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The Influence of Open Arm Ledges and Maze Experience in the Elevated Plus-Maze

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FERNANDES, C. AND S. E. FILE. *The influence of open arm ledges and maze experience in the elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **54**(1) 31-40, 1996.—In Experiment 1, rats were tested in a plus-maze, with or without small ledges on the open arms, after injection with vehicle or chlordiazepoxide (7.5 mg/kg). They were scored either on their first or second exposure to the maze; those scored on trial 2 had received a 5-min undrugged exposure to the maze 24 h earlier. This dose of chlordiazepoxide had a significant anxiolytic effect on trial 1 only in the maze without ledges, and on trial 2 only in the maze with ledges; thus, the presence of ledges differentially affected anxiolytic sensitivity on trials 1 and 2. The results of a factor analysis study (Experiment 2) confirmed that ledges had a differential effect when rats were repeatedly exposed to the maze. Thus, in the maze without ledges, the scores reflecting anxiolytic activity on trial 1 loaded on one factor, whereas the scores from trials 2 and 3 loaded on another independent factor. In the maze with ledges, the scores reflecting anxiolytic activity on trials 1, 2, and 3 loaded on three independent factors. Considering the published evidence and the results of the present study, we suggest that both types of plus-maze may be measuring the same type of anxiety with different sensitivities on trial 1 (e.g., generalised anxiety or fear of open spaces); different types of anxiety on trial 2 (without ledges—phobia/fear of heights; with ledges—not known), and trial 3 in the maze with ledges, yet another type of anxiety. The factor analysis results are also presented for ethological measures on the plus-maze, and for activity and exploration in the holeboard. Based on the factor loadings, a composite measure of anxiety on trial 1 is presented which will increase the sensitivity of the plus-maze to anxiolytic treatments. The measures of motor activity in the plus-maze load on a different factor from those derived from the holeboard, thus cautioning against considering all measures of motor activity as interchangeable.

Anxiety Phobia Plus-maze Holeboard Motor activity Benzodiazepines Factor analysis

THE widespread use of the elevated plus-maze has led to considerable diversity in its dimensions and construction (14). Several experimenters have found that with high doses of benzodiazepines a significant proportion of animals fall off the maze. To reduce this incidence, some have introduced small ledges along the open arms (4). The purpose of Experiment 1 was to determine the effect of the presence of ledges on the response of undrugged rats and those given a test dose of chlordiazepoxide on both trials 1 and 2 of the plus-maze. Rats with one previous 5-min trial in a plus-maze without ledges lose their sensitivity to the anxiolytic effects of benzodiazepines and barbiturates (6,9,11), and factor analysis has shown that the anxiolytic measures derived from trials 1 and 2 load on independent factors (12). Treit (20) has suggested that the controlling factor for trial 1 performance is fear of open spaces, and File et al. (12) suggested that on trial 2 it is the elevation of the arms. It is, therefore, possible that the changed sensitivity to anxiolytics is because trials 1 and 2 are measuring different forms of fear/anxiety.

File et al. (11) found an anxiolytic effect of chlordiazepoxide (7.5 mg/kg) on trial 1 in a maze without ledges, but no effect on trials 2 and 3. It is, therefore, possible that in this apparatus the nature of the fear/anxiety changes from trial 1 to trial 2, but thereafter remains stable. The results of Experiment 1 suggested that the presence of ledges reduced anxiolytic sensitivity on trial 1, but increased it on trial 2. The purpose of Experiment 2 was, therefore, to examine the factor loadings from traditional and ethological measures (1,4,18) in rats tested once in the holeboard and in a plus-maze, with or without ledges around the open arms, on three successive trials.

METHOD

Animals

Male hooded Lister rats (Charles River, Margate, UK), weighing approximately 200 g, were housed in groups of five, in a room adjacent to the testing rooms, maintained at 22°C, with lights (< 50 scotopic lx) on from 0700–1900 h. Food and water were freely available. All rats were handled prior to testing.

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EXPERIMENT 1

Drugs

Chlordiazepoxide hydrochloride (CDP, 7.5 mg/kg, Sigma, UK) was dissolved in distilled water; control (Veh) rats received water injections. All injections were IP in a volume of 2 ml/kg, 30 min before testing.

Apparatus

The plus-mazes were made of wood and had two open arms (50 × 10 cm) and two enclosed arms of the same size with walls 40 cm high, elevated 50 cm above the ground. The mazes were identical except for the addition of a perspex **ledge**, 0.5 cm high, around the perimeter of the open arms of one of the mazes. A camera was mounted vertically above each maze, and the behaviour was scored from a monitor in an adjacent room. Each rat was placed in the central square (10 × 10 cm), facing an enclosed arm, and allowed to freely explore the maze for 5 min. At the end of each trial, the maze was wiped clean with a damp cloth. The times spent on the open and enclosed arms were recorded by an observer blind to the drug treatment. (Four paws into, and two paws out of, an arm defining an arm entry and exit, respectively.) In addition, the percentage time spent on the open arms [open time/(open + closed time) × 100] was calculated. An increase in the percentage of time spent on the open arms is interpreted as an anxiolytic response, whereas the number of entries into closed arms provides a measure of general activity (8,17).

Procedure

Sixty-six rats were randomly allocated to be tested in a maze with (n = 34) or without (n = 32) open arm ledges. Within these groups they were then randomly allocated to a trial 1 or trial 2 test and to vehicle or chlordiazepoxide treatment; there were, thus, n = 8–9 rats/experimental group. All of the rats scored on trial 2 had a vehicle injection 30 min before exposure to the maze for 5 min on trial 1, with an inter-trial interval of 24 h. All testing took place under quiet conditions and low light (<50 scotopic lx) in an order randomized for drug treatment, between 0800–1400 h.

Statistics

The effect of open arm ledges on drug response and plus-maze experience was assessed with a three-way analysis of variance (ANOVA), with the presence of open arm ledges as one factor, drug treatment as a second factor, and test experience as the third. This was followed by post hoc Scheffé tests for individual group comparisons; it is the significances of these tests that are shown in Fig. 1.

Results

Considering the percentage time spent on the open arms, there was a significant effect of ledges, $F(1, 58) = 5.0$, $p < 0.05$, of drug treatment, $F(1, 58) = 11.2$, $p < 0.001$, and a significant ledge × drug × trial interaction, $F(1, 58) = 4.1$, $p < 0.05$. This was because chlordiazepoxide significantly increased the percentage of time spent on the open arms on trial 1 only in the plus-maze without ledges, and on trial 2 only in the plus-maze with ledges (see Fig. 1).

The presence of ledges significantly increased the number of closed arm entries, $F(1, 58) = 8.0$, $p < 0.01$, but there was no effect of chlordiazepoxide in either trial 1 or 2 of the plus-maze (see Table 1).

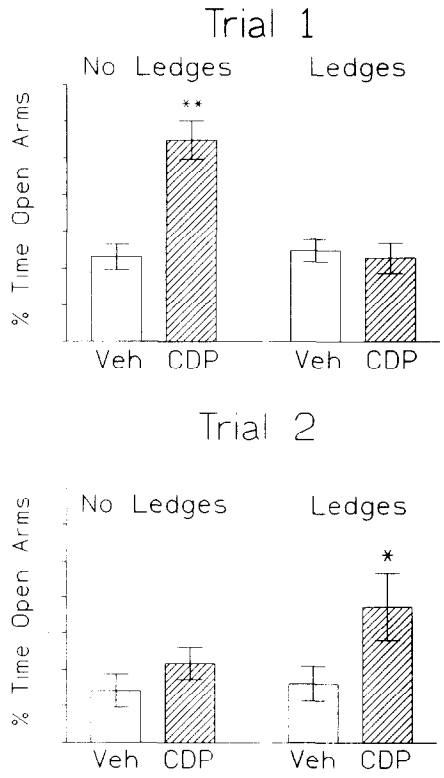

Fig. 1. Mean (±SEM) percentage of time spent on the open arms of a plus-maze, without or with ledges around the open arms, by rats on trial 1 or trial 2 in the plus-maze. Rats were injected IP with water (Veh) or chlordiazepoxide (CDP, 7.5 mg/kg), 30 min before testing. Those scored on trial 2 had received a 5-min undrugged exposure to the same maze 24 h earlier. *$p < 0.05$, **$p < 0.01$ compared with the respective control group.

EXPERIMENT 2

Apparatus

Holeboard. The holeboard was a wooden box 60 × 60 × 35 cm with four holes, each 6.5 cm in diameter, equally spaced in the floor. The number of head dips and the time spent head dipping was measured by the interruption of infrared beams from cells located immediately beneath the edges of the holes. Locomotor activity and rearing were measured by the interruption of infrared beams from cells located in the walls of the box, 4.5 and 12.5 cm, respectively, from the floor. The holeboard apparatus provides independent measures of motor activity and exploration (7,10).

The elevated plus-maze. Two plus-mazes one with and one without open arm ledges were used, as described in Experiment 1. The standard measures of the numbers of entries onto, and the times spent on, open and closed arms were recorded.

In addition to these standard spatiotemporal measures, the following "ethological" behaviours, based on those previously reported (4,18) were recorded (the closed arms and the central square were considered to be 'protected' areas of the maze, while the open arms were considered to be 'unprotected' areas): a) time spent on the central square; b) number of entries onto, and time spent on, the distal (end) part of the open arms; c) total number of rears (minimal rearing was seen in the unprotected areas so this measure was not expressed with

TABLE 1

MEAN (±SEM) NUMBER OF CLOSED-ARM ENTRIES MADE BY RATS ON TRIAL 1 OR TRIAL 2 IN A PLUS-MAZE WITHOUT OR WITH LEDGES AROUND THE OPEN ARMS

	Number of Closed-Arm Entries			
	Trial 1		Trial 2	
Drug Treatment	No Ledges	Ledges	No Ledges	Ledges
Veh	9.5 ± 0.8	10.9 ± 0.7	9.2 ± 1.2	11.7 ± 1.2
CDP	9.2 ± 1.3	11.4 ± 1.5	11.0 ± 1.0	9.7 ± 1.1

Rats were tested 30 min after an IP injection of water (Veh) or chlordiazepoxide (CDP, 7.5 mg/kg). Those scored on trial 2 had received a 5-min undrugged exposure to the same maze 24 h earlier.

regard to location); d) time spent scanning in unprotected areas of the maze (scrutinizing in any direction while on the unprotected areas, including stretch attend postures and flat-back approach behaviour but excluding head dipping); (e) numbers of protected and unprotected stretch attend postures (forward extension of head and shoulders followed by retraction to the original position); f) time spent in protected and unprotected flat-back approach behaviour (locomotor behaviour where the animal stretches to its full length and cautiously moves forward); g) number of closed arm returns (exiting a closed arm with the forepaws only and then returning/doubling back into the same closed arm); h) time spent scanning in protected areas of the maze (a collection of investigative behaviours made from protected areas, directed towards unprotected areas, of the plus-maze, which include stretch attend postures, flat-back approach behaviour, and closed arm returns); i) numbers of, and time spent in, protected and unprotected head dipping (scanning over the sides of the plus-maze); j) entry latency (time taken at the start of the trial to enter an arm); k) time spent self-grooming (face, paws, and whole body); and l) time spent immobile.

Procedure

A total of 100 rats were randomly allocated to two experimental groups (group A, no ledges; group B, ledges; 50 rats/group). On day 1 all animals were tested in the holeboard for 5 min. Immediately after the end of the holeboard test, animals were tested for 5 min in a plus-maze without (group A) or with (group B) open-arm ledges. These groups were retested in the same plus-maze to which they were initially exposed, on days 2 and 3, with an inter-trial interval of 24 h. All testing took place under quiet conditions and low light (< 50 scotopic lx), between 0800–1400 h.

Statistics

The behavioural parameters measured in each of the two plus-mazes were compared across trials 1, 2, and 3 by one-way analysis of variance (ANOVA), followed by post hoc Scheffé tests; it is the significances of these tests that are shown in Table 2.

The holeboard and plus-maze data were analysed separately for groups A and B by factor analyses using a principal component solution with an orthogonal rotation (varimax) of the factor matrix, which ensures that the extracted factors are independent of one another and should, therefore, reflect separate processes. The number of factors extracted for each analysis was selected using a combination of two criteria, the 75% variance rule (the relevant matrix variance is accounted for when the sum of the proportionate contributions of the eigenvalues exceeds 0.75) and the root curve analysis (the point of inflection of a plot of the eigenvalues from largest to smallest). In the tables, the factors for eigenvalues ≥1 are presented left to right in an order that corresponds to the decreasing size of the proportion of the original variance represented by each factor. The contribution of each behavioural variable to each factor is referred to as a factor loading. The higher the loading, the better the variable reflects a particular factor and, therefore, only factor loadings of >0.5 are reported in this study.

Results

The mean (±SEM) scores for the behavioural parameters measured in the plus-maze are presented in Table 2. The standard behavioural measures from the plus-maze show a typical spatial distribution with preference for the protected (closed arms and central square) compared with the unprotected (open arms) areas of the plus-maze. Although this pattern of distribution of behaviours is fairly constant with re-exposure to the maze, the extent of activity in the open and closed arms of the maze changes. There is a decrease in open arm activity with a corresponding increase in closed arm activity between trials 1 and 2, in both types of maze. The activity in the unprotected areas of the maze without ledges is not significantly reduced between trials 2 and 3, but in the maze with open arm ledges, there is a general increase in the time, and the percentage of time, spent in the open arms on trial 3 compared with trial 2. Time spent in the central square is reduced across trials in the maze without ledges, but there is a trend for this measure to increase on re-exposure to the maze with ledges. The number of closed and total arm entries remain fairly stable between trials 1 and 2 and then decrease between trials 2 and 3, in both mazes.

A similar distribution of behaviour, with preference for protected areas, was observed in the additional behaviours measured in the plus-maze (stretch attend postures, flat-back approach and scanning behaviour, head dipping) (see Table 2).

The subsequent factor analyses were performed on the data presented in Table 2, with the exception of the following variables: number of protected and unprotected stretch attend postures, number of closed-arm returns, entry latency to an arm, time spent grooming, and immobile. These behavioural parameters were excluded as they were of low incidence and/or had a skewed distribution, deviating from normality.

TABLE 2

MEAN (±SEM) BEHAVIOURAL PARAMETERS MEASURED IN THE ELEVATED PLUS-MAZES (TRIALS 1, 2, AND 3)

	No Ledges			Ledges		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
No. open arm entries	2.9 ± 0.3	1.8 ± 0.3*	1.4 ± 0.3*	4.4 ± 0.2	3.2 ± 0.3*	4.1 ± 0.5
Time in open arms (s)	29.4 ± 2.7	17.2 ± 3.4*	11.8 ± 3.4*	45.7 ± 3.0	31.2 ± 3.8*	46.0 ± 6.3†
No. closed arm entries	11.4 ± 0.4	10.6 ± 0.6	8.3 ± 0.6*†	11.5 ± 0.3	11.7 ± 0.5	9.3 ± 0.6*†
Time in closed arms (s)	183.4 ± 4.7	208.9 ± 6.3*	222.8 ± 8.3*	175.4 ± 4.4	181.3 ± 5.2	168.7 ± 8.9
Total No. arm entries	14.3 ± 0.6	12.4 ± 0.7	9.7 ± 0.8*†	15.8 ± 0.5	14.9 ± 0.6	13.4 ± 0.9*
% No. open arm entries	19.8 ± 1.4	12.2 ± 2.0*	9.5 ± 2.0*	26.7 ± 1.1	19.9 ± 1.7*	25.2 ± 2.7
% Time in open arms	14.2 ± 1.4	8.3 ± 1.7*	6.4 ± 1.8*	20.9 ± 1.4	14.9 ± 1.8	22.7 ± 3.1†
Time in central square (s)	87.2 ± 3.4	73.9 ± 4.0	65.4 ± 6.3*	78.9 ± 3.0	87.5 ± 3.6	85.4 ± 4.8
No. entries to distal	2.3 ± 0.2	1.2 ± 0.3*	0.7 ± 0.2*	3.5 ± 0.2	2.2 ± 0.3*	2.8 ± 0.4
Time in distal (s)	16.6 ± 1.8	6.8 ± 1.6*	4.3 ± 1.6*	24.6 ± 2.1	14.0 ± 2.3*	18.0 ± 2.8
Total No. of rears	12.7 ± 0.8	15.2 ± 0.9*	14.1 ± 0.9	15.8 ± 0.6	17.4 ± 0.8	14.8 ± 0.8†
Time in unprotected scan (s)	13.7 ± 1.4	7.2 ± 1.4*	5.8 ± 1.6*	21.8 ± 1.6	16.2 ± 1.9	20.6 ± 2.7
No. protected stretch attend posture	1.4 ± 0.1	1.7 ± 0.2	0.8 ± 0.1*†	1.3 ± 0.2	1.8 ± 0.2*	0.4 ± 0.1*†
No. unprotected stretch attend posture	0.2 ± 0.1	0.4 ± 0.1*	0.2 ± 0.0†	0.6 ± 0.1	0.9 ± 0.1	0.2 ± 0.1*†
Time protected flat-back approach (s)	8.4 ± 0.8	3.9 ± 0.5*	5.5 ± 1.0	4.7 ± 0.5	4.8 ± 0.5	3.8 ± 0.6
Time unprotected flat-back approach (s)	1.2 ± 0.3	0.8 ± 0.3	0.6 ± 0.3	2.0 ± 0.3	2.1 ± 0.3	1.3 ± 0.3
No. closed arm returns	0.7 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
Time in protected scan (s)	47.8 ± 2.1	35.3 ± 2.1*	36.5 ± 3.4*	41.4 ± 1.5	49.2 ± 1.9*	33.2 ± 2.0*†
No. protected head dips	5.8 ± 0.4	3.4 ± 0.4*	1.9 ± 0.3*†	3.5 ± 0.3	2.4 ± 0.3*	3.2 ± 0.3
Time in protected head dipping (s)	10.4 ± 1.2	6.1 ± 0.9*	2.9 ± 0.5*†	4.0 ± 0.4	2.7 ± 0.3	5.3 ± 0.6†
No. unprotected head dips	2.7 ± 0.4	2.0 ± 0.4	1.4 ± 0.4*	4.3 ± 0.4	2.4 ± 0.4*	4.9 ± 0.7†
Time in unprotected head dipping (s)	3.0 ± 0.5	3.1 ± 0.8	1.9 ± 0.7	3.2 ± 0.4	2.4 ± 0.6	6.5 ± 0.9*†
Entry latency (s)	0.6 ± 0.3	0.0 ± 0.0	0.9 ± 0.5†	1.8 ± 0.3	1.6 ± 0.4	4.0 ± 0.9*†
Time grooming (s)	1.1 ± 0.2	0.8 ± 0.1	1.6 ± 0.8	1.9 ± 0.5	1.8 ± 0.4	1.1 ± 0.3
Time immobile (s)	9.2 ± 2.2	11.0 ± 2.7	15.2 ± 3.1	2.9 ± 0.5	5.6 ± 0.9	8.6 ± 1.3*

*$p < 0.05$ compared with trial 1 data; †$p < 0.05$ compared with trial 2 data.

A) Factor analysis on the measures of behaviour in the holeboard. In both groups A and B, two clear independent factors emerged from a factor analysis of the holeboard data: factor 1 reflecting motor activity, and factor 2 reflecting exploration (see Table 3).

B) Factor analysis on the standard measures of behaviour in the plus-maze. As can be seen from Table 4, in both mazes two independent factors were extracted from the analysis of the standard measures. Factor 1, on which open arm activity loaded highly, was considered to be an index of anxiety. The

TABLE 3

ORTHOGONAL FACTOR LOADINGS FOR MEASURES OF BEHAVIOUR IN THE HOLEBOARD (ACCOUNTING FOR 77% OF THE TOTAL VARIANCE)

	Group A		Group B	
	Motor Activity	Exploration	Motor Activity	Exploration
No. of head dips		0.83		0.92
Time spent head dipping		0.86		0.88
Locomotor activity	0.87		0.80	
No. of rears	0.90		0.84	

TABLE 4

ORTHOGONAL FACTOR LOADINGS FOR TRADITIONAL MEASURES OF BEHAVIOUR IN PLUS-MAZES, WITHOUT OR WITH OPEN ARM LEDGES (ACCOUNTING FOR 88% AND 83% OF THE TOTAL VARIANCE, RESPECTIVELY)

	No Ledges		Ledges	
	Anxiety	Motor Activity	Anxiety	Motor Activity
No. open arm entries	0.92		0.94	
Time spent in open arms	0.95		0.94	
No. closed arm entries		0.93		0.66
Time spent in closed arms	−0.69	−0.63	−0.59	−0.74
Total No. arm entries		0.88	0.72	0.54
% No. open arm entries	0.96		0.91	
% Time spent in open arms	0.95		0.89	
Time spent in central square		0.76		0.91

number of entries into the closed arms, the total number of arm entries, and the time spent in the central square all contributed to factor 2. Factor 2, thus, appears to be reflecting both motor activity and/or exploration of the protected areas of the maze. The total number of arm entries also loaded on factor 1 (factor loading 0.31 in the plus-maze without ledges and 0.72 in the maze with ledges), indicating that this measure does not provide an independent measure of general activity in the maze.

As can be seen in Table 4, the number of closed arm entries is a better measure of activity than total number of arm entries, although the loading of the number of closed arm entries was much reduced in the maze with ledges. This suggests that the addition of open arm ledges reduces the value of this measure in assessing general activity in this type of maze. Factor analysis on both the behaviours measured in the plus-maze and the holeboard revealed four independent factors, separately reflecting anxiety in the plus-maze, activity in the plus-maze, activity in the holeboard, and exploration in the holeboard (see Table 7).

C) Factor analysis on the standard and ethological measures of behaviour in the plus-maze. In order to include the ethological measures it was necessary to exclude some of the standard ones and, thus, the percentage scores, the total number of arm entries, and the time spent in the closed arms were excluded from the subsequent analyses. The addition of ethological measures to the factor analysis of the plus-maze resulted in the emergence of four independent factors in the maze without ledges and five factors in the maze with ledges (see Table 5).

In both mazes factor 1 appeared to be reflecting anxiety, with the number of entries into, and time spent in, the open arms loading on this factor. Activity in the distal part of the open arms, time spent in unprotected scanning and unprotected head dipping (in the plus-maze without open arm ledges) also loaded on factor 1, suggesting that these additional measures may be used to assess anxiety in this version of the plus-maze.

The method of Bond and Lader (3) can be used to extract a composite measure of anxiety based on the factor loadings. Thus, based on the time scores, the measure for the maze without ledges would be: Σ (time open $\times$ 0.98) + (time distal $\times$ 0.95) + (time unprotected head dipping $\times$ 0.80) + (time unprotected scanning $\times$ 0.92).

In the maze without ledges, two independent factors (factors 2 and 4) emerged that appeared to relate to activity and/or exploration in the central square. Protected head dipping and the time spent in the central square loaded on factor 2 and the number of closed arm entries, time spent in protected scanning and flat-back approach, and the time spent in the central square all loaded on factor 4. It is possible that factors 2 and 4 reflect some type of decision-making or assessment of height and openness, respectively, from the central square. Factor 3 appeared to be a measure of motor activity, with contributions from total number of rears, time spent in unprotected flat-back approach, and a weak loading (factor loading 0.46) of number of closed arm entries.

The addition of open arm ledges to the plus-maze resulted in a shift in the pattern of factor loadings regarding unprotected head dipping from the anxiety factor to a separate factor (factor 3). The presence of open arm ledges may be reducing the level of anxiety/fear in the open arms so that unprotected head dipping no longer reflects anxiety but reflects exploration or assessment of the height of the maze. Two 'central square' factors were again identified in the maze with ledges, with factor 2 reflecting decision making/assessment of openness and factor 4, with a weak contribution from time spent in the central square (factor loading 0.40), reflecting decision-making/assessment of height. The number of closed arm entries and the total number of rears both contributed to factor 5, indicating that this factor measures motor activity.

D) Factor analysis on the measures of behaviour in trials 1, 2, and 3 of the plus-maze. Four factors emerged from the factor analysis on trials 1, 2, and 3 in the plus-maze without ledges (see Table 6). Factor 1 related to anxiety measured in both trials 2 and 3, and factor 2 reflected anxiety measured in trial 1. Factors 3 and 4 appeared to correspond to activity

TABLE 5

ORTHOGONAL FACTOR LOADINGS FOR TRADITIONAL AND ETHOLOGICAL MEASURES OF BEHAVIOUR IN PLUS-MAZES, WITHOUT OR WITH OPEN ARM LEDGES (ACCOUNTING FOR 79% AND 84% OF THE TOTAL VARIANCE, RESPECTIVELY)

	No Ledges				Ledges				
	Anxiety Factor 1	Decision (Height) Factor 2	Activity Factor 3	Decision (Open) Factor 4	Anxiety Factor 1	Decision (Open) Factor 2	Height Assessment Factor 3	Decision (Height) Factor 4	Activity Factor 5
No. open arm entries	0.94				0.87				
Time spent in open arms	0.98				0.95				
No. closed arm entries				0.70					0.65
Time spent in distal part of open arms	0.95				0.94				
No. entries to distal part of open arms	0.93				0.92				
Time spent in central square		0.65		0.58		0.73			
No. protected head dips		0.87						0.92	
Time spent protected head dipping		0.94						0.96	
No. unprotected head dips	0.86						0.78		
Time spent unprotected head dipping	0.80						0.92		
Time spent protected scanning				0.72		0.93			
Time spent unprotected scanning	0.92				0.96				
Total No. rears			0.88						0.80
Time protected flat-back approach				0.76		0.80			
Time unprotected flat-back approach			−0.58						

TABLE 6

ORTHOGONAL FACTOR LOADINGS FOR MEASURES OF BEHAVIOUR IN TRIALS 1, 2, AND 3 IN PLUS-MAZES, WITHOUT OR WITH OPEN ARM LEDGES (ACCOUNTING FOR 78% AND 77% OF THE TOTAL VARIANCE, RESPECTIVELY)

	No Ledges				Ledges				
	Anxiety Trials 2 and 3 Factor 1	Anxiety Trial 1 Factor 2	Activity Trials 2 and 3 Factor 3	Activity Trial 1 Factor 4	Anxiety Trial 2 Factor 1	Anxiety Trial 3 Factor 2	Anxiety Trial 1 Factor 3	Central Sq. Trials 2 and 3 Factor 4	Activity Trials 2 and 3 Factor 5
Plus-maze trial 1									
No. open arm entries		0.86					0.70		
Time spent open arms		0.95					0.67		
No. closed arm entries				0.82				−0.57	
Time spent in central square				0.80		0.69			
No. unprotected head dips		0.94					0.89		
Time spent unprotected head dipping		0.89					0.77		
Plus-maze trial 2									
No. open arm entries	0.81				0.75				
Time spent open arms	0.84				0.90				
No. closed arm entries			0.83						0.84
Time spent in central square			0.67					0.68	
No. unprotected head dips	0.88				0.90				
Time spent unprotected head dipping	0.80				0.86				
Plus-maze trial 3									
No. open arm entries	0.83					0.88			
Time spent open arms	0.87					0.91			
No. closed arm entries			0.74						0.60
Time spent in central square			0.55					0.68	
No. unprotected head dips	0.89					0.91			
Time spent unprotected head dipping	0.88					0.86			

in the protected areas of the maze, with the number of closed arm entries and time spent in the central square measured in trial 1 contributing to factor 4 and these same parameters measured in trials 2 and 3 loading together on factor 3.

In the maze with ledges, five independent factors were extracted in the factor analysis on repeated testing in the plus-maze, of which three factors (factors 3, 1, and 2) separately reflected anxiety measured in trials 1, 2, and 3, respectively (see Table 6). Time spent in the central square on trial 1 also loaded on factor 2, which related to the anxiety measured on trial 3. In contrast to the previous analysis of trial 1 alone (see Table 5), the measures of unprotected head-dipping now loaded on the anxiety factors for each trial. The two other factors (factors 4 and 5) appear to be reflecting central square and general activity, respectively.

DISCUSSION

Factor analyses using both the standard measures and the additional ethological measures revealed independent factors that related to **anxiety**, **activity**, and **assessment** behaviours in both plus-mazes. The results of these analyses generally agree with previous studies (4,7–9,12,13,16,18), in which the parameters measured in the plus-maze have been grouped into behaviours thought to reflect anxiety (4,7–9,12,13,16,18), activity (4,7–9,12,13,16,18), central square activity/exploration (4,12,18), risk assessment (4,13,18), and displacement (4). However, the considerable variation in the experimental procedures (e.g., species, strain, maze construction, handling, behaviours scored) between the various studies preclude detailed comparisons.

The strongest factor to emerge from analysis of the behaviour in the plus-maze on trial 1 was an anxiety factor, particularly in the maze without open arm ledges. A composite measure based on the anxiety factor, analogous to the human anxiety factor extracted from mood rating scales (3), was derived. This composite measure will prove useful in studies using rats tested on trial 1 in a plus-maze without ledges, enhancing the sensitivity of the plus-maze in detecting changes in anxiety, albeit at the cost of extensive behavioural scoring and analysis. A similar composite measure could be derived for trial 1 in a plus-maze with ledges from the factor loadings presented in Table 5. Although our studies have shown the importance of open arm ledges for rats tested in the plus-maze, this may not be applicable to mice. There are some differences in behaviour between mice and rats tested in the plus-maze, with mice showing higher levels of risk assessment behaviours than rats (18). Similar behavioural differences between mice and rats in response to exposure to cat have also been found (2).

Incorporation of the behaviours measured in the holeboard, which provides independent measures of exploration and motor activity [(7,8); this study], into the factor analysis on behaviour in both plus-mazes revealed independent factors, separately reflecting anxiety in the plus-maze, activity in the plus-maze, activity in the holeboard, and exploration in the holeboard (see Table 7). This suggests that the nature of the activity measured in the holeboard is different from that measured in the plus-maze, and that these behaviours are not interchangeable. Previous analysis of behaviour in the holeboard and the plus-maze (7,8,16) had indicated that motor activity measured in the plus-maze may be related to the activity measured in the holeboard. However, the total number of, and not the number of, closed arm entries were used in these analyses, a measure that does not provide an independent assessment of motor activity, as this behaviour loads on both the factors reflecting anxiety and activity [(4,7,8,18); this study].

The influence of the central square on the behaviours detected in the plus-maze is largely unknown, although it has been suggested that the exploratory behaviours seen in this area of the maze may relate to some kind of assessment and/or decision-making process (4,18,19,21). Two factors relating to central square activity were identified in the factor analysis performed in this study, and these factors were considered to separately reflect assessment and/or decision-making related to the openness and to the height of the maze viewed from the protected areas of the maze, on trial 1. Previous factor analyses have also identified factors related to central square activity (4,12,18), and this area may, thus, play an integral role in determining the avoidance of the open arms of the plus-maze.

One important inter-laboratory variation in plus-maze methodology is the presence of open arm ledges (14). Ledges have been added to the open arms to both encourage open arm exploration (18) and to prevent animals falling off the maze following drug administration (4). However, the results of the present study have shown that the inclusion of ledges on the open arms is not a trivial alteration in plus-maze construction. Comparison of the factor analyses on the behaviours measured in the plus-mazes without, and with, ledges found clear distinctions between the two mazes. The presence of ledges not only reduced the value of the number of closed arm entries as a measure of activity in the maze, again stressing the need for caution when interpreting activity in this test, but on trial 1 also shifted the loading of unprotected head-dipping from the factor reflecting anxiety to a separate factor. It is possible that there is a reduction in the nature/extent of the anxiety/fear presented by the open arms with ledges, and as a consequence, head-dipping behaviour no longer relates to anxiety but to a directed exploratory behaviour assessing the height of the maze.

A differential sensitivity to the anxiolytic effects of chlordiazepoxide in the plus-maze was found following the addition of ledges to the open arms of a plus-maze. On trial 1, the overall aversion of the open arms may be reduced by the presence of ledges, thereby shifting the sensitivity to anxiolytic drug effects, resulting in the lack of response to chlordiazepoxide in the plus-maze with ledges compared to the maze without ledges. Similarly, a shift in the sensitivity to the anxiolytic and anxiogenic effects of various benzodiazepine agonists and inverse agonists, with a reduction in baseline levels of behaviour, following the inclusion of open-arm ledges to a plus-maze has been found previously (15). However, there were no differences in the baseline level of behaviours detected in the plus-maze with ledges compared to the maze without ledges in Experiment 1, and as only one dose of chlordiazepoxide was tested, the effect of open arm ledges on the sensitivity of trial 1 to anxiolytic responses is not conclusive.

Following re-exposure to the plus-maze, chlordiazepoxide does not produce an anxiolytic effect in the maze without ledges, as found previously (6). It has been proposed that the nature of the anxiety measured in trial 2 of the plus-maze differs from that measured in trial 1 (12), and the anxiety detected on trial 2 is not sensitive to manipulation by benzodiazepine agonists. However, on trial 2 in the plus-maze with ledges, an anxiolytic response to chlordiazepoxide was seen on re-testing in the maze. This reversed pattern in sensitivities seen between the two mazes suggests that there is also a difference in the nature and/or sensitivity of the type of anxiety detected on trial 2 in a maze with, compared with a maze

TABLE 7

ORTHOGONAL FACTOR LOADINGS FOR TRADITIONAL MEASURES OF BEHAVIOUR IN PLUS-MAZES, WITHOUT OR WITH OPEN ARM LEDGES, AND IN THE HOLEBOARD (ACCOUNTING FOR 86% AND 84% OF THE TOTAL VARIANCE, RESPECTIVELY)

	No Ledges				Ledges			
	Anxiety Factor 1	Activity Plus-Maze Factor 2	Exploration Holeboard Factor 3	Activity Holeboard Factor 4	Anxiety Plus-Maze Factor 1	Exploration Holeboard Factor 2	Activity Holeboard Factor 3	Activity Plus-Maze Factor 4
No. open arm entries	0.93				0.97			
Time spent in open arms	0.95				0.96			
No. closed arm entries		0.90						0.61
Time spent in closed arms	−0.67	−0.64			−0.74			−0.54
Total No. arm entries		0.85			0.82			
% No. open arm entries	0.94				0.90			
% Time spent in open arms	0.94				0.94			
Time spent in central square		0.79						0.83
No. of head dips			0.80			0.88		
Time spent head dipping			0.83			0.87		
Locomotor Activity				0.85			0.79	
No. of rears				0.90			0.81	

without, open arm ledges. Although it is openness, rather than height, which is probably the most important determinant of the anxiety seen in trial 1 of the plus-maze (20), it has been suggested that the anxiety state measured in trial 2 reflects an acquired phobia/fear of heights learned on trial 1 of the plus-maze (9,12). Therefore, the addition of open arm ledges could have important ramifications for subsequent trials in the plus-maze if the open arm ledges alter the perception and/or experience of the anxiety on trial 1.

Subsequent factor analysis on the behavioural measures in trials 1, 2, and 3 of the plus-maze provides further evidence for the effect of ledges on the nature of the anxiety detected on re-exposure to the maze. In the maze without ledges, the parameters reflecting anxiety measured in trials 1 and 2 loaded on independent factors, whereas the anxiety measured in trial 3 of the plus-maze loaded on the same factor as the anxiety scores from trial 2. The emergence of only two factors relating to anxiety thus indicate that the type of anxiety detected on trial 2 is qualitatively different from that measured in trial 1, as found in previous studies (9,12), but that the type of anxiety detected in trial 2 remains unchanged for at least another trial in the maze without ledges. File et al. (11) found that although there were marked differences in the effect of chlordiazepoxide between trials 1 and 2 in rats tested in a plus-maze without ledges, trials 2 and 3 appeared similar with respect to the lack of effect of chlordiazepoxide.

The addition of ledges to the open arms of the maze resulted in the emergence of three independent factors reflecting anxiety, one factor for each of the group of anxiety parameters measured on trials 1, 2, and 3. In this maze, there is, therefore, a clear separation between the three trials, indicating that the type of the anxiety detected in the maze alters between each trial, for at least the first three exposures. This provides further evidence that the presence of ledges not only has a significant effect on the pharmacological sensitivity of the plus-maze, but may also influence the very nature of the anxiety detected on re-exposure to the plus-maze.

The measures of activity showed a similar separation into two independent factors, across trials, in the maze without ledges, with a factor relating to activity in trial 1 and another factor reflecting activity in trials 2 and 3. There was no clear activity factor for trial 1, in the maze with ledges, but activity measured in trials 2 and 3 in this maze loaded together on one factor. It is possible that a qualitative change in the nature of the activity measured in the plus-maze occurs following re-exposure to the maze. Although the number of closed arm entries only decreased in trial 3 in this study, a decrease in the extent of activity measured by an automated tracking system between trials 1 and 2 in the plus-maze has been reported (5). Whether this reduced activity is a result of habituation to the exploratory behaviour or due to some qualitative change in the general activity in the maze is uncertain.

This study has revealed the considerable complexity of the plus-maze and the important contribution of both the design of the apparatus (e.g., ledges) and the experience of the rat. In addition, it has also allowed us to derive a more sensitive measure of anxiolytic/anxiogenic activity in the plus-maze.

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REFERENCES

1. Blanchard, R. J.; Blanchard, D. C. Anti-predator defensive behaviors in a visible burrow system. J. Comp. Psychol. 103:70–82; 1989.
2. Blanchard, R. J.; Parmigiani, S.; Bjornson, C.; Masuda, C.; Weiss, S. M.; Blanchard, D. C. Anti-predator behavior of Swiss-Webster mice in a visible burrow system. Aggress. Behav. 21:123–136; 1995.
3. Bond, A.; Lader, M. H. The use of analogue scales in rating subjective feelings. Br. J. Med. Psychol. 47:211–218; 1974.
4. Cruz, A. P. M.; Frei, F.; Graeff, F. G. Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol. Biochem. Behav. 49:171–176; 1994.
5. Dawson, G. R.; Crawford, S. P.; Stanhope, K. J.; Iversen, S. D.; Tricklebank, M. D. One-trial tolerance to the effects of chlordiazepoxide on the elevated plus maze may be due to locomotor habituation, not repeated drug exposure. Psychopharmacology (Berlin) 113:570–572; 1994.
6. File, S. E. One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. Psychopharmacology (Berlin) 100: 281–282; 1990.
7. File, S. E. The biological basis of anxiety. In: Meltzer, H. Y.; Nerozzi, D., eds. Current practices and future developments in the pharmacotherapy of mental disorders. New York: Elsevier Science; 1991:159–165.
8. File, S. E. Behavioural detection of anxiolytic action. In: Elliott, J. M.; Heal, D. J.; Marsden, C. A., eds. Experimental approaches to anxiety and depression. London: John Wiley & Sons Ltd.; 1992:25–44.
9. File, S. E. The interplay of learning and anxiety in the elevated plus-maze. Behav. Brain Res. 58:199–202; 1993.
10. File, S. E.; Wardill, A. G. The reliability of the holeboard apparatus. Psychopharmacologia 44:47–51; 1975.
11. File, S. E.; Mabbutt, P. S.; Hitchcott, P. Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology (Berlin) 102:98–101; 1990.
12. File, S. E.; Zangrossi, H., Jr.; Viana, M.; Graeff, F. G. Trial 2 in the elevated plus-maze: A different form of fear? Psychopharmacology (Berlin) 111:491–494; 1993.
13. Handley, S. L.; Spooner, H. A.; McCreary, A. C.; McBlane, J. W. Factor analysis of behaviour in the elevated x maze. J. Psychopharmacol. 9:A32; 1995.
14. Hogg, S. Validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol. Biochem. Behav. 54(1):21–30; 1996.
15. Jones, G. H.; Cole, B. J. Are drug effects in the plus-maze dependent on the baseline level of fear? Behav. Pharmacol. 5:87; 1994.
16. Lister, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berlin) 92:180–185; 1987.
17. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open : closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
18. Rodgers, R. J.; Johnson, N. J. T. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. Pharmacol. Biochem. Behav. 52:297–303; 1995.
19. Rodgers, R. J.; Lee, C.; Shepherd, J. K. Effects of diazepam on behavioural and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. Psychopharmacology (Berlin) 106:102–110; 1992.
20. Treit, D.; Menard, J.; Royan, C. Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44:463–469; 1993.
21. Trullas, R.; Winslow, T. R.; Insel, T. R.; Skolnick, P. Are glutamatergic pathways involved in the pathophysiology of anxiety? In: Briley, M.; File, S. E., eds. New concepts in anxiety. New York: Macmillan; 1991:382–394.