

Ethopharmacological analysis of behaviour of rats using variations of the elevated plus-maze

V.Z. Anseloni and M.L. Brandão

Laboratório de Psicobiologia, FFCLRP, Campus USP, Av. Bandeirantes 3900, 14049-901, Ribeirão Preto, SP, Brasil

Correspondence to: M.L. Brandão at above address

Besides allowing better observation and recording of the behaviour of rodents, we have shown that the elevated plus-maze with transparent walls is as sensitive to anxiolytic and anxiogenic drug effects as a traditional apparatus with opaque walls. In this study we extend these observations with an ethopharmacological analysis of the behaviour of rats in the elevated plus-maze with transparent walls. A factor analysis of the behaviour of control (saline-treated) rats on the modified maze and on a standard maze revealed a characteristic distribution of the behavioural categories in six factors. For the modified maze, the first three factors, representing standard indices of anxiety, locomotion and risk assessment, were similar to the factor distribution reported by this and other studies using the standard elevated plus-maze test. Distinct features appeared on Factor 4, with the loading of rears in the modified maze together with the occurrence of the percentage of centre time and flat-back approach common to both mazes. Scanning and grooming, which are generally grouped on Factor 5 in the standard maze, appeared as isolated behavioural elements loading on Factors 5 and 6, respectively, in the modified maze. An ethopharmacological analysis of behaviour in the modified maze showed that midazolam (1 and 2 mg/kg) produced an anxiolytic profile, whereas pentylenetetrazol (5 and 10 mg/kg) produced an anxiogenic-like profile. These results point to the distinct features of the elevated plus-maze with transparent walls, which may be beneficial in the study of the different facets of anxiety and the mode of action of anxiolytic drugs in rats.

Keywords: Elevated plus-maze – Ethological analysis – Midazolam – Pentylenetetrazol – Rat – Transparent walls

INTRODUCTION

The elevated plus-maze has been used for over a decade to investigate and measure the behavioural, physiological and pharmacological aspects of anxiety (for reviews, see Handley and McBlane, 1993; Rodgers and Cole, 1994). Animals exposed to the elevated plus-maze show a sensitization of fear of the open arms (Montgomery, 1955) as well as behavioural and physiological manifestations of fear, such as freezing, defecation, and increases in plasma corticosteroids (Pellow *et al.*, 1985; Treit *et al.*, 1993). Primary or traditional indices used to report data obtained in this test are the number of entries into and time spent in the open and closed arms of the maze, or in the open, relative to the total entries or total time of the test. Anxiolytic drugs increase the number of entries into and the time spent in the open arms, whereas anxiogenic agents do the opposite (Handley and Mithani, 1984; Pellow *et al.*, 1985; Pellow and File, 1986; Trullas *et al.*, 1991). Some behaviours displayed by rats in the elevated plus-maze, such as grooming and rearing have not, however, been fully investigated

(Adamec and Shallow, 1993). Recently, we showed that rats still display open-arm avoidance when tested in an elevated plus-maze with transparent walls (versus traditional opaque), and that this modified apparatus allows more accurate recording of behaviour, especially in the enclosed compartments (Anseloni *et al.*, 1995). These findings are not surprising because: (1) elements of unfamiliarity, openness and elevation are still present in the modified apparatus; and (2) similar results have been reported in mice using a plus-maze with transparent walls (Cole and Rodgers, 1994; Rodgers and Johnson, 1995). Indeed, the use of this modified plus-maze made it possible for the latter authors to include successfully new ethological measures in the analysis of plus-maze behaviour. Furthermore, it has been found that minor changes in the construction material alter the sensitivity of this test (Griebel *et al.*, 1993; Fernandes and File, 1996). Variations in these parameters could contribute to the fact that the effects of drugs which act on GABAergic transmission are very clear whereas the effects of drugs

that act on 5-HT transmission are quite inconsistent (Chopin and Briley, 1987; Moser, 1989; Motta *et al.*, 1992; Handley and McBlane, 1993; Maissonnette *et al.*, 1993).

In the present study, we carried out an ethopharmacological analysis of the behaviour (expressed by traditional and novel ethological measures) of rats tested in the elevated plus-maze modified with transparent walls. We also undertook a factor analysis (Kim and Muller, 1978), of behaviour in the standard (enclosed) elevated plus-maze and the modified (transparent walls) plus-maze, in the belief that the elevated plus-maze may be measuring different aspects of anxiety (Green, 1991; File, 1992; Handley and McBlane, 1993; Treit *et al.*, 1993; Rodgers and Cole, 1994), and that this method may reveal how these different aspects are affected pharmacologically by anxiolytic and anxiogenic drugs which act on GABA transmission.

METHODS

Subjects

A total of 280 male Wistar rats weighing 250–300 g, from the animal house of the Campus of Ribeirão Preto of the University of São Paulo, were used. These animals were transported to a room adjacent to the test laboratory 72 h before the test, where they were housed in groups of six per cage under a 12:12 h light/dark cycle (lights on at 07.00 h) at $23 \pm 1^\circ\text{C}$, and given free access to food and water. The animals were taken to the test laboratory at least 1 h before testing.

Apparatus

Two types of apparatus were used: the widely used elevated plus-maze (standard) and a modified elevated plus-maze. Both mazes were made of wood with two open arms (50 × 10 cm) and two enclosed arms of the same size with 50 cm high walls. The level of illumination was 10 and 100 lux on the floor level of the walled arms of the transparent and opaque mazes, respectively. The mazes were configured so that arms of the same type were opposite to each other, and the whole maze was raised 50 cm from the floor. The walls of the closed arms of the standard maze were made of wood whereas those of the modified maze were made of sheets of transparent Plexiglas.

All testing was conducted during the mid-portion of the light phase of the light/dark cycle. The rats were placed individually in the centre of the maze facing a closed arm and allowed 5 min of free exploration. The behaviour of the animals was recorded by a videocamera positioned above the maze (standard) or

to its side (modified apparatus), allowing for the discrimination of all behaviours, with the signal relayed to a monitor in another room via a closed circuit TV camera. The maze was thoroughly cleaned after each test using damp and dry cloths. Each rat was tested only once.

Videotapes were subsequently scored by an observer using ethological analysis software (Observer) developed by Noldus (Netherlands). Using separate location and behaviour keys, this software allows the real-time scoring of videotapes of any behaviour by direct keyboard entry to a PC. Behaviours scored from videotape included traditional and novel plus-maze parameters. This software only records the next behavioural category after a 'stop' key is pressed, allowing for the recording of duration and frequency of prolonged behaviours such as grooming and rearing.

Ethological analysis

Behaviour on the standard elevated plus-maze and the modified maze was recorded and analysed in two large groups of untreated control animals ($n = 110$). The behaviour of each animal in the maze was analysed taking into account the standard measures recorded in each section of the maze (closed and open arms, central platform), comprising the frequency of open- and closed-arm entries (arm entry defined as all four paws into an arm), total arm entries and the amount of time spent by the animals in each section of the maze. These data were additionally used to calculate the percentage of open-arm entries, the percentage of time in the open arms and the percentage of time in the central platform. The ethological items recorded were grooming, rearing, peeping out, stretched-attend posture, flat-back approach, scanning, head dipping and end exploration. These categories were defined following work in rats (Blanchard *et al.*, 1991; Cruz *et al.*, 1994) and in mice (Rodgers and Johnson, 1995):

- *Grooming*: species-typical sequences beginning with the snout, progressing to the ears and ending with whole-body groom.
- *Rearing*: partial or total rising onto the hind limbs.
- *Scanning*: scrutinizing in any direction, including sniffing (olfactory exploration of maze floor and walls).
- *Head dipping*: exploratory movement of head/shoulders over sides of the maze and down towards the floor.
- *End exploration*: number of times the rat reached the end of an open arm.
- *Peeping out*: stretching the head/shoulders from the closed arms to the central platform.

- *Stretched-attend posture* (SAP): when the animal stretches to its full length and turns back to the anterior position.
- *Flat-back approach* (FBA): locomotion when the animal stretches to its full length and cautiously moves forward.

As a result of the relevance of thigmotactic cues provided by the walls of the maze (Treit *et al.*, 1993), stretched-attend, scanning and flat-back approach were further differentiated as 'protected' (i.e. occurring on/from the relative security of the closed arms or centre platform) or 'unprotected' (i.e. occurring on/from the open arms) (Rodgers and Johnson, 1995).

Drug treatment

Groups of animals ($n = 12$) were tested, in the modified maze only, following pretreatment with saline, midazolam (Sigma, USA; 1 or 2 mg/kg) or pentylene-tetrazol (Sigma; 5 or 10 mg/kg). Midazolam and pentylene-tetrazol were dissolved in saline solution (0.9%) shortly before use. Saline also served as a vehicle control. Compounds were administered (1 ml/kg, i.p.) 30 min (midazolam) and 5 min (pentylene-tetrazol) before testing.

Statistical analysis

Data are reported as means \pm SEM. Comparisons of the exploratory activity of rats submitted to the two types of mazes were performed by *t*-test (independent samples, $n = 110$). Results of experiments with midazolam and pentylene-tetrazol were analysed by one-way analyses of variance (ANOVA). Duncan post-hoc comparisons were carried out if significant overall F-values were obtained ($n = 12$ for each group).

Factor analyses were performed using datasets obtained from the standard plus-maze and from the maze with transparent walls. These factor analyses were performed using the method of best fit to the observed correlations and the varimax method (principal component solution with orthogonal rotation) of the factor matrix. This combined method guarantees that the extracted factors are independent and should, therefore, reflect separate processes. Two criteria were used to select the number of factors extracted, the 75% variance rule (Kaiser test) and the Scree plot test (the point at which the smooth decrease in eigenvalues levels off to the right). The factor loading reflects the contribution of each variable to each factor. In this study, therefore, loadings of less than 0.4 were discarded. A value of 1.0 would be a perfect reflection, whereas a loading of less than 0.4 indicates a poor

reflection of the factor. The factors extracted from this analysis are independent, and are therefore likely to reflect different processes.

RESULTS

Open-arm avoidance remained a salient feature of the behaviour of rats submitted to the modified plus-maze. The animals still preferred to stay in the closed arms of the apparatus with transparent walls (see Table I). However, the modified apparatus clearly influenced the performance of the rats relative to their exploratory activity in the standard plus-maze. Statistically significant increases were seen for open-arm entries ($t = 4.04$, $p < 0.01$), total number of entries ($t = 2.53$, $p < 0.01$), percentage of open-arm entries ($t = 3.72$, $p < 0.01$), percentage of time spent in the open arms ($t = 7.04$, $p < 0.01$). Whereas the percentage of closed time was significantly lower in the modified maze ($t = 6.13$, $p < 0.01$) the number of entries into the closed arms ($t = 1.66$, NS) and the percentage of time spent in the centre of the maze ($t = 0.56$, NS) were not significantly different.

Regarding the ethological profile in the modified maze, the flat-back approach was equally distributed between the open and closed arms. Grooming, stretched-attend postures, rearing and scanning were predominantly seen in the closed arms ($> 60\%$) and head dipping occurred mostly in the open arms. The comparative analysis of the occurrence of these ethological behavioural items showed that it changed according to the type of maze. Scanning ($t = 3.66$, $p < 0.01$), flat-back approach ($t = 6.02$, $p < 0.01$) and end-exploration ($t = 2.85$, $p < 0.01$) were more prominent in the modified maze, whereas rearing ($t = 3.68$, $p < 0.01$), stretched-attend postures ($t = 6.16$, $p < 0.01$) and peeping out ($t = 7.17$, $p < 0.01$) were higher in the standard maze. Head dipping and grooming did not differ significantly between mazes. All these comparisons are shown in Table I.

As already reported by other authors using the standard maze (Motta *et al.*, 1992; Cruz *et al.*, 1994; Anseloni *et al.*, 1995) the traditional measures were also affected by midazolam and pentylene-tetrazol in the modified maze. Midazolam increased the percentage of open-arm entries [$F(2,23) = 7.60$, $p < 0.01$] and caused a selective increase [$F(2,23) = 10.81$, $p < 0.01$] in the percentage of time spent in the open arms, and in the total number of entries [$F(2,23) = 3.55$, $p < 0.05$], whereas no significant effect could be detected in closed-arm entries [$F(2,23) = 1.74$, NS]. On the other hand, the injection of pentylene-tetrazol decreased both the percentage of open-arm entries

TABLE I. Standard and ethological measures of behaviour displayed by male Wistar rats in the standard and modified elevated plus-mazes (mean \pm sem)

Behavioural item	Standard	Modified
Open-arm entries	2.20 \pm 0.16	3.15 \pm 0.16**
Closed-arm entries	4.09 \pm 0.17	4.38 \pm 0.14
Total arm entries	6.26 \pm 0.32	7.53 \pm 0.26**
% Open-arm entries	19.72 \pm 1.05	25.01 \pm 0.96**
% Open time	9.60 \pm 1.14	20.68 \pm 1.51**
% Closed time	82.06 \pm 1.44	70.40 \pm 1.50**
% Centre time	9.00 \pm 1.03	8.00 \pm 0.52
Rearing	15.67 \pm 0.66	12.83 \pm 0.40**
Head dipping	8.15 \pm 0.63	9.84 \pm 0.49
Stretched-attend postures (SAP)	13.20 \pm 0.99	6.39 \pm 0.49**
Scanning	21.50 \pm 1.06	33.12 \pm 1.61**
Grooming	5.67 \pm 0.26	4.91 \pm 0.33
Flat-back approach	1.44 \pm 0.14	3.14 \pm 0.22**
Peeping out	4.60 \pm 0.30	2.11 \pm 0.16**
End exploration	1.51 \pm 0.24	2.56 \pm 0.22**
Protected SAP	3.90 \pm 0.24	2.47 \pm 0.20**
Protected scanning	15.44 \pm 0.59	12.88 \pm 0.53**
Protected flat-back	0.81 \pm 0.10	1.48 \pm 0.13**

** $p < 0.01$

[$F(2,23) = 5.14$, $p < 0.05$] and the time spent in these arms [$F(2,23) = 5.73$, $p < 0.01$]. Pentylentetrazol did not change the total number of entries [$F(2,23) = 1.54$, $p > 0.05$] or closed-arm entries [$F(2,23) = 0.95$, $p > 0.05$] of the animals in the modified maze.

Regarding the ethological measures, the administration of midazolam increased end-arm exploration

[$F(2,23) = 13.69$, $p < 0.01$] and head dipping [$F(2,23) = 4.51$, $p < 0.01$], reduced the frequency of rearing [$F(2,23) = 2.75$, $p < 0.01$], grooming [$F(2,23) = 2.06$, $p < 0.01$], stretched-attend posture [$F(2,23) = 4.64$, $p < 0.01$] and flat-back approach [$F(2,23) = 4.42$, $p < 0.01$] and did not alter scanning [$F(2,23) = 1.18$, NS] and peeping-out [$F(2,23) = 1.53$, NS]. Pentylentetrazol decreased head dipping [$F(2,23) = 4.31$, $p < 0.01$] and increased peeping out [$F(2,23) = 6.13$, $p < 0.01$] and stretched-attend posture [$F(5,77) = 4.64$, $p < 0.01$]. Other ethological measures, such as end-exploration [$F(2,23) = 0.76$, NS], scanning [$F(2,23) = 1.32$, NS], rearing [$F(2,23) = 0.91$, NS], grooming [$F(2,23) = 1.08$, NS] and flat-back approach [$F(2,23) = 1.27$, NS] were not changed by pentylentetrazol. All these effects are depicted in Figure 1.

Factor analysis

The factors for eigenvalues of 1 or more in Tables II, III and IV left to right are presented in an order that corresponds to the decreasing size of the proportion of the original variance accounted for by each factor.

The analysis shown in Table II took into account all standard plus-maze measures, i.e. total entries, open entries, closed entries, percentage of open entries and percentage of open time. Two factors emerged from both elevated plus-maze datasets. Highly loaded measures on Factor 1 comprised open entries, percentage of open entries and percentage of open time. Total arm entries loaded only moderately on this

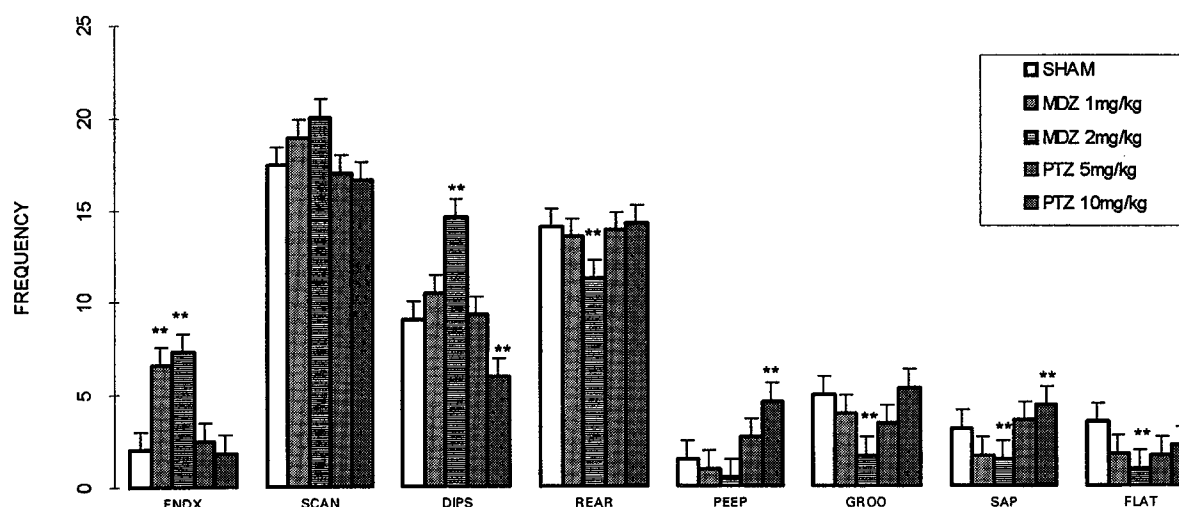


FIG.1. Effects of midazolam (1 and 2 mg/kg, i.p.) and pentylentetrazol (5 and 10 mg/kg, i.p.) on the ethological measures of the behaviour of rats on the elevated plus-maze with transparent walls. Data are presented as means \pm SEM). * $p < 0.01$ compared to the control group. (Duncan test after ANOVA). ENDX, end exploration; SCAN, scanning; DIPS, head dipping; REAR, rearing; PEEP, peeping out; GROO, grooming; SAP, stretched-attend posture; FLAT, flat-back approach.

TABLE II. Orthogonal factor loadings for standard plus-maze measures in the control groups of Wistar rats tested on the modified and standard elevated plus-mazes¹

	Modified		Standard	
	F1	F2	F1	F2
Open entries	0.80		0.75	
% Open time	0.77		0.70	
% Open entries	0.91		0.96	
Closed entries		0.96		0.95
Total arm entries	0.41	0.88	0.40	0.82

Factor loadings of < 0.4 are not included. The two factors accounted for 100% of the total variance in the modified maze and 90% of the total variance in the standard maze. Criteria: Eigenvalue > 1.

TABLE III. Orthogonal factor loadings for standard plus-maze measures, time spent on the central platform, and all ethological measures, in Wistar rats tested on the standard elevated plus-maze¹

	F1	F2	F3	F4	F5
Open entries	0.72	0.66			
% Open time	0.84				
% Open entries	0.74				
End exploration	0.77				
Head dipping	0.75				
% Closed time	-0.78				
Stretched-attend posture			0.83		
Closed entries		0.81			
Total arm entries	0.54	0.68			
% Centre time			0.47		
Grooming				-0.52	
Scanning				0.61	
Flat-back approach			0.79		
Rearing					0.88
Peeping out					
Protected SAP			0.90		
Protected flat-back			0.69		
Protected scanning				0.84	

¹Factor loadings of < 0.4 are not included. Criteria: Eigenvalue > 1. These principal components accounted for 79% of the total variance.

factor. In contrast, closed entries and total arm entries loaded highly on Factor 2.

Considering traditional and ethological measures, five and six factors emerged from the analysis of the behaviour of the animals exposed to the standard and modified plus-maze, respectively. The first three factors were roughly similar in the standard (Table III) and modified plus-maze (Table IV). Factor 1 showed high loadings of open-arm entries, percentage of open time and percentage of closed time (negative loading).

TABLE IV. Orthogonal factor loadings for standard plus-maze measures, time spent on the central platform, and all ethological measures, in Wistar rats tested on the modified elevated plus-maze¹

	F1	F2	F3	F4	F5	F6
Open entries	0.61	0.60				
% Open time	0.88					
% Open entries	0.78					
End exploration	0.80					
Head dipping	0.75					
% Closed time	-0.86					
Stretched-attend posture			0.93			
Closed entries		0.77				
Total arm entries	0.44	0.82				
% Centre time			0.45			
Grooming	-0.52					0.44
Scanning					0.83	
Flat-back approach				0.76		
Rearing				-0.54		
Peeping out						
Protected SAP			0.91			
Protected flat-back				0.70		
Protected scanning					0.94	

¹Factor loadings of < 0.4 are not included. Criteria: Eigenvalue > 1. These principal components accounted for 79% of the total variance.

The ethological measures, end exploration, head dipping and grooming (negative loadings) also loaded highly on this factor, whereas total arm entries and percentage of open entries loaded only moderately on this factor. Open-arm entries, closed entries and total arm entries loaded heavily on Factor 2, and percentage of centre time also loaded moderately on this factor. Stretched-attend posture (and its protected form) loaded highly on Factor 3. Differences in loadings in both mazes started to be observed from Factor 4. In the standard maze, percentage of centre time, percentage of closed time and grooming loaded on Factor 4 and total rears, protected scanning and head dipping loaded on Factor 5. Otherwise, using the modified maze, the flat-back approach (and its protected form) and rearing loaded on Factor 4. Scanning (and its protected form) loaded highly on Factor 5 whereas only grooming loaded on Factor 6.

DISCUSSION

A comparison between the exploratory activity of rats exposed to the standard or the modified plus-maze

showed a robust difference in the expression of all standard and ethological measures examined in this study, with the exception of closed arm entries, percentage of centre time, head dipping and grooming (see Table I). Some of these differences were unexpected (e.g. increased open-arm activity in the modified test). These differences are probably caused by the elevated plus-maze construction, which can alter the reactivity of rodents and influence their performance in this model of anxiety (Rodgers and Johnson, 1995). As closed arms are more illuminated in the modified test, because the walls are transparent, openness and height are more salient. Consequently, closed arms became more stressful so that animals spent less time in them. In fact, a high level of illumination serves as an additional anxiogenic stimulus in the test, as has been shown by other authors (File, 1980; Morato and Castrechini, 1989; Griebel *et al.*, 1993). Nevertheless, in the present study rats still preferred the closed arms, despite the baseline increase in open-arm exploration in the modified maze. In this context, it is important to note that in an elevated plus-maze with one standard open arm and one with a clear sheet of Plexiglas secured vertically along a single edge, the animals preferred the latter (Treit *et al.*, 1993). These results led to the suggestion that the preference of rats for the Plexiglas-edge open arms is derived from the possibility of thigmotaxis. This may mean that the avoidance of the open arms occurs primarily because they do not allow the rats to engage in thigmotactic behaviour, which is a natural defensive response of the rat that remains near to vertical surfaces, thereby avoiding predators (Treit and Fundytus, 1988; Treit *et al.*, 1993).

The elevated plus-maze has been one of the most useful tests for detecting anxiolytic and anxiogenic drug effects, and for investigating their mechanisms of action (Trullas *et al.*, 1991; File, 1992; Handley and MacBlane, 1993). Standard anxiolytic drugs, such as diazepam, increase the percentage of entries and the time spent in the open arms of the maze. Our results are consistent with these reports, as midazolam produced a clear anxiolytic effect in our modified maze reducing avoidance of the open arms without changing the locomotor activity of the animals in the closed arms. On the other hand, anxiogenic drugs, such as FG 7142, a β -carboline, and pentylenetetrazol, administered in subconvulsant doses, have been found to decrease the percentage of entries into and the time spent in the open arms of the maze (Dorow *et al.*, 1983; Pellow and File, 1986; Cruz *et al.*, 1994). These drug effects have been attributed to an inhibition of GABA function (Schofield, 1992). Our results are consistent with these reports, as pentylenetetrazol produced

a very clear anxiogenic profile in the present test, enhancing avoidance of the open arms of the maze. Regarding the ethological measures, the effects produced by both drugs were also consistent with what has been reported in the literature (Cole and Rodgers, 1993, 1994; Cruz *et al.*, 1994). Overall, midazolam reduced stretched-attend postures and the flat-back approach (effects that may be due to a reduced fear of leaving safe areas of the maze) and increased total head-dips, indicating an enhanced tendency to explore actively the potentially dangerous areas (Cole and Rodgers, 1993). In general, pentylenetetrazol changed these 'risk assessment' behaviours in the opposite direction. Importantly, scanning, in the way it is operationally defined here, was not affected by either midazolam or pentylenetetrazol, suggesting that this response may not be directly linked to anxiety.

Two separate factor analyses were performed on the datasets recorded from the two mazes used in this work, to characterize the factor structure of the observed behaviours. The factor structure and factor loadings observed in the analysis with the traditional measures were only found to be very similar in both apparatuses, and replicate factor analyses reported by File (1992), Lister (1987) and Rodgers and Johnson (1995). This analysis showed that these measures could be accommodated by two factors. The standard anxiety indices (open entries, percentage of open entries, percentage of open time) loaded heavily on Factor 1 for both maze conditions. This factor is taken as an anxiety factor. In contrast, total arm entries and closed arm entries loaded heavily on Factor 2, which is considered to represent locomotor activity. The total number of entries also loaded moderately on Factor 1. This finding is consistent with previously reported results, which indicate that this measure is not a pure index of motor activity, as it may be contaminated by anxiety (File, 1992; Cruz *et al.*, 1994; Rodgers and Johnson, 1995).

Factor analysis on both the standard and the ethological measures revealed different distributions in the behavioural categories. Overall, the factor structure for the modified maze comprised a total of six factors, one more than that of the standard apparatus. The first three factors followed a distribution that conforms with that observed in the present study and reported by other factorial studies using the standard maze (Cruz *et al.*, 1994; Rodgers and Johnson, 1995; Fernandes and File, 1996). Standard indices such as open entries, percentage of open time and percentage of open entries loaded heavily on Factor 1, which is supposed to be inversely related to anxiety. Importantly, ethological measures, such as head dipping and end exploration, previously interpreted as indicative

of anxiety (Cruz *et al.*, 1994; Rodgers and Johnson, 1995), were also found to load highly on this factor. As occurred in the simpler analysis, total entries also loaded on Factor 1, although less heavily, confirming that this item may not be considered a reliable index of locomotor activity. Measures expressing locomotor activity such as closed entries and total arm entries loaded on Factor 2. Open-arm entries also loaded on Factor 2, in agreement with reports from other authors (Cruz *et al.*, 1994). As noted by those authors these findings seem logical, because entering onto an open arm involves motor activity. Stretched-attend postures and the flat-back approach (and their protected forms) loaded on Factor 3. Scanning (and its protected form), which has been associated to risk assessment in previous studies, loaded on Factor 4. Grooming also loaded moderately on this factor. These two latter factors have somewhat different distributions in the modified test. The flat-back approach (and its protected variant) and rearing heavily loaded on Factor 4. Scanning and grooming which are grouped together on Factor 4 in the standard plus-maze, loaded independently on Factors 5 and 6, respectively, in the modified test.

In the modified maze, Factor 4 may reflect an anxiety factor not only linked to risk assessment as has been reported by studies using the standard maze. The flat-back approach may, in fact, be related to decision making (Rodgers and Johnson, 1995). Furthermore, it is possible that in our present experimental conditions, rearing, which also loaded on this factor, may represent attempts to avoid threatening situations associated with the height clues present in the apparatus. In this context, it is worth mentioning that aversive stimulation of some structures associated with the genesis and elaboration of fear states/generalized anxiety, such as the dorsomedial hypothalamus, produces high scores for rearing, in contrast with the dorsal periaqueductal gray, which is related to explosive behaviour/panic attacks (see Brandão *et al.*, 1986, for a review). It thus makes behavioural sense that rearing loaded on the same factor as the flat-back approach, when the decision to avoid or escape the threatening situation was already made, than, for instance, with stretched-attend postures, when the decision to escape is still being made. As a matter of fact, in the present study midazolam also reduced the occurrence of rearing and the flat-back approach.

It has been suggested that although scanning, including sniffing, appears to be a function of defence-related factors it mainly reflects foraging/searching for consumatory objects (Blanchard *et al.*, 1991). The present results are consonant with this idea, insofar as

this behavioural item was not affected by benzodiazepines. Grooming may represent displacement in situations induced by environmental stimuli such as novelty and handling (Archer, 1973). Therefore, the differences in factor structure between the mazes with opaque and transparent walls raise the possibility that they measure different aspects of anxiety.

The present ethopharmacological study, together with the factor analysis, provide new evidence for the behavioural and pharmacological validity of the elevated plus-maze with transparent walls. We believe that the modified elevated plus-maze: (1) allows an accurate behavioural analysis during the whole period of the test, even when animals are in the closed arms; and (2) provides new behavioural measures to facilitate the study of different aspects of anxiety, and the mode of action of different classes of anxiolytic drugs.

REFERENCES

- Adamec RE and Shallow T (1993) Lasting effects on rodent anxiety of a single exposure to a cat. *Physiology and Behavior*, **54**, 101–109.
- Anseloni VCZ, Motta V, Lima G and Brandão ML (1995) Behavioral and pharmacological validation of the elevated plus maze constructed with transparent walls. *Brazilian Journal of Medical and Biological Research*, **28**, 597–601.
- Archer J (1973) Tests for emotionality in rats and mice: a review. *Animal Behavior*, **21**, 205–235.
- Blanchard DC, Blanchard RJ and Rodgers RJ (1991) Risk assessment and animal models of anxiety. In: *Animals Models in Psychopharmacology* (Eds B Olivier, J Mos and JL Slanegen), pp. 117–134, Birkhauser, Bale.
- Brandão ML, DiScala G, Bouchet MJ and Schmitt P (1986) Escape behavior produced by the blockade of glutamic acid decarboxylase (GAD) in mesencephalic central gray and medial hypothalamus. *Pharmacology, Biochemistry and Behavior*, **24**, 497–501.
- Chopin P and Briley M (1987) Animal models of anxiety: the effect of compounds that modify 5-HT neurotransmission. *Trends in Pharmacological Sciences*, **8**, 383–388.
- Cole JC and Rodgers RJ (1993) An ethological analysis of the effects of chlordiazepoxide and bretazenil (Ro-6028) in the murine elevated plus-maze. *Behavioural Pharmacology*, **4**, 573–580.
- Cole JC and Rodgers RJ (1994) Ethological evaluation of the effects of acute and chronic buspirone treatment in the murine elevated plus-maze test: comparison with haloperidol. *Psychopharmacology*, **14**, 288–296.
- Cruz APM, Frei F and Graeff FG (1994) Ethopharmacological analysis of rat behavior on the plus-maze. *Pharmacology, Biochemistry and Behavior*, **49**, 171–176.
- Dorow R, Horowski R, Paschelke G, Amin M and Braestrup C (1983) Severe anxiety induced by FG 7142, a β -carboline for benzodiazepine receptors. *Lancet*, **9**, 98–99.
- Fernandes C and File S (1996) The influence of open arm ledges and maze experience in the elevated plus-maze. *Pharmacology, Biochemistry and Behavior*, **54**, 31–40.

- File SE (1980) The use of social interactions as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. *Journal of Neuroscience Methods*, **2**, 219–238.
- File SE (1992) Behavioural detection of anxiolytic action. In: *Experimental approaches to anxiety and depression* (Eds JM Elliot, DJ Heal and CA Marsden), pp. 25–44. John Wiley and Sons Ltd, Chichester.
- Green S (1991) Benzodiazepines, putative anxiolytics and animal models of anxiety. *Trends in Pharmacological Sciences*, **14**, 101–104.
- Griebel G, Moreau J-L, Jenck F, Martin JR and Misslin R (1993) Some critical determinants of the behaviour of rats in the elevated plus-maze. *Behavioural Processes*, **29**, 37–48.
- Handley SL and McBlane JW (1993) 5-HT drugs in animal models of anxiety. *Psychopharmacology*, **112**, 13–20.
- Handley SL and Mithani S (1984) Effects of alpha-adrenoceptor agonists in a maze-exploration model of "fear"-motivated behavior. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **327**, 1–5.
- Kim J and Muller CW (1978) *Factor Analysis, Statistical Methods and Practical Issues*, pp. 49–54. Sage Publications, California.
- Lister RG (1987) The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology (Berlin)*, **92**, 180–185.
- Maisonnette S, Morato M and Brandão ML (1993) Role of resocialization and of 5-HT_{1A} receptor activation on the anxiogenic effects induced by isolation in the elevated plus-maze. *Physiology and Behavior*, **54**, 753–758.
- Montgomery KC (1955) The relationship between fear induced by novel stimulation and exploratory behaviour. *Journal of Comparative and Physiological Psychology*, **48**, 254–260.
- Morato de Carvalho S and Castrechini P (1989) Effects of floor and environmental activity in the elevated plus-maze. *Brazilian Journal of Medical and Biological Research*, **22**, 707–710.
- Moser PC (1989) An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. *Psychopharmacology*, **99**, 48–53.
- Motta V, Maisonnette S, Morato S and Brandão ML (1992) Effects of blockage of 5-HT₂ receptors and activation of 5-HT_{1A} receptors on the exploratory activity of rats in the elevated plus-maze. *Psychopharmacology (Berlin)*, **107**, 135–139.
- Pellow S and File SE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacology, Biochemistry and Behavior*, **24**, 525–529.
- Pellow S, Chopin P, File SE and Briley M (1985) Validation of open:closed arm entries in the elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, **14**, 149–167.
- Rodgers RJ and Cole JC (1994) The elevated plus-maze: pharmacology, methodology and ethology. In: *Ethology and Psychopharmacology* (Eds SJ Cooper and CA Hendrie), pp. 10–44. John Wiley and Sons, Chichester.
- Rodgers RJ and Johnson NJT (1995) Factor analysis of spatio-temporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacology, Biochemistry and Behavior*, **52**, 297–303.
- Scholfield CN (1992) Antagonism of γ -aminobutyric acid and muscimol by picrotoxin, bicuculline, strychnine, bemegride, leptazol, D-tubocurarine and theophylline in the isolated olfactory cortex. *Archives of Pharmacology*, **318**, 274–280.
- Treit D and Fundytus M (1988) Thigmotaxis as a test for anxiolytic activity in rats. *Pharmacology, Biochemistry and Behavior*, **31**, 958–962.
- Treit D, Menard J and Royan C (1993) Anxiogenic stimuli in the elevated plus-maze. *Pharmacology, Biochemistry and Behavior*, **44**, 463–469.
- Trullas R, Winslow TR, Insel TR and Skolnick P (1991) Are glutamatergic pathways involved in the pathophysiology of anxiety? In: *New Concepts in Anxiety* (Eds M Briley and SE File), pp. 382–394. MacMillan, London.

(Received 15 January 1997; accepted as revised
14 May 1997)