

Research report

Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety

Leandro J. Bertoglio, Antonio P. Carobrez *

Departamento de Farmacologia/CCB, Universidade Federal de Santa Catarina, Florianópolis SC 88040-900, Brazil

Received 9 July 1999; received in revised form 27 September 1999; accepted 27 September 1999

Abstract

Studies have shown an increased open arm avoidance in rats re-exposed to the elevated plus-maze (EPM), which suggests a qualitative shift in emotional states from an unconditioned (Trial 1) to a learned (Trial 2) form of fear response, but a precise source of aversion has not been determined. Using rats submitted to the EPM or various EPM-derived configurations, this study was designed to investigate what previous maze experiences in Trial 1 are required to increase avoidance of open arms in EPM Trial 2. Results obtained from rats submitted to the EPM or EPM-derived configurations confirmed the increased open arms avoidance in Trial 2. Rats confined to either open or enclosed arms failed to show the increased avoidance of open arms in Trial 2. The results are discussed in terms of the minimum prerequisite in Trial 1 to elicit an avoidance learning response to open arms in Trial 2, and also the implications of an acquired fear response in rats for the study of the biological basis of anxiety. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Anxiety; Learning; Elevated plus-maze; Retest; Risk assessment behavior; Rat

1. Introduction

A series of experiments conducted in 1955 studied the relationship between fear and exploratory behavior in an elevated Y-maze composed of a varying numbers of open and enclosed arms [23]. Based on the fact that rats would explore the enclosed arms with a higher frequency than the open arms, one of the Montgomery's main conclusions was that in this model, novel stimulation elicited both the exploratory and the fear drive, thus generating approach-avoidance conflict behavior. Thirty years later, Handley and Mithani [16], in an attempt to study the involvement of the noradrenergic systems in anxiety, developed a symmetrically constructed elevated X-maze consisting of two open and two enclosed arms, based on earlier observations by Montgomery [23]. Their assumptions were that the

response by rats to this type of maze would be sensitive to the anxiolytic and anxiogenic drugs in rats, and therefore the effects of anxiolytic drugs should be assessed in terms of preference for the open arms as simple ratios (open arm/total entries percentage). These predictions were subsequently confirmed (see [29] for a review) and validated for both rats [26] and mice [21]. The elevated plus-maze test (EPM) is the most popular of all currently available animal models of anxiety, based on the study of spontaneous behavior [4,8]. The consistency and relevance of this model can be assessed in 900 published papers over the past 15 years [8,16,17,19,21,26,29,39].

Rats are normally cautious when exploring open spaces and in the EPM, after an initial overall exploration, they avoid the open arms starting around the third minute of trial 1 [18], and instead remain in the two enclosed arms of the maze [2,20,26]. Although rats forced to stay in the open arms of the EPM show fear-like reactions such as freezing, defecation, and

* Corresponding author. Fax: +55-48-2224-164.

E-mail address: adepadua@mbx1.ufsc.br (A.P. Carobrez)

increased production of plasma corticosterone [26], the precise source of aversion has not been determined [17]. Treit et al. [39] showed that in the EPM, the lack of thigmotaxis in the open arms might be the main avoidance-promoting feature, rather than height or novelty.

As pointed out by Rodgers and Cole [29], there are two major groups of variables influencing behavior and/or drug response in the EPM: (1) organismic variables such as, species, genetic strain [27,28], gender [19,28,37] and age [22]; and (2) procedural variables such as housing [22], prior handling [1,9], prior stress [38,41], and lighting levels [15,24], which have all been reported to have significant effects on basal anxiety.

Although early results regarding the influence of previous maze experience in EPM performance showed that repeated testing did not modify baseline open arm entries or time [3,11,21,26], nowadays there is an agreement that the retest of rats and mice in the EPM increases the open arm avoidance, therefore suggesting an anxiogenic tendency [31,34,39]. File et al. [11] described a phenomenon named “one trial tolerance” due to the lack of anxiolytic effect by benzodiazepines in the EPM for rats submitted to a single prior exposure to the apparatus. The main line of reasoning to explain the retest effect includes the fact that a 300 s prior experience in the EPM is able to release endogenous inverse agonists that bind to and alter the state of benzodiazepine receptors in brain areas, further inducing desensitization of benzodiazepine receptors [13]. This pharmacological evidence would reflect an enhancement in memory processes, therefore supporting the notion that a prior undrugged EPM experience induces a qualitative shift in anxiety/fear reaction from an unconditioned to an acquired phobic response [18,33,39].

An unanswered question related to test–retest or Trial 1–Trial 2 data in the EPM refers to the key feature in the avoidance learning process. The purpose of this study was to investigate what previous maze experiences in Trial 1 were required to alter baseline EPM results in Trial 2, confirming the hypothesis of a qualitative shift in anxiety/fear response.

2. Materials and methods

2.1. Subjects

The subjects were 149 male Wistar rats weighing 250–300 g, aged 12–14 weeks at the time of testing, housed in groups of five or six per cage (50 × 30 × 15 cm), under a 12 h light:12 h dark cycle, in a temperature-controlled environment (23 ± 1°C) and with free access to food and water. The period of adaptation to laboratory conditions was of 48 h prior to testing. The experimental sessions were conducted during the light phase of the cycle, between 12:00 and 17:00 h.

2.2. Apparatus

2.2.1. Elevated plus-maze

This equipment was made of wood and consisted of two opposite open arms, 50 × 10 cm (surrounded by 1 cm high Plexiglas), 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.

2.2.2. EPM-derived mazes

The elevated T-maze (ETM) consisted of an adaptation of the EPM where one enclosed arm was blocked off forming a T-shaped device. The elevated L-maze (ELM) consisted of an adaptation of the EPM with one enclosed and one open arm blocked, resulting in an L-shaped device.

The open arm confinement (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 of one open or one enclosed arm, respectively, in the EPM. The “open arm plus central platform” (OACP) was obtained through barriers installed in the exits of the two enclosed arms and one open arm.







2.3. Procedures




The experiments were carried out in a dimly-lit room (44-lux). Behavior was recorded by videocamera. 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).

In Trial 1, 95 naive rats were randomly assigned to one of the experimental groups described in Table 1. Rats submitted to the EPM, ETM or ELM were placed at the end of the enclosed arm. Rats from the OACP group were first placed on the central platform. Rats from OAC and EAC groups were isolated inside a single open or enclosed arm respectively. In all groups, the test duration of Trial 1 was set at 300 s.

Forty-eight hours after Trial 1, Trial 2 was conducted with all rats tested in the EPM for 300 s. Maze-naive (MN) rats, only exposed to the EPM once, were used as the control. The parameters analyzed in Trial 2 comprised the frequency of open and enclosed arm entries, and the amount of time spent by the rats in the center 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), percentage time spent in open arms (% OT, (open arm time/300) × 100) and percentage time spent in the central platform (%CT, (central time/

Table 1
Code and group assignments for the first trial (300 s duration)

Figure	Code	Group	N
	MN	Maze-naive - Control	27
	EPM	Elevated plus-maze	15
	ETM	Free exploration in the elevated T-maze	10
	ELM	Free exploration in the elevated L-maze	10
	OAC	Single open arm confinement in the EPM	11
	EAC	Single enclosed arm confinement in the EPM	11
	OACP	Open arm plus central platform confinement in the EPM	11

 = Open arms
  = Enclosed arms
  = Blocked arm

300) × 100). In addition, aborted attempts to reach the open arms were counted as frequency of tries [35]. Risk assessment (RA) behavior was interpreted according to the formula: RA = [frequency of tries/(300 – time spent in open arms) × 60].

In addition, data from 81 MN rats (including 27 rats from the control group) submitted to the elevated plus-maze test (300 s) were analyzed through factor analysis techniques in order to detect structure in the relationships between variables.

2.4. Statistics

Data obtained from results in the EPM in Trial 2, according to procedures detailed in Table 1, were analyzed by single-factor (group) analysis of variance

Table 2
Factor analysis for variables detected in maze-naive rats submitted to the elevated plus-maze test^a

	Factor 1	Factor 2	Factor 3
Percentage open arm entries	–0.85		0.42
Percentage open arm time	–0.97		
Enclosed arm entries			–0.96
Percentage central platform		–0.89	
Risk assessment behavior	–0.48	–0.75	
Percentage of total variance represented	39.6	27.0	23.5

^a Factor loading lower than 0.4 has not been included. The three factors account for 90.1% of the total variance.

(ANOVA). Further comparisons were performed using the Newman–Keuls test. The level of statistical significance adopted was $P < 0.05$.

Data from 81 MN rats were analyzed using a principal component solution with orthogonal rotation (varimax) of the factor matrix. The number of factor matrices corresponded to those reaching eigenvalues equal or greater than 1. All statistical analyses were performed using the Statistica[®] software package.

3. Results

Table 2 shows the factor analysis including all five parameters studied in MN rats submitted to the EPM. Three factors were detected accounting for 90.1% of total variance. The results showed that high factor loading were detected for %OE and %OT on factor 1, for %CT on factor 2 and for enclosed arm entries on factor 3. RA presented a moderate loading on factor 1 and a higher loading on factor 2. Factor 2 high loading for RA and %CT suggests this factor to be related to the decision-making process. Based on the factor analysis presented here (Table 2) and in the literature [18,30], RA data obtained according to our methodology matches the available data on stretch attend postures, %CT and enclosed arm returns published elsewhere [18].

Data illustrated in Figs. 1 and 2 assesses the effects of Trial 1 maze experience in Trial 2 (EPM, 300 s performance). ANOVA of data from Trial 2 showed a

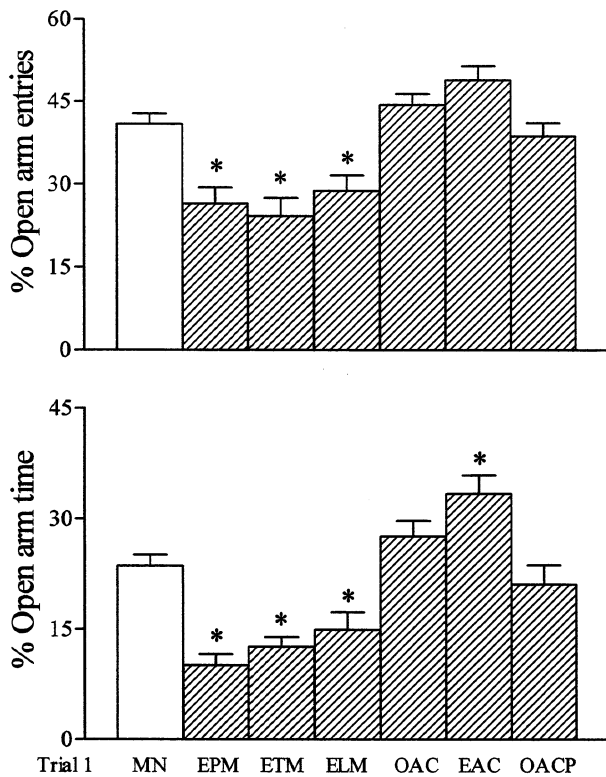


Fig. 1. Changes in number of entries (upper panel) and time spent (lower panel) in open arms by rats tested in the elevated plus-maze in Trial 2. Data are represented as mean \pm S.E.M. X-axis labels for groups refer to Table 1. * Significantly different from MN group (post-hoc Newman–Keuls test, $P < 0.05$).

marked group effect for both %OE ($F(6,88) = 12.0$; $P < 0.00001$) and %OT ($F(6,88) = 16.4$; $P < 0.00001$). Post-hoc Newman–Keuls tests revealed that prior exposure to the EPM, ETM or ELM test significantly ($P < 0.05$) reduced %OE and %OT in Trial 2, whereas previous exposure to the EAC increased only %OT. Trial 1 exposure to the OAC and OACP failed to alter %OE and %OT values in EPM Trial 2 (Fig. 1).

ANOVA of RA behavior data showed a marked Trial 1 maze-experience effect in the Trial 2 EPM test ($F(6,88) = 6.8$; $P < 0.00001$). The Newman–Keuls test revealed a significantly ($P < 0.05$) increased RA in Trial 2 for groups ETM, ELM, OAC and EAC, while prior EPM or OACP tasks failed to significantly alter this parameter (Fig. 2, top).

Locomotor activity, represented by enclosed arm entries data (Fig. 2, middle), showed also a Trial 1 group effect revealed by ANOVA ($F(6,88) = 5.5$; $P < 0.0001$). Further comparisons using the Newman–Keuls test showed an increased ($P < 0.05$) enclosed arm entries in Trial 2 EPM for the group previously submitted to the ETM.

No previous maze experience in Trial 1 altered the time on the central platform in the EPM in Trial 2 (Fig. 2, bottom).

4. Discussion

A crucial theme in behavioral pharmacology is the carryover effect, a phenomenon related either to long-term drug effects or to repeated and sequential experimental approaches in the same animal. Although long-term drug-effect problems can be partially resolved by increasing the time between experiments, the same does not apply to sequential experimental approaches if there is a learning component in the first trial that modifies performance in subsequent trials. This later affirmation appears to fit the EPM model of anxiety when comparing the performance of the same animals in Trial 1 and on subsequent trials [10,18,39]. It

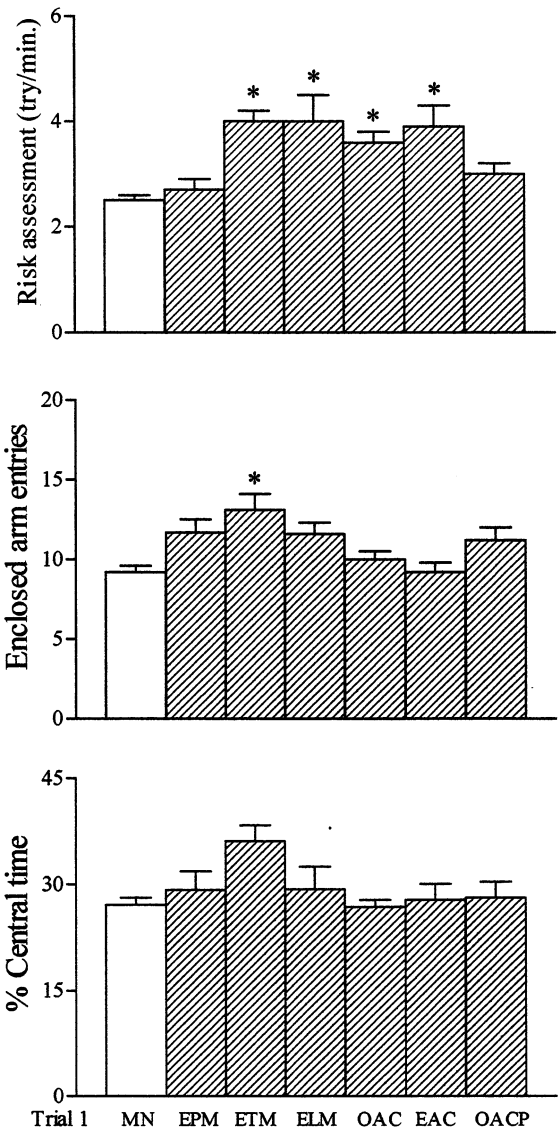


Fig. 2. Changes in risk assessment behavior (top), enclosed arm entries (middle) or percentage central time (bottom) in rats tested in the elevated plus-maze on Trial 2. Data are represented as mean \pm S.E.M. X-axis labels for groups refer to Table 1. * Significantly different from MN group (post-hoc Newman–Keuls test, $P < 0.05$).

has been claimed by Rodgers et al. [32] that the retest in the EPM is associated with behavioral changes [33], indicative of aversive learning, which are independent of manipulations of extra-maze cues.

The results obtained in the present study agree with the suggestion that previous maze experience modifies Trial 2 performance in the EPM. Rats submitted to the EPM in Trial 1 showed a significant reduction in %OE and %OT in the EPM in Trial 2. Risk assessment behavior, %CT, and enclosed arm entries were not modified in Trial 2 in comparison with control, showing that although rats were not exiting to open arms, there were as many movements toward open arms as in Trial 1. These results suggest that rats in Trial 2 were still motivated to explore the open arms in spite of a high avoidance response to this arm (Fig. 2). Fernandes and File [7] have shown that the addition of ledges affects the outcome of pharmacological manipulations; however, a factor analysis reported in the same study concluded that these differences are probably due to the expression of different types of fear/anxiety in mazes constructed with or without ledges, rather than just to a change in sensitivity to pharmacological manipulation.

Elevated maze models of anxiety for rodents are generally constructed to include at least two different surroundings with graded aversive meanings to the animal [14,16,21,26,36]. This feature seems to be the minimal prerequisite for the approach-avoidance conflict elicited in the EPM, and the strategy outlined in this study to test this assertion was to evaluate the influence of the ratio "number of open:enclosed arms". Two different EPM configurations, ETM and ELM, were used in Trial 1. The results showed that in both groups (ETM and ELM), the %OE and %OT were reduced in the EPM in Trial 2 in comparison with the control, revealing a response profile, which was quite similar to that of the EPM group. Although %CT was not modified, there was a significant increase in risk assessment behavior, suggesting that in the ETM and ELM configurations, although derived from the EPM model, the lack of room for locomotor activity actually stimulates the rat to attempt a further exploration of the maze.

The fact that EPM, ETM or ELM experience in Trial 1 was able to influence EPM performance in Trial 2 suggests that the existence of at least two different aversive environments is the key feature in the Trial 1 avoidance learning process, rather than the ratio number of open:enclosed arms attempts. To assess this suggestion, rats were confined in open (group OAC) or enclosed (group EAC) arms, or in the open arm plus central platform (group OACP) in Trial 1 and further placed in EPM in Trial 2. Rats submitted to OAC or OACP in Trial 1 performed in a manner equal to the MN group regarding %OE and %OT in Trial 2. Rats

from EAC groups showed a significantly increased %OT. In all cases, Trial 1 experience failed to increase open arm avoidance in Trial 2, in agreement with the suggestion that at least two different environments are needed in order to modify EPM performance in Trial 2. The fact that EAC group significantly increased %OT and also risk assessment behavior in EPM in Trial 2 suggests that a previous minor aversive exposure could motivate the rat's exploratory activity towards the open arm. On the other hand, the lack of influence from OAC and OACP on EPM on Trial 2 suggests that the high aversion present in the open arms and the impossibility of escape caused a deficit in the avoidance learning process, detected in Trial 2. Although it is difficult to grade the intensity of the stress, it has been shown that corticosterone levels are raised in rats confined to the open arms of the EPM [26]. Stress and corticosterone can block long-term potentiation induction, a feature that can be related to learning processes [5,25,40]. Taken together, both facts could explain the impaired learning in OAC, regarding data taken from the literature showing that prior stress can enhance anxiety [17,29]. A small enclosed environment represented by expansion of the open arms encompassing the central platform was not sufficient to further stimulate the rats to explore the EPM, as indicated by the maintenance of risk assessment equal to that of the control subjects.

Recent behavioral studies agree with the proposal that for rodents, the test–retest in the EPM results in a qualitative shift in emotional state. More specifically, factor analysis on anxiolytic measures in the EPM in Trials 1 and 2 loads in independent factors [12,18]. It is interesting to point out that anxiety measures obtained from rats submitted to EPM in Trial 3 load in the same factors as in Trial 2. Treit et al. [39] showed that open-arm avoidance increases in the second trial, but that no habituation occurs in subsequent exposures in up to 18 trials. This learned emotional response of remarkable endurance has recently been shown using the elevated T-maze model of anxiety, one of the experimental approaches used in Trial 1, where rats maintain increased avoidance to the open arms for up to 3 months [35]. This wealth of evidence reinforces the suggestion that an initial unconditioned fear in Trial 1 would shift to a learning avoidance in Trial 2 [10,33].

A curious and still unclear fact regarding the EPM is the precise source of aversion to the open arms. Falter et al. [6] showed that the height of the maze did not modify the EPM exploration. Treit et al. [39] suggested that rather than the height, the lack of thigmotaxis in the open arms was the main avoidance-promoting feature. File et al. [10] proposed that the experience in the open arms, including exploration and head-dipping over the edges, is the crucial factor in the avoidance learning process. However, the present study does not

support this proposition, mainly because of the results obtained using the OAC group. In agreement with the study from Falter et al. [6], the rat's confinement to an open arm in Trial 1 does not modify EPM performance in Trial 2, regardless of the amount of time spent exploring it. This fact invalidates the hypothesis that any activity exclusive to the open arms is the main avoidance-promoting feature, but suggests that the learning process may involve the cognitive ability to choose among different levels of aversive-bound situations. It seems that in maze models of anxiety, the pairing of a highly aversive meaning with the impossibility of exhibiting thigmotaxis in the open arm, and the association with a less aversive (thigmotaxis-positive) environment, might be the key feature in the aversive learning process, which changes the qualitative emotional state. The increased risk assessment behavior in the OAC group suggests a familiarity with the maze, supporting the notion that the high aversion elicited in the open arms impairs associative avoidance learning.

In conclusion, the facts presented in this paper confirm that the test–retest in the EPM involves behavioral changes, suggesting a qualitative shift in emotional state. Our results also support the idea that the existence of at least two environments with different levels of aversion is the key feature in the Trial 1 avoidance learning process, rather than the ratio of open:enclosed arms. In addition, the results suggest that the learning process may involve the cognitive ability to choose among different levels of aversive-bound situations.

Most treatable anxiety disorders are composed of an acquired phobic response, which in most of cases impairs the occupational life of a person. This clinical fact, and the improvement of our knowledge in animal models of anxiety, will certainly converge in order to provide a better understanding of the biological basis of anxiety. It remains to be determined whether the learned fear responses obtained in Trial 2 are susceptible to the same pharmacological or cognitive treatments as are phobias in humans.

Acknowledgements

The Brazilian Government CNPq (Proc. 521864/96-8) supported this research, from which Leandro J. Bertoglio received a student fellowship and Antonio P. Carobrez a research fellowship.

References

[1] Andrews N, File SE. Handling history of rats modifies behavioral effects of drugs in the elevated plus-maze test of anxiety. *Eur J Pharmacol* 1993;235:109–12.

- [2] Brett RR, Pratt JA. Chronic handling modifies the anxiolytic effects of diazepam in the elevated plus-maze. *Eur J Pharmacol* 1990;178:135–8.
- [3] Critchley MAE, Njung'e K, Handley SL. Prevention of 8-OH-DPAT anxiogenic effect by ipsapirone and 5-HT₁ antagonist beta-blockers. *Br J Pharmacol* 1988;94:389P.
- [4] Dawson GR, Tricklebank MD. Use of elevated plus-maze in the search for novel anxiolytic agents. *Trends Pharmacol Sci* 1995;16:33–6.
- [5] Diamond DM, Fleshner M, Ingersoll N, Rose GM, Falter U, Gower AJ, Gobert J. Psychological stress impairs spatial working memory: relevance to electrophysiological studies of hippocampal function. *Behav Neurosci* 1996;110:661–72.
- [6] Falter U, Gower AJ, Gobert J. Resistance of baseline activity in the elevated plus-maze to exogenous influences. *Behav Pharmacol* 1992;3:123–8.
- [7] Fernandes C, File SE. The influence of open arm ledges and maze experience in the elevated plus-maze. *Pharmacol Biochem Behav* 1996;49:21–30.
- [8] File SE. Behavioral detection of anxiolytic action. In: Elliott JM, Heal DJ, Marsden CA, editors. *Experimental Approaches to Anxiety and Depression*. New York: John Wiley & Sons Ltd, 1992:25–44.
- [9] File SE, Andrews N, Wu PY, Zharkovsky A, Zangrossi H. Modification of chlordiazepoxide's behavioral and neurochemical effects by handling and plus-maze experience. *Eur J Pharmacol* 1992;218:9–14.
- [10] File SE, Gonzalez LE, Gallant R. Role of the basolateral nucleus of the amygdala in the formation of a phobia. *Neuropsychopharmacology* 1998;19:397–405.
- [11] File SE, Mabbitt PS, Hitchcott PK. Characterization of phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. *Psychopharmacology* 1990;102:98–101.
- [12] File SE, Zangrossi H, Viana M, Graeff FG. Trial 2 in the elevated plus-maze: a different form of fear? *Psychopharmacology* 1993;111:491–4.
- [13] Gonzales LE, File SE. A five minute experience in the elevated plus-maze alters the state of the benzodiazepine receptor in the dorsal raphe nucleus. *J Neurosci* 1997;17:1505–11.
- [14] Graeff FG, Viana M, Tomaz C. The elevated T-maze, a new experimental model of anxiety and memory: effect of diazepam. *Braz J Med Biol Res* 1993;26:1–4.
- [15] Handley SL, McBlane JW. 5HT drugs in animal models of anxiety. *Psychopharmacology* 1993;112:13–20.
- [16] Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of "fear"-motivated behaviour. *Naunyn Schmiedeberg's Arch Pharmacol* 1984;327:1–5.
- [17] Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol Biochem Behav* 1996;54:21–30.
- [18] Holmes A, Rodgers RJ. Responses of Swiss–Webster mice to repeated plus-maze experience: further evidence for qualitative shift in emotional state? *Pharmacol Biochem Behav* 1998;60:473–88.
- [19] Imhof JT, Coelho ZMI, Schmitt ML, Morato GS, Carobrez AP. Influence of gender and age on performance of rats in the elevated plus-maze apparatus. *Behav Brain Res* 1993;56:177–80.
- [20] Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus-maze for evaluation of memory in mice: effects of nootropics, scopolamine, and electroconvulsive shock. *Psychopharmacology* 1990;101:525–32.
- [21] Lister R. The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 1987;92:180–5.

- [22] Lopes da Silva N, Ferreira VMM, Carobrez AP, Morato GS. Individual housing from rearing modifies the performance of young rats on the elevated plus-maze apparatus. *Physiol Behav* 1996;60:1391–6.
- [23] Montgomery KC. The relation between fear induced by novelty stimulation and exploratory behaviour. *J Comp Physiol Psychol* 1955;48:254–60.
- [24] Morato S, Castrechini P. Effects of floor surface and environmental illumination on exploratory activity in the elevated plus-maze. *Braz J Med Biol Res* 1989;22:707–10.
- [25] Pavlides C, Watanabe Y, Magariños AM, McEwen BS. Opposing roles of type I and type II adrenal steroid receptors in hippocampal long-term potentiation. *Neuroscience* 1995;68:387–94.
- [26] Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* 1985;14:149–67.
- [27] Ramos A, Mellerin Y, Mormede P, Chaouloff F. A genetic and multifactorial analysis of anxiety-related behaviours in Lewis and SHR intercrosses. *Behav Brain Res* 1998;96:195–205.
- [28] Rodgers RJ, Cole JC. Influence of social isolation, gender, strain, and prior novelty on plus-maze behavior in mice. *Physiol Behav* 1993;54:729–36.
- [29] Rodgers RJ, Cole JC. The elevated plus-maze: pharmacology, methodology and ethology. In: Cooper SJ, Hendrie CA, editors. *Ethology and Psychopharmacology*. Chichester: Wiley, 1994:9–44.
- [30] Rodgers RJ, Johnson NJT. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacol Biochem Behav* 1995;52:297–303.
- [31] Rodgers RJ, Shepherd JK. Influence of prior maze experience on behavior and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. *Psychopharmacology* 1993;113:237–42.
- [32] Rodgers RJ, Johnson NJ, Carr J, Hodgson TP. Resistance of experientially-induced changes in murine plus-maze behaviour to altered retest conditions. *Behav Brain Res* 1997;6:71–7.
- [33] Rodgers RJ, Johnson NJT, Cole JC, Dewar CV, Kidd GR, Kimpson PH. Plus-maze retest profile: importance of initial stages of trial 1 and response to post-trial cholinergic receptor blockade in mice. *Pharmacol Biochem Behav* 1996;54:41–50.
- [34] Rodgers RJ, Lee C, Shepherd JK. Effects of diazepam on behavioral and antinociceptive responses to the elevated plus-maze experience. *Psychopharmacology* 1992;106:102–10.
- [35] Sanson LT, Carobrez AP. Long-lasting inhibitory avoidance acquisition in rats submitted to the elevated T-maze model of anxiety. *Behav Brain Res* 1999;101:59–64.
- [36] Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CT. Behavioural and pharmacological characterization of the elevated “zero-maze” as an animal model of anxiety. *Psychopharmacology* 1994;116:56–64.
- [37] Steenbergen HL, Farabollini F, Heinsbroek RPW, van de Poll NE. Sex-dependent effects of aversive stimulation on holeboard and elevated plus-maze behavior. *Behav Brain Res* 1991;43:159–65.
- [38] Steenbergen HL, Heinsbroek RPW, van Hest A, van de Poll NE. Sex-dependent effects of inescapable shock administration on shuttlebox-escape performance and elevated plus-maze behavior. *Physiol Behav* 1990;48:571–6.
- [39] Treit D, Menard J, Royan C. Anxiogenic stimuli in the elevated plus-maze. *Pharmacol Biochem Behav* 1993;44:463–9.
- [40] Xu L, Anwyl R, Rowan MJ. Behavioural stress facilitates the induction of long-term depression in the hippocampus. *Nature* 1997;387:497–500.
- [41] Zangrossi H, File SE. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. *Brain Res Bull* 1992;29:381–8.