, of control behavioral profiles of maze-naive (EPM-1) and maze-experienced (EPM-2) rats, from Experiment 1, as well as of rats confined either to an open (OAC) or enclosed (EAC) arm, from Experiment 2. ANOVA revealed a significant main effect of the group for %OT, as well as a tendency (P=.058) for the %OE parameter. Further analysis showed decreased %OT only in the EPM-2 group (maze-experienced rats). Confinement either to an open or to an enclosed arm in Trial 1 failed to alter the behavioral profile of rats submitted to the EPM in Trial 2, when compared to the maze-naive (EPM-1) group (Table 1).

3.1. Experiment 1—MDZ effects in maze-naive and maze-experienced rats

The two-factor ANOVA results, from maze-naive or maze-experienced rats pretreated with MDZ (0.125–1.0 mg/kg) and submitted to the EPM, showed significant maze experience vs. drug treatment interactions for both enclosed arm entries [$F(8,98) = 5.4$, $P < .001$] and %OT [$F(8,98) = 9.6$, $P < .00001$] parameters. In addition, significant main effects (but no interactions) of the maze-experience and drug treatment factors were identified for %OE [$F(4,98) = 7.7$, $P < .00001$ and $F(2,98) = 62.2$, $P < .00001$], for %OT [$F(4,98) = 11.2$, $P < .00001$ and $F(2,98) = 197.2$, $P < .00001$], and for enclosed arm entries [$F(4,98) = 20.1$, $P < .00001$ and

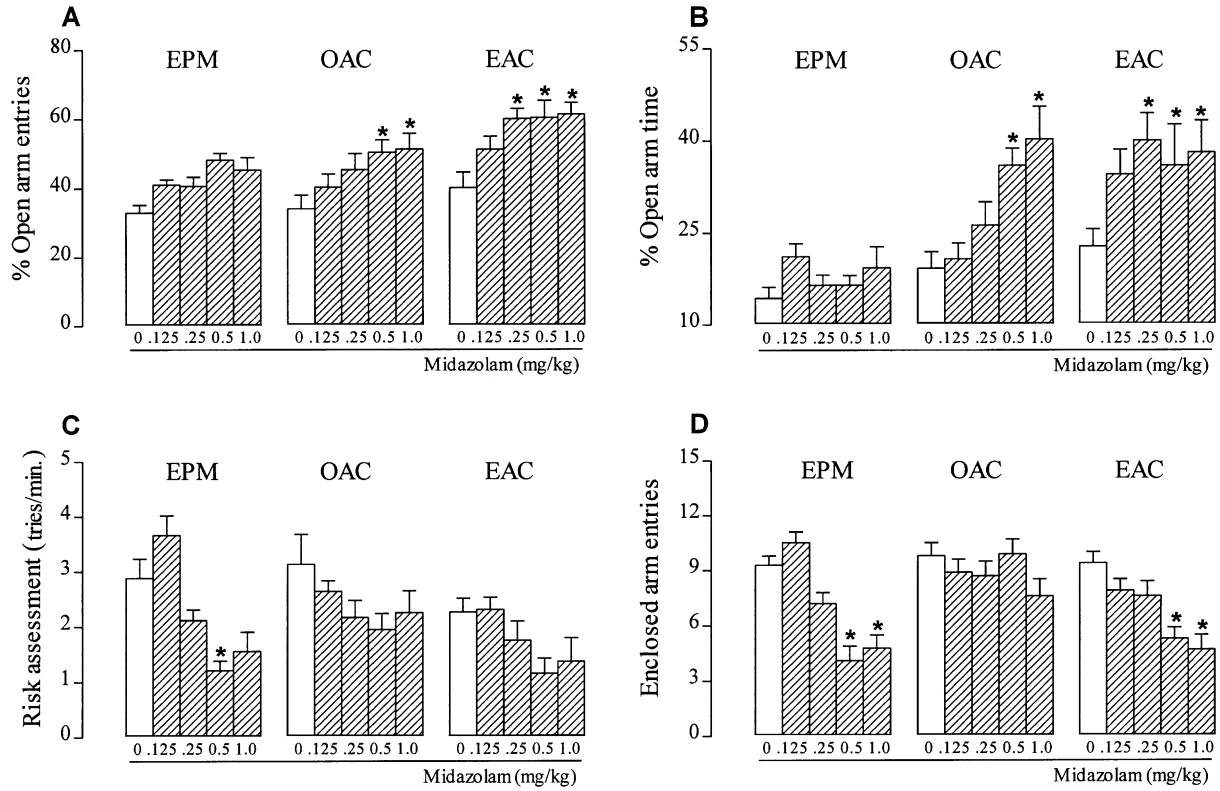

Fig. 2. Trial 2, midazolam effects on the percentage of entries (A) and of time spent (B) in open arms, on risk assessment behavior (C), as well as on the enclosed arm entries (D), in rats submitted to the EPM, revealed by two-factor (Trial-1-experience vs. drug treatment) ANOVA followed by post hoc Newman–Keuls test ($P < .05$). During Trial 1, one of these groups was allowed to freely explore the EPM for 5 min, a second was confined to an open (OAC) arm, and the third was confined to an enclosed (EAC) arm for the same period. Data are presented as mean ± S.E.M. * = Statistical difference from respective control group.

$F(2,98) = 5.0$, $P < .05$] parameters, respectively. The analysis showed only significant maze experience effects for risk assessment [$F(4,98) = 7.4$, $P < .00001$] parameter.

Data illustrated in Fig. 1 show the effects of MDZ in maze-naive and maze-experienced rats. Comparisons using the Newman–Keuls test revealed that prior treatment with MDZ, at the doses of 0.25–1.0 mg/kg, increased ($P < .05$) open arm exploration, represented by %OE and %OT parameters, in maze-naive rats. In maze-experienced rats, both OAA (decreased %OT) and abolished MDZ anxiolytic were observed. RA behavior was decreased by pretreatment with 0.5 mg/kg MDZ only in maze-naive rats. In contrast, MDZ (at the higher doses) decreased enclosed arm entries independent of the maze experience.

3.2. Experiment 2—Effects of prior maze experience on MDZ efficacy

The two-factor ANOVA results, from rats pretreated with MDZ (0.125–1.0 mg/kg) and submitted to the EPM in Trial 2, showed significant Trial-1-experience vs. drug treatment interactions for both enclosed arm entries [$F(8,148) = 5.4$, $P < .001$] and %OT [$F(8,148) = 2.2$, $P < .001$] parameters. In addition, significant main effects (but no interactions) of the Trial-1-experience and drug treatment factors were identified for %OE [$F(4,148) = 17.4$, $P < .00001$ and $F(2,148) = 11.2$, $P < .00001$], for %OT [$F(4,148) = 26.6$, $P < .00001$ and $F(2,148) = 5.8$, $P < .001$], for risk assessment behavior [$F(4,148) = 5.3$, $P < .01$ and $F(2,148) = 10.2$, $P < .00001$], and for enclosed arm entries [$F(4,148) = 16.4$, $P < .00001$ and $F(2,148) = 16.6$, $P < .00001$], respectively.

Data illustrated in Fig. 2 show the effects of MDZ in rats submitted to the EPM in Trial 2. Although the Newman–Keuls test failed to show MDZ effects in both %OE and %OT in the EPM group, prior treatment with MDZ increased ($P < .05$) open arm exploration in both OAC and EAC groups, at the doses of 0.5–1.0 and 0.25–1.0 mg/kg, respectively. In addition, MDZ (at the doses of 0.5 and 1.0 mg/kg) decreased enclosed arm entries in both EPM and EAC groups, while decreased RA behavior was only observed in the EPM group pretreated with 0.5 mg/kg MDZ and submitted to the EPM in Trial 2.

4. Discussion

It has been demonstrated that prior maze experience increases the OAA (Bertoglio and Carobrez, 2000, in press; Fernandes and File, 1996; Gonzalez and File, 1997; Griebel et al., 1993; Holmes and Rodgers, 1998, 1999; Rodgers and Shepherd, 1993; Rodgers et al., 1996; Treit et al., 1993), alters the nature of the behavioral responses elicited in a subsequent exposure to the maze (File and Zangrossi, 1993; Holmes and Rodgers, 1998; Rodgers and Shepherd, 1993), and compromises the anxiolytic effects of BZs (File et al., 1990; Holmes and Rodgers, 1999; Lister, 1987; Rodgers and Shepherd, 1993). Taking into account these findings and considering that the precise source of open arm aversion in the EPM still remains unclear (Hogg, 1996), the aim of the present study was to evaluate how prior maze experience (Trial 1) alters behavioral and pharmacological responses (MDZ effects) in rats submitted to the EPM in Trial 2.

Present results from Experiment 1 showed that MDZ increased open arm exploration, represented by %OE and %OT, in maze-naive rats (Fig. 1A,B), while in maze-experienced rats, both an increased OAA and a loss of MDZ anxiolytic effects (OTT) were observed. Our results also support the idea that BZs anxiolytic efficacy is compromised by prior maze experience, while the behavioral/emotional response elicited in a subsequent exposure to the EPM, against which BZs are ineffective (File and Zangrossi, 1993; File et al., 1993), may be qualitatively different to that of Trial 1 (Holmes and Rodgers, 1998; Rodgers and Shepherd, 1993). Results from factor analysis also agree with this hypothesis since the primary indices of anxiety (%OE and %OT) from Trials 1 and 2 load on independent factors (File and Zangrossi, 1993; File et al., 1993; Holmes and Rodgers, 1998). As the general locomotor activity for control rats, represented by enclosed arm entries, remained unaltered in Trial 2 (Table 1), our results failed to support the suggestion that OTT may be reflecting merely locomotor habituation (Dawson et al., 1994). However, decreased general locomotor activity was observed in rats treated with MDZ at the highest dose used, independent of the maze experience (Fig. 1D).

File et al. (1990) have proposed prior open arm experience as the crucial factor in the loss of BZs efficacy (OTT) in a subsequent exposure of rodents to the EPM, while Holmes and Rodgers (1999) attribute this phenomenon to prior experience of the enclosed arms. Our results from Experiment 2 confirmed that administration of MDZ prior to Trial 2 failed to alter both %OE and %OT in the EPM group (prior EPM-experience in Trial 1). Nevertheless, MDZ retained its anxiolytic effects in Trial 2 for rats confined either to an open (OAC group) or to an enclosed (EAC group) arm for 5 min during Trial 1 (Fig. 2), suggesting that prior experience in the whole apparatus may be involved in reduced MDZ effects in a subsequent exposure to the EPM.

As pointed out by Bertoglio and Carobrez (2000), the key feature in the Trial 1 avoidance learning process (OAA), in elevated maze models, might be related to the existence of at least two different aversive environments (open and enclosed arm). We also showed that the behavioral profile of rats confined to an open or enclosed arm, in Trial 1, is similar to that of maze-naive rats, regarding %OE and %OT, in Trial 2. Based on these results, and considering that MDZ retained its efficacy in both rats confined either to an open (OAC group) or enclosed (EAC group) arm in Trial 1 (Fig. 2), our results suggest that OAA might be contributing to a qualitative shift in the nature of the behavioral and pharmacological responses in a subsequent exposure to the

EPM. Moreover, it seems that these changes are guided by the initial acquired information on the whole apparatus.

In summary, the results of the present study confirmed that MDZ increased open arm exploration in maze-naive rats, while in maze-experienced rats, both OAA and OTT phenomenon to the MDZ effects were observed. However, as MDZ retained its efficacy in Trial 2 for both rats confined to an open or enclosed arm in Trial 1, it seems that a minimal prior experience in the whole maze may be involved with this loss of MDZ anxiolytic effects. Moreover, the results also suggest that the key experience in Trial 1 is not just of the open arm per se, but the relative safety/danger of different parts of the maze. Only such comparative experience can lead to the acquisition of OAA in favor of enclosed arm preference. As far as OAA is acquired early in Trial 1 (Bertoglio and Carobrez, in press; Holmes and Rodgers, 1998), further experiments are currently assessing whether BZs anxiolytic effects seen in Trial 2 are dependent on the time of exposure to the EPM, in Trial 1.

Acknowledgments

The Brazilian Government-CNPq supported this research; Leandro J. Bertoglio was in receipt of a doctoral fellowship and Antonio P. Carobrez a research fellowship. The authors thank Roche, Brazil for the kind gift of midazolam and Gareth Cuttle for English corrections on the manuscript.

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