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Factor Analysis of Spatiotemporal and Ethological Measures in the Murine Elevated Plus-Maze Test of Anxiety

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RODGERS, R. J. AND N. J. T. JOHNSON. *Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety.* PHARMACOL BIOCHEM BEHAV **52**(2) 297–303, 1995.—Recent research employing the elevated plus-maze to assess anxiety in rodents has incorporated a variety of behavioral elements in addition to the standard parameters of entries onto and time spent in the aversive open arms. In the present study, we have used a large database comprising the behavioral profiles of 90 undrugged mice to examine the relationship between the standard spatiotemporal measures and a range of specific behaviors related to the defensive repertoire of the mouse. A factor analysis applied to the standard measures revealed two factors related to anxiety and locomotor activity. The simple addition of center time (an infrequently recorded measure) to the analysis yielded a third factor, most probably related to decision making. A large-scale factor analysis applied to all measures further confirmed the existence of factors related to anxiety, locomotor activity, and decision making, and revealed three further factors thought to represent risk assessment, vertical activity, and exploratory behavior. Thus, the inclusion of ethological measures not only confirmed prior knowledge based on a very limited range of measures, but also demonstrated the existence of additional behavioral dimensions. The potential applications of this knowledge are discussed.

Elevated plus-maze Anxiety Factor analysis Structure of behavior Mice

THE ELEVATED plus-maze paradigm is currently one of the most widely used animal models in behavioral pharmacology (13,15,25). The test is based on the aversion of rodents for open spaces (31), has been validated for rats (20) and mice (18), and is reported to be bidirectionally sensitive to manipulations designed to influence anxiety [for recent reviews, see (15,25)]. The primary indices of anxiety in this model are spatiotemporal measures related to the frequency of visits to the open arms (relative to total arm entries) and the amount of time spent in these aversive areas of the apparatus (relative to session duration). However, as with any animal model, the utility of the plus-maze is dependent upon rate-dependency factors, with low anxiety baselines less than optimal for detecting antianxiety effects and high baselines virtually useless for detecting anxiety enhancement (23).

Although such problems can be partially surmounted by the selection of appropriate genetic strains and optimal test conditions [e.g., (15,25)], a number of authors [e.g., (11, 19,22)] have argued that test sensitivity, reliability, and ecological validity may be improved by focussing upon what the animals actually do in the maze, as well as their physical location. In this context, and stemming from the elegant work of the Blanchards on antipredator defense in rodents [e.g., (5)], several research groups have begun to routinely score aspects of defensive behavior (e.g., risk assessment) in the maze as well as the more usual spatiotemporal measures [e.g., (3, 26,30)]. It, therefore, becomes important to consider the relationship(s) between the various measures now recorded in this very popular animal model of anxiety. One way to approach this particular issue is through the application of factor analytical tools.

Factor analysis has been used in plus-maze research for two principal reasons: a) to identify the relationship between specific test indices and factors/dimensions such as anxiety and locomotor activity, and b) to assess whether different animal models are measuring the same type of anxiety. In the first plus-maze factor analysis to be published, Lister (18) found that the standard test measures (in mice) could be ac-

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commodated by two factors thought to represent anxiety (% open entries, % open time) and activity (total arm entries). Virtually identical results were subsequently obtained in hooded Lister rats using the same parameters (13). The latter author also found that the addition of the absolute values for open/closed/total arm entries and for time spent in open/ closed arms simply confirmed the two-factor structure of behavior in the maze. Both File (13) and Belzung et al. (4) have reported that different measures of anxiety (e.g., as recorded in the plus-maze, social interaction, Vogel conflict, light/dark exploration, hole-board, free-exploration, and neophobia tests) correlate very poorly with one another. Indeed, they can actually yield separate anxiety factors [e.g., plus-maze anxiety, social interaction anxiety, Vogel anxiety; (13)], thereby confirming the growing view that the inherent inconsistency in the behavioral pharmacology of anxiety arises at least in part from the fact that different tests are tapping into different facets of anxiety. More recently, File et al. (14) have employed factor analysis to confirm earlier suggestions (28) that the anxiety produced upon reexposure to the maze is qualitatively different to that produced on initial exposure: thus, trial 1 and trial 2 standard indices of anxiety were found to load on entirely separate factors. Although other interpretations are possible (10), the implied qualitative difference in trial 2 anxiety may explain the relative benzodiazepine insensitivity of maze-experienced rodents, i.e., one-trial tolerance (12,14,28).

In the present study, we have used factor analytic techniques (principal components analysis) to identify the structure of behavior in an ethological version of the murine elevated plus-maze test. Subjects were tested under conditions identical to those routinely employed in our pharmacological studies [e.g., (6–8,26,27,29)]. The results both confirm and extend earlier observations by delineating the relationship between the standard spatiotemporal and newer ethological measures, and by identifying additional dimensions of plus-maze behavior not previously recognized.

METHOD

Subjects

Subjects were 90 male DBA/2 mice (Biomedical Services, University of Leeds), aged 12–15 weeks at the time of testing. They were housed in groups of 10 per cage (45 × 28 × 13 cm) in a temperature-controlled environment (21 ± 1°C) in which a 12 h reversed light cycle was in operation (lights off: 0700 h). Food and water were freely available with the exception of the brief test periods. All mice were experimentally naive.

Apparatus and Procedure

The elevated plus-maze was a modification of that developed and validated for NIH Swiss mice by Lister (18). Two open arms (30 × 5 × 0.25 cm) and two enclosed arms (30 × 5 × 15 cm) extended from a common central platform (5 × 5 cm), and the entire apparatus was elevated by a single central support to a height of 60 cm above floor level. The floor of the maze was constructed of black Plexiglas while the side and end walls of the enclosed arms were made from clear Plexiglas. Exploration of the open arms was encouraged by the inclusion of a slight edge (0.25 cm) around their perimeter, and by testing under dim red light (4 × 60 W indirect).

All testing was conducted during the midportion of the dark phase of the LD cycle. To facilitate adaptation, mice were transported to the test laboratory at least 1 h prior to testing. Thirty minutes before testing, each mouse received an intraperitoneal injection (10 ml/kg) of drug vehicle (saline, saline/Tween 80 or 0.5% methyl cellulose). Testing commenced with the placement of a subject on the central platform of the maze facing an open arm. A standard 5-min test duration was employed, and the maze was thoroughly cleaned between subjects using damp and dry cloths. Behavior was recorded by videocamera and relayed to a monitor and VCR in an adjacent laboratory. To enhance the discriminability of certain behaviors (e.g., grooming and sniffing), the camera was postitioned above the maze and at an angle of approximately 50°.

Videotapes were subsequently scored by a highly trained observer using ethological analysis software (Hindsight) developed by our colleague, Scott Weiss. Using separate location and behavior keys, this software permits the real-time scoring of videotapes (of any behavioral test) by direct keyboard entry to a PC. The datafiles thus created are compatible with a variety of powerful statistical packages, including that used in the present study (Statistica; StatSoft Inc., Tulsa). Parameters scored from videotape were the standard spatiotemporal measures and a range of specific behaviors related to the defensive repertoire of the mouse (6–8,23–29).

Standard measures comprised 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 open and closed sections of the maze. These data were used to additionally calculate % open entries (i.e., open entries/total entries × 100) and % time spent in open and closed arms (e.g., open time/300 × 100). To these standard parameters, the following items were added: % time spent on the central platform (midtime/300 × 100); rearing frequency, and rearing duration (all rearing occurred against the walls of the enclosed arms); the frequency of discrete behaviors such as head dipping (exploratory movement of head/shoulders over sides of the maze), stretched attend postures (SAP; an exploratory posture in which the mouse stretches forward and retracts to original position without locomoting forward), and closed arm returns (exiting a closed arm with only the forepaws, and returning/doubling back into the same arm); and the duration (s) of prolonged behaviors such as sniffing (olfactory exploration of maze floor and walls, and occasional air sampling), grooming (species-typical sequence beginning with snout, progressing to ears, and ending with whole body groom), and flat back approach behavior (exploratory locomotion where the animal stretches to its full length and cautiously moves forward).

In view of the importance of thigmotactic cues provided by the walls of the maze (31), head dipping, stretched attend, sniffing, and flat back approach were further differentiated as a function of whereabouts on the maze they were displayed. In accord with previous reports [e.g., (6)], the closed arms and central platform were together designated protected areas of the maze (i.e., offering relative security), while the open arms were designated unprotected areas. Thus, the above behavioral items are given both as percent protected scores (i.e., %p; protected/total × 100) as well as behavior totals.

Statistics

Data were analyzed by factor analysis using a principal components solution with orthogonal rotation (varimax) of the factor matrix. This method ensures that the extracted factors are independent of one other. Factor pattern matrices were identified using a combination of the Kaiser criterion (factors must have eigenvalues ≥ 1) and the Cattell Scree test (on a simple line plot, the point at which the smooth decrease in eigenvalues levels off to the right) (17). The factor loading of each behavioral item indicates how well that item correlates with the factor; thus, a loading of ± 1.0 indicates a perfect (positive/negative) correlation, whereas a loading of less than 0.4 would suggest that the item is rather weakly linked to the factor. As such, only loadings greater than 0.4 are reported. To facilitate comparisons with earlier studies, three separate factor analyses were performed: a) on the standard measures, b) on the standard measures plus percent time spent on the central platform, and c) on the entire dataset, i.e., standard measures, percent time center, and all ethological measures.

RESULTS

Behavioral Profile

Table 1 summarizes the dataset upon which the present factor analyses were performed. Subjects showed high levels of arm entries, rearing, stretched attend postures, and sniffing, and relatively low levels of head dipping, closed arm returns, flat back approach, and grooming. On arm entry data, a clear preference for closed arms (approximately 2 : 1) was apparent while, on percent time data, a fairly typical rank order preference of closed arms (51%) > center platform (32%) > open arms (17%) was evident. Flat back approach behavior was equally distributed between protected and unprotected areas of the maze, head dipping and stretched attend postures were shown predominantly from protected areas (>60%), and sniffing occurred overwhelmingly in protected areas of the maze (>90%).

TABLE 1

ETHOLOGICAL DESCRIPTION OF BEHAVIOR DISPLAYED BY MALE DBA/2 MICE (N = 90) IN A 5-MIN ELEVATED PLUS-MAZE TEST

Behavioral Item	Mean + SE
Total arm entries	16.62 ± 0.52
Open arm entries	5.84 ± 0.34
Closed arm entries	10.78 ± 0.36
Total rears	14.14 ± 0.64
Total head dips	2.56 ± 0.26
Total stretched attend postures	18.09 ± 0.81
Closed arm returns	1.21 ± 0.16
Rearing duration (s)	21.04 ± 1.10
Sniff duration (s)	71.81 ± 7.01
Flat back approach duration (s)	9.64 ± 0.84
Grooming duration (s)	10.72 ± 1.50
Percent open entries	33.88 ± 1.49
Percent open time	17.44 ± 1.31
Percent centre time	31.68 ± 0.93
Percent closed time	50.87 ± 1.32
Percent protected head dips	63.50 ± 4.31
Percent protected stretched attend	64.80 ± 2.79
Percent protected sniff	90.27 ± 1.44
Percent protected flat back approach	52.55 ± 4.06

This dataset was used in the three factor analyses reported in Tables 2–4.

TABLE 2

ORTHOGONAL FACTOR LOADINGS FOR THE STANDARD PLUS-MAZE MEASURES IN MALE DBA/2 MICE

Variable	Factor 1	Factor 2
Total arm entries	–	0.92
Open arm entries	−0.85	0.42
Closed arm entries	–	0.95
Percent open entries	−0.92	–
Percent open arm time	−0.93	–
Percent closed arm time	0.78	–

Factor loadings of < 0.4 have not been included. The two factors account for 86.7% of the total variance.

Factor Analysis 1

This analysis included all standard plus-maze measures, i.e., total entries, open entries, closed entries, % open entries, % open time, and % closed time. Two clear factors emerged, accounting for 86.7% of the total variance (Table 2). The variables that loaded highly on Factor 1 were the number of open entries, % open entries, percent open time, and percent closed time. It should be noted that total entries also loaded moderately on this factor (loading not listed as below threshold: −0.39), while all measures except percent closed time loaded negatively. Variables with high loadings on Factor 2 were closed arm entries and total entries, while open arm entries only modestly loaded in this factor (0.42).

Factor Analysis 2

Kaiser and Scree plot analyses indicated that the simple inclusion of % time spent on the central platform of the maze resulted in the emergence of three factors, which together accounted for 91.2% of the total variance (Table 3). Factors 1 and 2 remained very much the same as in the first analysis, including the moderate loading (−0.42) of total entries on Factor 1. The new Factor 3 showed a very high loading for percent time spent on the central platform as well as a fairly high (but negative) loading for percent time spent in the enclosed arms.

Factor Analysis 3

This final analysis included all spatiotemporal and behavioral measures currently taken in our laboratory. These com-

TABLE 3

ORTHOGONAL FACTOR LOADINGS FOR THE STANDARD PLUS-MAZE MEASURES, PLUS PERCENT TIME SPENT ON THE CENTRE PLATFORM

Variable	Factor 1	Factor 2	Factor 3
Total arm entries	−0.42	0.88	–
Open arm entries	−0.86	–	–
Closed arm entries	–	0.96	–
Percent open entries	−0.91	–	–
Percent open arm time	−0.92	–	–
Percent closed arm time	0.75	–	−0.62
Percent centre time	–	–	0.93

Factor loadings of < 0.4 have not been included. The three factors account for 92.0% of the total variance.

prise some 19 items, of which 5 may be said to be strongly derivational (% open entries, %pDips, %pSAP, %pSniff, and %pflat back). Kaiser and Scree plot analyses identified six factors, which together accounted for 76.1% of the total variance in this much extended dataset (Table 4). Factor 1 again showed high loadings for open entries, % open entries, % open time, and % closed time (note positive loading). The ethological measures of closed arm returns, and the protected forms of head dipping, stretched attend postures, sniffing, and flat back approach also loaded highly on this factor. Total arm entries (−0.44) and time spent on the central platform (0.42) loaded only moderately on this factor.

Closed arm entries, total entries, and total flat back approach loaded heavily on Factor 2, while total stretched attend postures and total sniffing loaded very highly on Factor 3. Time spent on the central platform loaded highly on a separate factor (Factor 4) which, as for Factor Analysis 2, was negatively associated with time spent in the closed arms. Two ethological items, grooming (−0.63) and closed arm returns (−0.44), also loaded on Factor 4. Rearing (frequency and duration) loaded very highly on Factor 5, indicating at least a partial separation of horizontal (Factor 2) and vertical motor activity. Grooming, total entries, and closed arm entries also loaded moderately on this factor. Finally, total head dips loaded very highly on Factor 6, which, to a very much lesser degree (on cutoff at 0.40), also loaded total stretched attend postures.

DISCUSSION

When exposed to an elevated plus-maze for 5 min, male DBA/2 mice exhibit high levels of activity, characterized by significant amounts of locomotion, rearing, stretched attend postures, and olfactory investigation. Other consistently observed, but lower-frequency behaviors were flat back approach, grooming, head dipping, and closed arm returns. In accordance with the model (18,20,31) and with this specific inbred mouse strain [e.g., 23,32)], behavior was unequally distrubuted in the maze with subjects a) making twice as many closed arm entries as open arm entries and b) showing a rank order temporal preference of closed > center > open, with a threefold closed/open arm differential. This unambiguous open arm avoidance confirms the aversive qualities of the open arms. Exploratory/information-gathering behaviors (i.e., sniffing, head dipping, stretched attend postures) were predominantly shown from the relative security of protected areas of the maze, confirming previous observations [e.g., (8,23,24)]. In contrast, flat-back approach [seen at the start of a test session and directed from the center platform towards both open and closed arms; (6)] was equally distributed between protected and unprotected areas.

Three separate factor analyses were performed on this dataset to characterize the factor structure of the observed behaviors. The first analysis showed that the standard spatiotemporal measures could be accommodated by two factors. Interestingly, the factor structure and factor loadings outlined in Table 2 are virtually identical to those reported for male NIH Swiss mice (18), male hooded Lister rats (13,14), and male Wistar rats (9). As the standard anxiety indices (open entries; % open entries; % open time) loaded heavily on Factor 1, this factor is taken to be an anxiety factor. The fact that time spent in the closed arms also loads highly (but positively) on this factor is entirely consistent with this interpretation. In contrast, total entries and closed arm entries load heavily on Factor 2, which, correspondingly, is interpreted as a locomotor activity factor. As noted by several other authors [e.g., (1,9,13,16,18)], total entries cannot be taken as a pure index of locomotor activity. This is because this item also loads, albeit less heavily (−0.39), on the anxiety factor (Factor 1). However, it would appear that closed arm entries could be

TABLE 4

ORTHOGONAL FACTOR LOADINGS FOR STANDARD PLUS-MAZE MEASURES, PLUS TIME SPENT ON THE CENTRAL PLATFORM, PLUS ALL ETHOLOGICAL MEASURES

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Total arm entries	−0.44	0.65	—	—	0.43	—
Open arm entries	−0.83	—	—	—	—	—
Closed arm entries	—	0.77	—	—	0.43	—
Total rears	—	—	—	—	0.88	—
Rear duration	—	—	—	—	0.91	—
Total head dips	—	—	—	—	—	0.86
Total SAP	—	—	0.73	—	—	0.40
Closed arm returns	0.49	—	—	−0.44	—	—
Sniff duration	—	—	0.80	—	—	—
Flat back duration	—	0.58	—	—	—	—
Groom duration	—	—	—	−0.63	−0.46	—
Percent open entries	−0.86	—	—	—	—	—
Percent open time	−0.95	—	—	—	—	—
Percent centre time	0.42	—	—	0.77	—	—
Percent closed time	0.65	—	—	−0.63	—	—
Percent protected dips	0.52	—	—	—	—	—
Percent protected SAP	0.86	—	—	—	—	—
Percent protected sniff	0.70	—	—	—	—	—
Percent protected flat back	0.62	—	—	—	—	—

Factor loadings of < 0.4 are not included. The six factors account for 76.1% of the total variance. SAP = stretched attend posture.

used as a relatively pure index of locomotor activity as it loads highly and exclusively on Factor 2 [see also (9,13)]. In addition to loading highly on Factor 1, the absolute number of open arm entries also loaded moderately on Factor 2 (0.42). Although at apparent variance with the findings of File (13), this finding is consistent with a recent report by Cruz et al. (9) and, in agreement with the latter authors, and we consider this to be a logical loading in that open arm entries do contribute to total entries and, therefore, to general activity on the maze.

The inclusion of time spent on the central platform [a variable infrequently recorded in the plus-maze: (25)] resulted in the emergence of a third factor (Table 3). The loadings for Factors 1 (anxiety) and 2 (locomotor activity) remained very much the same as in the first analysis. The exceptions were the appearance of total entries on Factor 1 (increasing loading from −0.39 to −0.42), and the loss of open entries from Factor 2 (decreasing loading from 0.42 to 0.35). Against a relatively arbitrary loading threshold of 0.4, these swings must be considered marginal at best. By far the most important difference between the first and second analyses was the emergence of a third factor, loading highly on center time and moderately (and negatively) on closed-arm time. A similar factor has been recently been identified in the rat plus-maze (9,14). As it has previously been argued that time center must reflect decision making, perhaps related to approach/avoid conflict (9,28,32), we choose to interpret Factor 3 as reflecting these processes.

The final and large-scale analysis incorporated all plus-maze parameters currently recorded in our laboratory, including the various ethological measures related to murine defensive behavior. Six factors emerged from this investigation of the data (Table 4), three of which appear to correspond directly with the factors identified in the simpler analyses. Factor 1 (anxiety) retained very high loadings on traditional spatiotemporal measures as well as its moderate loading for total arm entries. Importantly, several ethological measures (closed arm returns and protected forms of head dipping, SAP, sniffing, and flat back approach), previously interpreted as indicative of anxiety [e.g., (27,29)], were also found to load moderately/highly on this factor. We believe that the latter items are related by virtue of reflecting a reluctance to leave the security of the closed arms. The inclusion in Factor 2 (locomotor activity) of the duration of flat back approach is entirely consistent with the forward (if cautious) locomotor form/function of this behavior. Factor 4 seems to be homologous with the third factor (decision making, approach/avoid) identified in the intermediate analysis (for comparison, see Table 3). Consistent with the latter profile, center time loaded very highly on this factor, which is again seen to be associated with a moderate negative loading for closed-arm time. The negative loadings for closed-arm returns and grooming tend to confirm its relationship with decision making, i.e., decisions having been made. By definition, the former occurs exclusively in closed arms while the latter almost always occurs in secure areas of the maze (unpublished observations).

The full analysis revealed three additional factors that need to be considered. Total SAP and total sniffing were the only measures to load highly on Factor 3. SAP are considered a primary index of risk assessment, a group of behaviors that are thought to facilitate information gathering in potentially dangerous environments, and which have been found to be particularly sensitive to anti- and proanxiety drugs [e.g., (3,5,21,25,30)]. The finding that this measure coloads with olfactory investigation/sniffing (arguably a very important means of information gathering in a macrosmatic species), suggests that Factor 3, indeed, represents risk assessment. It is, therefore, important to note that neither of these measures loads on Factor 2, the locomotor activity factor. Factor 5 is clearly related to vertical activity, with very high loadings for rearing frequency and duration. The observation that total entries and closed arm entries also loaded (to exactly the same degree, 0.43) on Factor 5 makes behavioral sense in that rearing occurs in its highest frequency against the side and end walls of the closed arms. Although it is not clear at present why grooming should load negatively on this factor, one possibility is that it reflects response competition with vertical and horizontal activity reducing opportunities to groom. Finally, total head dipping was found to load strongly and exclusively on Factor 6 and, as this behavior has traditionally been used as a primary index of exploratory behavior (13,18), Factor 6 may be taken to reflect exploration. Although total SAP also loaded on Factor 6, its numerical loading was just at the threshold level of 0.40 and very much lower than its loading on Factor 3 (0.73).

In a very recent factor analysis of behavior in the rat elevated plus-maze which, like the present study, employed some ethological measures in addition to more usual spatiotemporal measures, Cruz and colleagues (9) identified four distinct factors with loadings greater than 0.4: Factor 1 (anxiety), Factor 2 (activity), Factor 3 (decison making); Factor 4 (displacement). They found that the ethological elements of scanning and end exploring loaded strongly on Factor 1, along with the standard anxiety measures. This pattern is not only consistent some of our own findings (e.g., the high Factor 1 loading of protected head dipping), but also with the fact that the elements in question are measures directly related to open-arm activity. Further similarity between the studies is apparent in that center time loaded highly on a factor other than the main anxiety factor. However, there are some differences in factor structure between the studies, e.g., Cruz et al. (9) found rearing to load along with measures of locomotor activity on Factor 2, while grooming had the highest loading on Factor 4 (displacement). In addition, they found that their measure of risk assessment to load on three factors, a coloading that was considered ambiguous and, therefore, problematic. However, given that all three factors (1, 3, and 4) could arguably be said to measure different aspects of anxiety (avoidance of danger; decision making, approach–avoid conflict), coloadings for risk assessment should perhaps not be too surprising. Furthermore, the definition of risk assessment employed is surprisingly restrictive, "exiting an enclosed arm with forepaws and head only, and investigating the surroundings (this behavior was often, but not necessarily, accompanied by body stretching)" (p. 172). There are several problems with the definition (a point alluded to by the authors themselves), the most serious of which is that risk assessment (e.g., SAP) directed from the center platform towards the open (or, indeed, closed) arms is completely excluded from the analysis. For mice, if not for rats, this form of risk assessment is very much more common than that seen from enclosed arms towards other parts of the maze.

Whether the differences in factor structure between the present study and that of Cruz et al. (9) reflect a true species difference is unclear at the present time. What is clear is that the general construction of the mazes differ (e.g., the enclosed arms of the rat maze are opaque, whereas ours are transparent) and test procedures differ (e.g., they place their subjects on the central platform facing a closed arm, whereas we place them facing an open arm; they handle their subjects daily prior to testing, ours are unhandled; they test during the light phase, we test during the dark, etc). Furthermore, as acute handling/injection has been reported to alter plus-maze behavior in rats (2), it is possible [though not very likely: see uninjected control profiles in (23) and (24)] that any differences between our findings and those of Cruz et al. (9) may be attributable to our use of data from vehicle-treated animals. As the plus-maze is very sensitive to methodological factors [e.g., (15,25)], any one (or combination) of these variables could account for the differences observed. Furthermore, as factor analyses are sensitive to the number of variables and to the ratio of variables to subjects (17), this may well represent another source of uncontrolled variation. Suffice it to say that both studies point to multiple dimensions of behavior in the elevated plus-maze test, dimensions that may be used profitably in the study of drug effects.

In conclusion, the adoption of an ethological approach to the scoring of behavior in the elevated plus-maze clearly confirms and extends our present understanding of this test procedure. Not only does the behavioral profile comprise factors related to anxiety and locomotor activity as previously thought, but it also includes dimensions that appear to be related to vertical activity, exploration, risk assessment, and decision making. These new insights into the structure of plus-maze behavior provide a) a more concrete conceptual framework for the use of ethological scoring of this test; b) a more effective means of interpreting the effects of drugs and other manipulations in the test (in particular, the difficult issue of behavioral specificity); and, if desired for routine screening purposes; c) a more rational basis for the selection of a subset of items most suited to represent the different dimensions of plus-maze behavior. An intriguing aspect of the present work (and that of Cruz and colleagues) concerns the possibility that different behaviors observed in the same model may provide windows on to different aspects of anxiety, e.g., approach/avoid conflict, decision making, and overt avoidance behavior. Further studies on this issue are clearly warranted.

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