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Anxiolytic effects of ethanol and phenobarbital are abolished in test-experienced rats submitted to the elevated plus maze

L.J. Bertoglio, A.P. Carobrez*

Departamento de Farmacologia, CCB, Universidade Federal de Santa Catarina, Rua Ferreira Lima 82, Florianópolis, SC 88015-420, Brazil

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Abstract

Prior test experience compromises the anxiolytic efficacy of benzodiazepines (BZs) either in rats or mice, a phenomenon not exclusive to the elevated plus-maze (EPM) animal model of anxiety, which is referred to as ''one-trial tolerance.'' However, it remains to be determined whether a similar event occurs when testing other drugs that also possess binding-sites on the $GABA_A$ receptor, such as ethanol and barbiturates. In the present study, we have addressed this issue using maze-naive and maze-experienced (free exploration of the EPM 48 h earlier for 5 min) rats pretreated with ethanol (1.0–1.4 g/kg) or phenobarbital (20–60 mg/kg) and submitted to the EPM. The results confirmed the anxiolytic profile of both drugs, represented by increased open arm exploration and decreased risk assessment behavior, in maze-naive rats. However, in maze-experienced rats, neither ethanol nor phenobarbital anxiolytic effects were observed, suggesting that prior maze experience compromised the drugs' anxiolytic activity. Thus, the ''one-trial tolerance'' phenomenon might also be extended to other drugs that bind to the $GABA_A$ receptor complex.

Keywords: Anxiety; Elevated plus-maze; Prior test experience; One-trial tolerance; Ethanol; Phenobarbital; Rat

1. Introduction

The elevated plus-maze (EPM) is a widely used test, which employs both rats and mice for the study of drug effects on anxiety (Hogg, 1996; Rodgers, 1997), where prior test experience not only increases open arm avoidance (Bertoglio and Carobrez, 2000; Espejo, 1997; Fernandes and File, 1996; Gonzalez and File, 1997; Holmes and Rodgers, 1998, 1999; Treit et al., 1993), but also alters the nature of the behavioral responses elicited in a subsequent exposure to the EPM (File and Zangrossi, 1993; Rodgers and Shepherd, 1993; Rodgers et al., 1992). In fact, minute-by-minute analysis of the behavioral profile has revealed that progressive open arm avoidance starts around the second minute of Trial 1 and persists throughout Trial 2 (Bertoglio and Carobrez, 2002a; Holmes and Rodgers, 1998; Rodgers et al., 1996).

In addition to these observations, it has been reported that the anxiolytic efficacy of benzodiazepines (BZs) and barbiturates, which modulate the γ-aminobutyric acid (GABA) neurotransmission system via the type A ($GABA_A$) receptor complex, is reduced (or even abolished) in maze-experienced rodents (Bertoglio and Carobrez, 2002b; File, 1993; File and Zangrossi, 1993; File et al., 1993; Gonzalez and File, 1997; Holmes and Rodgers, 1999; Rodgers and Shepherd, 1993; Rodgers et al., 1992; Treit et al., 1993). This phenomenon, referred to as ''one-trial tolerance'' (File et al., 1990), was first described by Lister (1987) and has been found to be independent of the drug state in Trial 1, of intertrial interval and of the material from which the maze is constructed (Espejo, 1997; File, 1993; File et al., 1990; Lister, 1987; Rodgers and Shepherd, 1993). A number of hypotheses have been proposed to explain this loss of BZs effectiveness in a subsequent exposure to the EPM, including locomotor habituation (Dawson et al., 1994), sensitization of fear of the open arms (Rodgers and Shepherd, 1993), an altered state of the BZs receptors (Gonzalez and File, 1997) and a qualitative shift in the emotional state elicited (Holmes and Rodgers, 1998; Rodgers and Shepherd, 1993), against which BZs are ineffective (File and Zangrossi, 1993; File et al., 1993). Furthermore, File et al. (1990) have suggested prior open arm experience as the crucial factor in the loss of BZs efficacy (''one-trial tolerance'' phenomenon) in a subsequent exposure of rodents to the EPM,

* Corresponding author. Tel.: +55-48-331-9491; fax: +55-48-222-4164.
E-mail address: adepadua@farmaco.ufsc.br (A.P. Carobrez).

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while Holmes and Rodgers (1999) attribute this phenomenon to prior experience of the enclosed arms. Recently, however, it has been reported that prior experience in the whole apparatus might be involved in the loss of BZ (midazolam) anxiolytic activity (Bertoglio and Carobrez, 2002b). On the other hand, several studies using rats have argued that the "one-trial tolerance" phenomenon 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 duration of the EPM trials for both rats (File et al., 1993) and mice (Holmes and Rodgers, 1999).

Although the "one-trial tolerance" phenomenon with BZs 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 test (Holmes et al., 2001), it remains to be determined whether prior test experience abolishes the anxiolytic efficacy of ligands that also possess binding-sites on the $GABA_A$ receptor complex. In the present study, we have addressed this issue using maze-naive and maze-experienced rats pretreated with ethanol or phenobarbital and submitted to the EPM. It is appropriate to mention that these pioneer drugs, which induce a clear anxiolytic activity in rats submitted to the EPM, were used as a starting point to understand how prior test experience changes the pharmacological response (loss of anxiolytic effects) observed in subsequent exposure of rodents to the EPM. Regarding the inclusion of phenobarbital, it was based on the fact that there are neither evaluation of ethologically derived measures related to risk assessment behavior, which has proved very sensitive to changes in anxiety (Cole and Rodgers, 1993), nor a dose–response curve using this drug.

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 subjects were reared in the above conditions from weaning and, 48 h before the experiment, they were moved to an adjacent room under the same light cycle and regimen conditions.

2.2. Drugs

Ethanol (Vetec, Brazil) was prepared by dilution in 0.9% saline (which, alone, served as a vehicle control) to a concentration of 20% w/v. An injectable solution of sodium phenobarbital (Fenocris, Pharmacon, Brazil), initially at a concentration of 100 mg/ml, was also diluted in 0.9% saline to a concentration of 20, 40 or 60 mg/ml. The solutions were administered intraperitoneally in an injection volume of 6.0 and 1.5 ml/kg, respectively. Studies employing ethanol usually adopt a considerable volume injection intraperitoneally, which can vary from 8.6 to 15.0 ml/kg (Ferreira et al., 2000; White et al., 2002), likely due to the ethanol irritant propriety. However, as an abdominal discomfort/distress might be produced by this volume injection, in the present study was employed a moderated injection volume of ethanol. Further, to avoid abdominal discomfort/distress, we injected the ethanol (or vehicle) volume through two consecutive injections. Regarding the dose selection, it was based in previous studies that examined the anxiolytic profile of ethanol (Ferreira et al., 2000) and phenobarbital (Johnston and File, 1989; Pellow et al., 1985) in rats.

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.

2.4. Procedures

The experiments were carried out in a low illumination (44 lux) conditions room, during the diurnal phase (between 12:00 and 16:00 h). Behavior was recorded by videocamera. A monitor and a video-recording system were installed in an adjacent room. A trained observer (intrarater reliability $\geq .90$) scored the parameters from the videotape. After each trial, the maze was cleaned with wet and dry towels.

2.5. Experiment 1: effects of ethanol in maze-naive and maze-experienced rats

Among the 102 rats used, 48 were maze-naive, while 54 had been preexposed, 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 (vehicle; 1.0, 1.2 or 1.4 g/kg ethanol; $n = 10–16$) and submitted to the EPM for 5 min. The injection-test interval employed was 30 min.

2.6. Experiment 2: effects of phenobarbital in maze-naive and maze-experienced rats

Among the 94 rats used, 43 were maze-naive, while 51 had been preexposed, without drug treatment, to the EPM (maze-experienced group) 48 h earlier for 5 min. Within

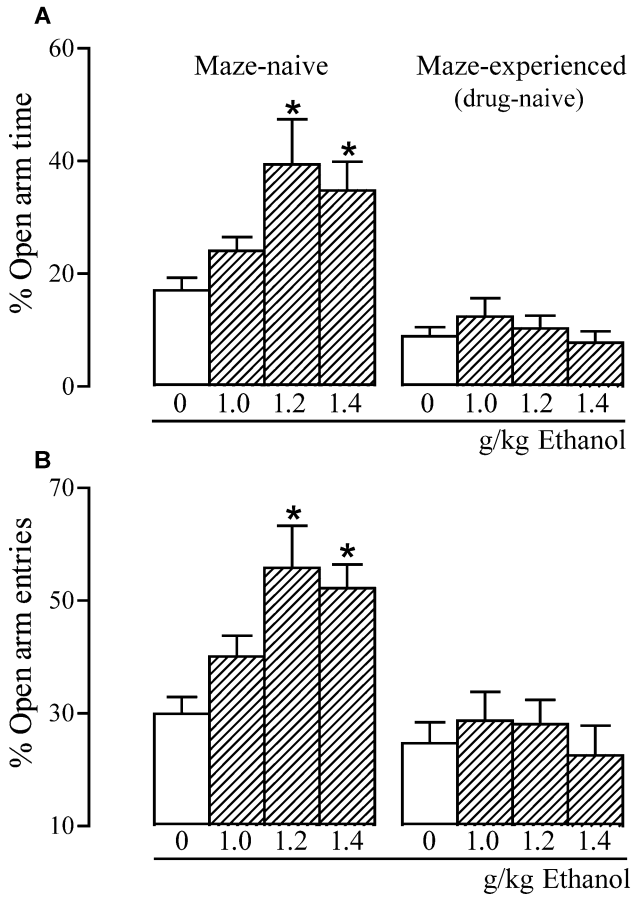

Fig. 1. Ethanol (1.0–1.4 g/kg) effects on the percentage of time spent (A) and entries (B) in the open arms, in both maze-naive and maze-experienced (drug naive) rats submitted to the elevated plus-maze, revealed by two-factor (maze experience vs. drug treatment) ANOVA, followed by post-hoc Newman–Keuls test ($P<.05$). Data are presented as mean ± S.E.M. * Statistical difference from vehicle control group. See Table 1 for complementary data.

each group, the animals were randomly allocated to different treatment conditions (vehicle; 20, 40 and 60 mg/kg phenobarbital; $n=10–14$) and submitted to the EPM for 5 min. The injection-test interval employed was 30 min.

2.7. Behavioral analysis

Behavioral measures 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] as well as the percentage time spent in open (%OT; open arm time/300 × 100) arms, enclosed (enclosed arm time/300 × 100) arms and on the central platform (%CT; central platform time/300 × 100). We also included ethologically derived measures related to the defensive pattern of risk assessment (RA) behavior, which has been proven very sensitive to changes in anxiety (Cole and Rodgers, 1993; Cruz et al., 1994; Griebel et al., 1997), in the analysis. Thus, the number of tries (exploratory posture where the rat stretches forward and then retracts to its original position without actually moving forward) to reach the open arms, performed by rats from the central platform or enclosed arms (protected areas of the maze), was recorded. In addition, to estimate the frequency of tries per minute performed by rats from protected areas of the maze the following formula was applied: {[number of tries/(300 − time spent in open arms)] × 60}, as described elsewhere (Bertoglio and Carobrez, 2000, 2002b).

2.8. Statistics

Data obtained from both experiments were analyzed by two-factor (maze experience vs. drug treatment) analyses of variance (ANOVA), followed by Newman–Keuls tests. The level of statistical significance adopted was $P<.05$. All statistical analyses were performed using the Statistica software (StatSoft, Tulsa, OK).

2.9. Ethics

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

3. Results

3.1. Experiment 1: effects of ethanol in maze-naive and maze-experienced rats

Data illustrated in Fig. 1 and Table 1 show the effects of ethanol (1.0–1.4 g/kg) in maze-naive and maze-experienced

Table 1
Effects of ethanol (1.0–1.4 g/kg) on general locomotor activity (enclosed arm entries), on the decision-making process (% central platform time), on risk assessment behavior (number of tries and tries per minute), as well as on the percentage of time spent in enclosed arms (% enclosed arm time) in maze-naive and maze-experienced rats submitted to the EPM ($n=10–16$)

	Vehicle	1.0 g/kg	1.2 g/kg	1.4 g/kg
Maze-naive				
Enclosed arm entries	8.2 ± 0.6	9.1 ± 0.5	6.7 ± 0.9	8.3 ± 0.7
% central platform time	31.8 ± 1.8	30.4 ± 2.3	18.8 ± 2.6	21.3 ± 2.5
Number of tries	8.0 ± 0.7	7.5 ± 0.9	4.3 ± 1.4 *	4.4 ± 0.5 *
Tries per minute	1.9 ± 0.2	1.9 ± 0.2	1.2 ± 0.4	1.4 ± 0.2
% enclosed arm time	51.1 ± 3.1	45.5 ± 3.6	41.8 ± 6.8	43.9 ± 5.0
Maze-experienced				
Enclosed arm entries	8.0 ± 0.8	8.5 ± 0.9	8.7 ± 0.8	9.5 ± 0.9
% central platform time	30.7 ± 2.9	29.4 ± 2.9	30.1 ± 4.2	34.3 ± 3.9
Number of tries	9.4 ± 0.9	9.2 ± 0.8	9.6 ± 1.6	11.4 ± 1.3
Tries per minute	2.1 ± 0.2	2.2 ± 0.2	2.1 ± 0.3	2.5 ± 0.3
% enclosed arm time	60.3 ± 3.8	58.2 ± 5.0	59.5 ± 4.5	57.9 ± 4.8

Data are presented as mean ± S.E.M. See Fig. 1 for complementary data.
* $P<.05$ vs. vehicle group.

rats submitted to the EPM. The two-factor ANOVA results showed a significant maze experience vs. drug treatment interaction for %OT [$F(3,94)=3.6$, $P<.02$], for %OE [$F(3,94)=4.8$, $p<.01$], for %CT [$F(3,94)=3.2$, $P<.03$], as well as for number of tries [$F(3,94)=3.2$, $P<.03$] parameters. The analysis also showed main effects of the maze experience factor for tries per minute [$F(1,94)=11.2$, $P<.001$] parameter. Further comparisons using the Newman–Keuls test revealed that prior treatment with ethanol at a dose of 1.2 or 1.4 g/kg increased ($P<.05$) %OT and %OE parameters (Fig. 1A,B), as well as decreasing ($P<.05$) the number of tries in maze-naive rats (Table 1). No statistical differences regarding the enclosed arm entries, %CT, tries per minute and percentage of enclosed arm time parameters (Table 1) were observed in maze-naive rats. In addition, post-hoc comparisons failed to show any statistically significant changes for all parameters evaluated in maze-experienced rats (Fig. 1 and Table 1).

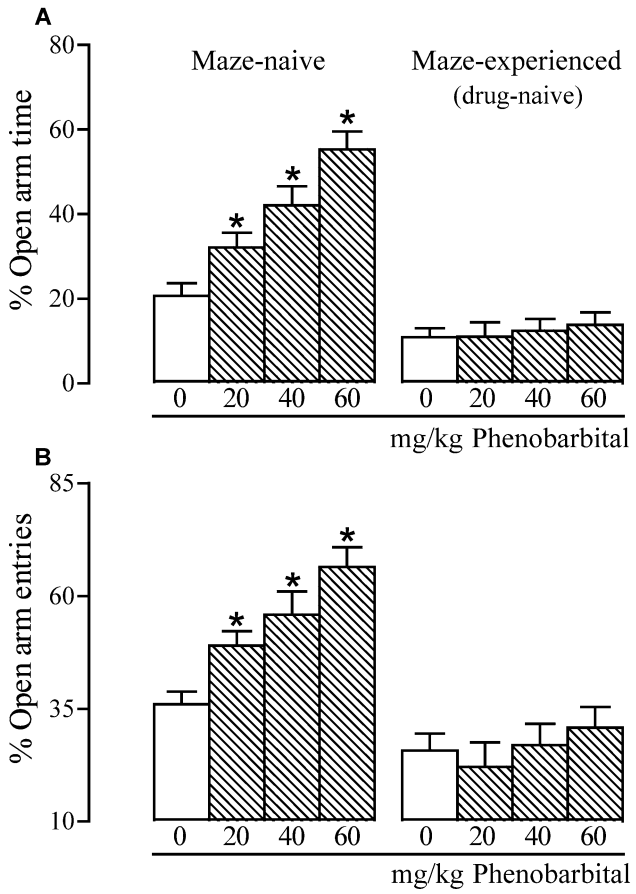

Fig. 2. Phenobarbital (20–60 mg/kg) effects on the percentage of time spent (A) and entries (B) in the open arms, in both maze-naive and maze-experienced (drug naive) rats submitted to the elevated plus-maze, revealed by two-factor (maze experience vs. drug treatment) ANOVA, followed by post-hoc Newman–Keuls test ($P<.05$). Data are presented as mean±S.E.M. * Statistical difference from vehicle control group. See Table 2 for complementary data.

Table 2
Effects of phenobarbital (20–60 mg/kg) on general locomotor activity (enclosed arm entries), on the decision-making process (% central platform time), on risk assessment behavior (number of tries and tries per minute), as well as on the percentage of time spent in enclosed arms (% enclosed arm time) in maze-naive and maze-experienced rats submitted to the EPM ($n=10–14$)

	Vehicle	20 mg/kg	40 mg/kg	60 mg/kg
Maze-naive				
Enclosed arm entries	8.1±0.6	10.0±0.6	8.7±1.2	7.0±1.0
% central platform time	30.7±2.5	21.4±2.2	24.6±2.5	22.7±2.0
Number of tries	8.7±1.0	5.1±1.1*	3.7±0.7*	3.7±0.8*
Tries per minute	2.2±0.3	1.5±0.3	1.2±0.2	1.5±0.2
% enclosed arm time	48.5±4.3	46.3±4.6	33.2±3.9*	21.9±2.8*
Maze-experienced				
Enclosed arm entries	9.3±0.7	9.2±0.7	10.9±1.1	10.4±0.8
% central platform time	32.7±3.0	40.2±4.3	37.0±2.5	33.5±3.0
Number of tries	9.4±0.9	10.2±1.2	10.2±1.1	10.1±1.0
Tries per minute	2.1±0.2	2.3±0.3	2.3±0.2	2.3±0.2
% enclosed arm time	56.3±4.1	48.7±5.4	50.5±3.7	52.6±4.4

Data are presented as mean±S.E.M. See Fig. 2 for complementary data.
* $P<.05$ vs. vehicle group.

3.2. Experiment 2: effects of phenobarbital in maze-naive and maze-experienced rats

Data illustrated in Fig. 2 and Table 2 show the effects of phenobarbital (20–60 mg/kg) in maze-naive and maze-experienced rats submitted to the EPM. The two-factor ANOVA results showed a significant maze experience vs. drug treatment interaction for %OT [$F(3,86)=7.4$, $P<.0002$], for %OE [$F(3,86)=2.7$, $P<.05$], for number of tries [$F(3,86)=3.5$, $P<.02$] and for percentage of enclosed arm time [$F(3,86)=3.8$, $P<.02$] parameters. The analysis also showed main effects of the maze experience factor for %CT [$F(1,86)=26.8$, $P<.00001$], for tries per minute [$F(1,86)=13.3$, $P<.0005$] and for enclosed arm entries [$F(1,86)=5.7$, $P<.02$] parameters. Further comparisons using the Newman–Keuls test revealed that prior treatment with phenobarbital at a dose of 20, 40 or 60 mg/kg increased ($P<.05$) %OT and %OE (Fig. 2A,B), as well as decreased ($P<.05$) the number of tries and percentage of enclosed arm time (only at the dose of 40 or 60 mg/kg) in maze-naive rats (Table 2). No statistically significant differences regarding the enclosed arm entries, %CT and tries per minute parameters (Table 2) were observed in maze-naive rats. In addition, post-hoc comparisons failed to show any statistically significant changes for all parameters evaluated in maze-experienced rats (Fig. 2 and Table 2).

4. Discussion

The results of Experiment 1 in the present study showed that ethanol, at the doses of 1.2 and 1.4 g/kg, increased open arm exploration, represented by %OT and %OE parameters,

in maze-naive rats. These results agree with previous studies that examined the anxiolytic profile of acute administration of ethanol either in naive rats (Criswell et al., 1994; Ferreira et al., 2000; Prunell et al., 1994) or mice (Cole et al., 1999; Hale et al., 1990; LaBuda and Hale, 2000; Lister, 1987) submitted to the EPM. In addition, as reported for mice by Cole et al. (1999), decreased risk assessment behavior, here represented mainly by the number of tries performed from the relatively safer areas of the EPM, was observed in maze-naive rats submitted to the EPM after treatment with ethanol at 1.2 or 1.4 g/kg, reinforcing the suggested ethanol anxiolytic effect. These effects were observed in the absence of significant changes in general locomotor activity, represented by enclosed arm entries.

In contrast to results obtained in maze-naive rats, our results showed a loss of ethanol anxiolytic efficacy in maze-experienced rats, suggesting that the anxiolytic effect of ethanol is compromised by the prior maze experience. Interestingly, Cole et al. (1999) reported similar results in mice pretreated with ethanol (1.75 g/kg) on Day 10, after 9 days of saline injections and EPM exposure. Thus, as previously reported for the BZs, such as chlordiazepoxide (File et al., 1990; File and Zangrossi, 1993; Holmes and Rodgers, 1999), diazepam (Rodgers et al., 1992; Rodgers and Shepherd, 1993; Treit et al., 1993) and midazolam (Bertoglio and Carobrez, 2002b; Gonzalez and File, 1997), the "one-trial tolerance" phenomenon may also be observed for the ethanol anxiolytic effect. By contrast, Boerngen-Lacerda and Souza-Formigoni (2000), using mice with prior exposure to the locomotor activity and open field tests, as well as to the EPM test on 3 consecutive days and submitted to the EPM after pretreatment with ethanol (2.0 g/kg), reported the anxiolytic efficacy of ethanol. In our view, these discrepancies could be related to some important methodological differences between our study and theirs, such as EPM trial duration (5 vs. 3 min), prior maze experience (EPM test vs. locomotor activity, open field and EPM tests), animal species (rats vs. mice), as well as age (13–15 vs. 6–7 weeks) of the subjects tested. Furthermore, in spite of the fact that each study employed distinct animal species, similar maze dimensions were used. However, the majority of studies (Cole et al., 1999; Espejo, 1997; Holmes and Rodgers, 1998, 1999) have adopted a scaled down version of the EPM when mice are used in the experiments, as initially proposed by Lister (1987). Taking into account these methodological differences, we could not conclude if these contradictory results may be reflecting, exclusively, an influence of prior maze experience effect on the anxiolytic effect of ethanol. Whatever the case, in order to avoid any misunderstanding regarding the "one-trial tolerance" phenomenon and the development of tolerance to ethanol's effects, it is appropriate to recall that tolerance, which can be temporally divided into rapid (minutes to hours), short-term changes in response to continuous acute ethanol exposure (acute tolerance) and delayed (hours to days) long-term changes in response to chronic ethanol exposure (chronic tolerance), is defined as a reduction in the intensity of the effect of a drug over time and is usually associated with repeated exposure to that drug (Chandler et al., 1998). For instance, some animal studies have demonstrated the development of tolerance to the anxiolytic effect of ethanol after repeated exposure to the drug (Criswell and Breese, 1989; Koob et al., 1987). It is also worth mentioning that ethanol interacts with receptors of the *N*-methyl-D-aspartate (NMDA) type for the excitatory amino acid transmitter glutamate (Crews et al., 1996), which has been shown to be involved in the mediation of anxiety-like behavior (Bennett and Amrick, 1986; Guimaraes et al., 1991). Therefore, a reduced NMDA receptor activation combined with increased $GABA_A$ activity could form the molecular basis for the anxiolytic effects of ethanol (Chandler et al., 1998). It is also interesting to consider the study from LaBuda and Fuchs (2002), in which they showed that the anxiolytic action of ethanol requires the presence of normal catecholamine activity.

The results of Experiment 2 showed that all doses of phenobarbital (20–60 mg/kg) tested lead to increased open arm exploration in maze-naive rats. Our results confirmed previous studies that showed anxiolytic activity of phenobarbital in naive rats submitted to the EPM (File, 1993; Johnston and File, 1989). Nevertheless, Pellow et al. (1985) failed to show any significant change in open arm exploration after acute administration of phenobarbital (25 or 35 mg/kg) in rats submitted to the EPM. Decreased risk assessment behavior (number of tries) was observed in maze-naive rats after phenobarbital treatment, reinforcing the suggestion of an anxiolytic effect of phenobarbital. On the other hand, the analysis failed to show any anxiolytic profile for all doses of phenobarbital tested in maze-experienced rats. File (1993) reported similar results in maze-experienced rats submitted to the EPM after phenobarbital (35 mg/kg) treatment. These results support the idea that prior maze experience also compromises the anxiolytic effect of phenobarbital. Furthermore, these effects were observed in the absence of significant changes in general locomotor activity, represented by enclosed arm entries.

Gonzalez and File (1997) have proposed an altered (or even desensitized) state of the BZ receptor as a basis for the "one-trial tolerance" phenomenon, whereas Chacur et al. (1999) do not entirely support this suggestion since they reported that prior maze experience induced immediate increases in [^{3}H]flunitrazepam binding to BZ receptors in several amygdaloid and hippocampal nuclei of rats, while no significant effects on [^{3}H]muscimol binding to the GABA sites of the same brain structures were observed (Chacur et al., 1999). However, as the present results extended the "one-trial tolerance" phenomenon either to the anxiolytic activity of ethanol or phenobarbital, which bind to the GABA but not the BZ binding-site, we suggest that adaptive changes might be occurring not only at the BZ binding site but also in the whole $GABA_A$ receptor complex, perhaps leading to the loss of the anxiolytic activity of

these drugs observed in a subsequent exposure to the EPM. Further, it is tempting to speculate that prior maze experience somehow alters the subunit composition of the $GABA_A$ receptor-chloride channel, rendering it insensitive to allosteric modulation of a range of ligands acting at different sites, such as BZs, ethanol and barbiturates.

In spite of the obvious influence of prior maze experience on the anxiolytic effect of either ethanol (Experiment 1) or phenobarbital (Experiment 2), no significant differences were observed in control group scores. The former result diverges from many reports showing increased open arm avoidance (decreased %OT and %OE parameters) in a subsequent exposure to the EPM (Espejo, 1997; Fernandes and File, 1996; Holmes and Rodgers, 1998; Treit et al., 1993). However, as the current experimental design incorporated saline injection prior to maze testing, an injection effect per se may have concealed an expected experience-induced shift in EPM behavioral baseline. Similar unexpected results have been reported by Rodgers and Shepherd (1993), as well as by Holmes and Rodgers (1999). As the general locomotor activity, represented by enclosed arm entries, remained unaltered for all maze-experienced groups (Tables 1 and 2), our results failed to support the suggestion that ''one-trial tolerance'' phenomenon may be reflecting merely locomotor habituation (Dawson et al., 1994).

In summary, the present results confirmed the anxiolytic profile of both ethanol and phenobarbital, represented by increased open arm exploration and decreased risk assessment behavior, in maze-naive rats. However, in maze-experienced rats, neither ethanol nor phenobarbital exhibited anxiolytic effects, suggesting that prior maze experience alters their pharmacological actions (anxiolytic efficacy) in a subsequent exposure to the EPM. Moreover, the present results showed that the ''one-trial tolerance'' phenomenon might also be observed for other drugs that bind to other sites of the $GABA_A$ receptor complex in addition to the BZs. Thus, the ''one-trial tolerance'' phenomenon seems to involve adaptive changes of the $GABA_A$ receptor-chloride channel complex not restricted to the BZs binding site. One possibility is that prior maze experience might modify the subunit composition of the $GABA_A$ receptor, rendering it insensitive to allosteric modulation by ethanol, phenobarbital and BZs. However, it remains to be established whether the ''one-trial tolerance'' phenomenon may also be extended to other $GABA_A$ receptor ligands (e.g., neurosteroids), as well as to other non-$GABA_A$-related anxiolytic drugs.

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