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Ethopharmacological Analysis of Rat Behavior on the Elevated Plus-Maze

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CRUZ, A. P. M., F. FREI AND F. G. GRAEFF. *Ethopharmacological analysis of rat behavior on the elevated plus-maze.* PHARMACOL BIOCHEM BEHAV 49(1), 171–176, 1994. – Behavioral categories were measured in rats left on an elevated plus-maze for 5 min, in addition to the traditional measures. Four independent factors emerged from a factor analysis. The variables that loaded highly and positively on Factor 1, seemingly related with anxiety, were: number of entries onto open arms, time spent on open arms, percentage of open/total arm entries, percentage of time on open arms, scanning over the edge of an open arm, and open arm end-exploring. The time spent on enclosed arms loaded highly, but negatively on the same factor. Risk-assessment from an enclosed arm also loaded negatively on Factor 1. Number of enclosed arm entries, total number of arm entries and rearing loaded highly on Factor 2, probably related to motor activity. However, the total number of entries also loaded on Factor 1, being thus a mixed index. Similarly, the number of open arm entries loaded on both Factors 1 and 2. As expected, the variables having high loads on Factor 1 were changed to one direction by administration of two anxiolytics (nitrazepam and midazolam) and to the opposite direction by two anxiogenic drugs (pentylenetetrazol and FG 7142). Such pattern of drug effects was not observed with the remaining variables.

Elevated plus-maze Rats Factor analysis Anxiolytics Anxiogenic drugs

THE elevated plus-maze, consisting of two open arms crossed with two enclosed arms all being elevated from the ground, is widely used for studying anxiolytic drugs and the neurobiological mechanisms of anxiety (2,4,7,9,12,14,15,19,20,24). This model is based on the natural fear of open and elevated alleys (17). As a consequence, rats on the elevated plus-maze tend to avoid the open arms and stay more on the enclosed arms. Anxiolytic drugs increase the number of entries onto and the time spent on open arms, whereas anxiogenic agents do the opposite (2,14,19,20). When confined to the open arms, rats show behavioral and physiological manifestations of fear, such as freezing, defecation, and increases in plasma corticosteroids (20). Recently reported results indicate that open space (or, more specifically, the impossibility of performing thigmotaxis) rather than height is the main cause of fear of the open arms (27).

The ratio (14) and the percentage (20) of open per total arm entries have been used as indexes of anxiety. Often the percentage of time spent on the open arms is also reported (20). Truly, these indexes relate negatively with anxiety, since they are typically increased by anxiolytic while being decreased by anxiogenic drugs (2,14,19,20).

Because changes in motor activity may also influence exploratory behavior in the elevated plus-maze, the total (open + enclosed) number of entries has been used to reflect this factor (20). However, several studies using factor analysis suggest that this is not entirely true, since total entries also load importantly on the anxiety factor. On the other hand, the number of enclosed arm entries loads highly and exclusively on the factor associated with exploration and, thus, may substitute for total number of entries as an index of motor activity (11).

While on the elevated plus-maze, rats display a variety of behaviors that are amenable to ethoexperimental analysis (6). It is possible that certain behavioral categories reflect anxiety, motor activity, or other factors even better than the traditional indexes of exploration. Although systematic studies using this approach have been performed in the mouse (21), only some specific behavioral elements have been investigated in the rat (1,18). As a consequence, the first experiment of the present

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study is aimed at measuring the behavior of undrugged rats on the elevated plus-maze, using both the traditional indexes and behavioral parameters selected from an ethogram developed for this experimental situation. These two types of measures were then included in a factor analysis for estimating the loadings of the different variables on the independent factors that emerged from the analysis.

To validate the above approach, the effect of drugs was measured on both the traditional parameters and the ethological categories. The drugs chosen were two benzodiazepine receptor agonists (28), nitrazepam (NTZ) and midazolam (MDZ), and two agents that have been reported to induce anxiety in laboratory animals and in humans, the β-carboline FG 7142 (FG) and pentylenetetrazol (PTZ) (10,19,20,23).

METHOD

Animals

Male Wistar rats weighing 190–240 g and having free access to food and water were housed in groups of five on a 12-h light cycle. The experimental sessions were conducted during the light phase of the cycle, between 1400 and 1800. Rats were daily handled for 5 min during the three last days before the experiments.

Apparatus

The elevated plus-maze was made of wood, according to the specifications of Pellow et al. (20). The apparatus consisted of two opposed open arms measuring 50 × 10 cm, crossed at right angle with two opposed arms of the same size. The latter were enclosed by walls 40-cm high, except for the entrance. The four arms delimited a central area of 10 cm^2. The whole apparatus was elevated 50-cm above the floor. To avoid rats falling down, a rim of Plexiglas 1-cm high was made to surround the open arms. Illumination was provided by a 60-W red light bulb suspended 120-cm above the maze. The experimental sessions were recorded by a vertically mounted videocamera, linked to a monitor and VCR in an adjacent room. Videotapes were later analysed by an observer unaware of treatment conditions.

Drugs

The following drugs were used: nitrazepam (Roche, Brazil), midazolam (Roche, Brazil), pentylenetetrazol (Sigma, USA) and *N*-methyl-β-carboline-3-carboxamine (FG 7142, Ferrosan, Denmark). Before injection, MDZ and PTZ were dissolved in sterile saline, while NTZ and FG were suspended in saline containing 2% Tween 80. All drugs were injected IP in a volume of 1.0 ml/kg.

Procedure

Experiment 1. Each rat (n = 30) was placed in the central square facing an enclosed arm, and allowed to freely explore the elevated plus-maze for 5 min. Before the next rat was introduced, the maze was cleaned with a solution of 20% ethanol and dried. The standard measures (number of entries onto open and enclosed arms, and time spent on open and enclosed arms), as well as the time spent on the central platform (26) were recorded. In addition, the time displaying the following behavioral categories was measured: (a) *scanning:* protruding the head over the edge of an open arm and scrutinizing in any direction (it includes head-dipping); (b) *risk-assessment:* exiting an enclosed arm with the forepaws and head only, and investigating the surroundings (this behavior was often, but not necessarily, accompanied by body stretching); (c) *rearing:* rising on the hind limbs; (d) *self-grooming:* cleaning any part of the body surface with the tongue, teeth, and/or forepaws; and (e) *end-exploring:* number of times the rat reached the end of an open arm. Since it was difficult to distinguish relaxed immobility from freezing with the angle of observation used, these categories were not included in the analysis.

Experiment 2. To measure the effect of drugs on the above variables, rats were randomly allocated to the following experimental groups: 1) injected with 20 mg/kg PTZ 5 min before being placed on the elevated plus-maze, n = 10; 2) injected with 1.5 mg/kg FG 30 min before the experimental session, n = 8; 3) injected with 1.5 mg/kg NTZ 30 min before the session, n = 8; and 4) injected with 2.5 mg/kg MDZ 30 min before the session, n = 8. In addition, there were three control groups: 1) rats injected with saline 30 min before the session (for MDZ); 2) rats injected with saline 5 min before the session (for PTZ); and 3) rats injected with Tween vehicle 30 min before the session (for NTZ and FG). The doses of the drugs used were chosen on the basis of reported results showing that they caused clear anxiolytic or anxiogenic effects with a minimum impairment of motor activity.

Data Analysis

For each animal, the total number of entries (open + enclosed arms), the percentage of open arm entries (100 × open/total) and the percentage of time spent on the open arms (100 × open/(open + enclosed)) were calculated. The data from Experiment 1 were submitted to factor analysis using a principal component solution with an orthogonal rotation (varimax) of the factor matrix. Using the smallest eigenvalue criterion (mineigen = 1), four factors emerged, explaining 85.67% of the total sample variance. The data from Experiment 2 were analyzed either by the Student's t-test or by one way analysis of variance (ANOVA). Whenever ANOVA was significant, the Dunnett's test for multiple comparisons of individual groups with control was performed. The level of statistical significance adopted was $p < 0.05$.

RESULTS

Experiment 1

The factor loading for each behavioral parameter provides an estimate of how well that variable reflects a particular factor. A value of 1.0 would be a perfect reflection, whereas loading of <0.4 indicates a poor reflection of the factor. Since the factors extracted from this analysis are independent, they are likely to reflect different processes.

Table 1 shows the loading of each measure taken in the elevated plus-maze on the independent factors that came out of the factor analysis. The variables that loaded highly on Factor 1, likely to represent the reverse of anxiety, were: number of entries onto open arms, time spent on open arms, percentage of open arm entries, and percentage of time on open arms. The time spent on enclosed arms loaded highly and negatively on this factor. Among the behavioral categories rated, scanning and end-exploring loaded heavily and positively on Factor 1, whereas risk-assessment loaded negatively on the same factor, although it also loaded on Factors 3 and 4.

The measures loading highest on Factor 2, seemingly related to motor activity, were: number of enclosed arm entries,

TABLE 1
ORTHOGONAL FACTOR LOADINGS FOR UNDRUGGED RATS ON THE ELEVATED PLUS-MAZE

Factors	1	2	3	4
No. open	0.81	0.47	–	–
No. enclosed	–	0.96	–	–
Total entries	0.60	0.82	–	–
% open entries	0.92	–	–	–
Time open	0.96	–	–	–
Time center	–	–	0.94	–
Time enclosed	−0.90	–	–	–
% time open	0.93	–	–	–
Scanning	0.85	–	–	–
End-exploring	0.74	–	–	–
Risk-assessment	−0.60	–	−0.47	−0.60
Rearing	–	0.71	–	–
Self-grooming	–	–	–	−0.81

Only loadings > 0.40 are shown.

total number of arm entries, and rearing. Yet, the total number of entries also loaded considerably on Factor 1. On the other hand, the number of open arm entries also loaded above criterion on Factor 2, though less heavily than on Factor 1.

The time spent at the central square of the maze had a high loading on Factor 3 only, while the variable loading highest on Factor 4 was self-grooming.

Experiment 2

The effect of drugs on the traditional indexes of exploration in the elevated plus-maze is shown in Figs. 1 and 2. It may be seen that the variables with high positive loads on Factor 1 of the above factor analysis (absolute and relative number of entries onto and of time spent on open arms) were significantly decreased by the anxiogenic agents PTZ and FG, while being increased by the anxiolytics NTZ and MDZ. In contrast, the time spent on enclosed arms, that loaded negatively on Factor 1, was significantly decreased by the two anxiolytics and increased by PTZ; the increase caused by FG did not reach significance level.

The total number of arm entries—an index that loaded mainly on Factor 2, but also considerably on Factor 1—was significantly decreased by both PTZ and FG, and increased by NTZ. A similar trend was shown by MDZ, but the difference from control lacked statistical significance. In turn, the number of enclosed arm entries was not affected by the drug treatments, except for PTZ, that significantly decreased it. Finally, the time spent on the central square of the maze was significantly decreased by NTZ only.

Figure 3 shows that scanning and end-exploring, two behavioral categories that loaded highly and positively on Factor 1, were significantly decreased by the anxiogenic drugs while being increased by the anxiolytics. Conversely, risk-assessment, which loaded negatively on this factor (but also on Factors 3 and 4), was significantly decreased by NTZ and MDZ. However, this parameter was not significantly affected by PTZ and FG.

Rearing, a variable that loaded exclusively on Factor 2, was significantly decreased by both PTZ ($p < 0.01$) and FG ($p < 0.05$), but not significantly affected by the anxiolytics. Finally, no significant change in grooming (negatively loaded on Factor 4) was determined by any of the drugs tested.

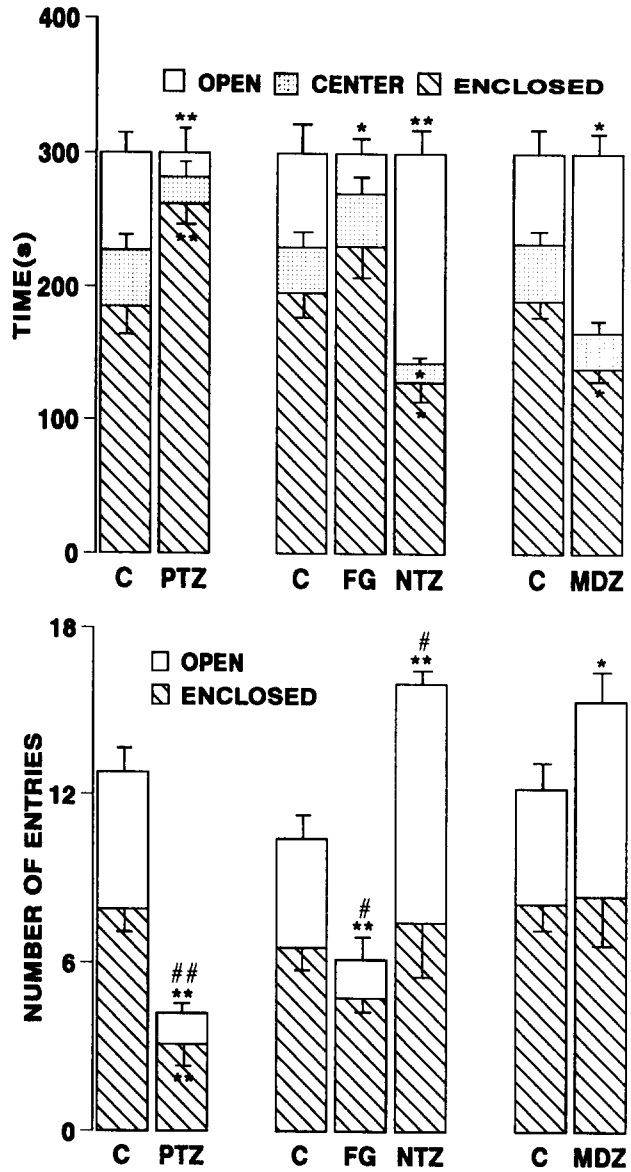

FIG. 1. Effects of two anxiolytics, nitrazepam (NTZ) and midazolam (MDZ), and two anxiogenic drugs, pentylenetetrazol (PTZ) and FG 7142 (FG), on absolute measures of exploration in the elevated plus-maze. Bars represent the mean and vertical lines the SEM. Rats were injected IP with NTZ (1.5 mg/kg), MDZ (2.5 mg/kg), FG (1.5 mg/kg), or their respective control vehicles 30 min before the experimental session. PTZ (20 mg/kg) and its control were injected IP 5 min before the test. *$p < 0.05$, **$p < 0.01$ drug vs. control group. The symbol # was used instead of * for the total number of entries; n = 10 for the PTZ group and its control; n = 8 for the remaining groups.

DISCUSSION

Regarding the traditional parameters measured in the elevated plus-maze, the present factor analysis largely agrees with that previously reported by File (11). In both studies, time on open arms as well as percentage of open arm entries and of time on open arms loaded highly and exclusively on Factor 1, supposed to be inversely related to anxiety. However, an important difference occurred concerning the absolute num-

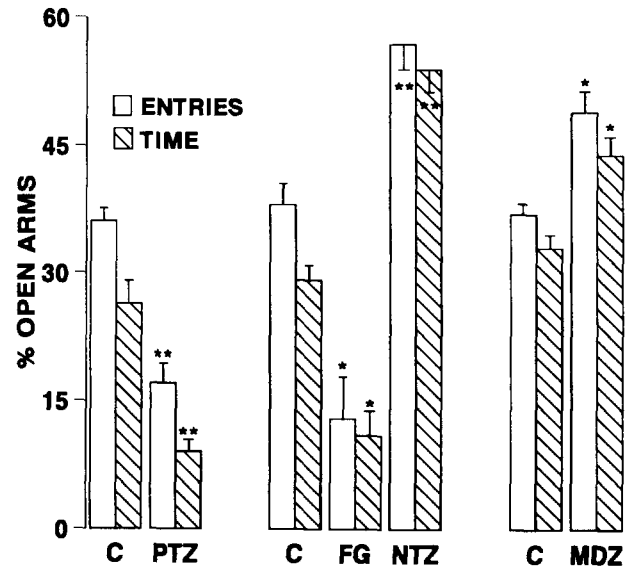

FIG. 2. Effect of anxiolytic and anxiogenic drugs on relative indexes of exploration in the elevated plus-maze. Other specifications in Fig. 1 legend.

ber of open arm entries. In File's (11) results this measure loaded exclusively on Factor 1, whereas in the present study it also loaded on Factor 2, that is believed to reflect general motor activity (see Table 1). Although the cause of this difference may be only guessed (e.g., rat strain is one possibility), the present results make behavioral sense, since entering onto an open arm involves motor activity. On the other hand, time spent on the open arm is far less influenced by motility, and indeed it appeared as a noncontaminated index of anxiety in both the present and File's (11) analyses. Another way of

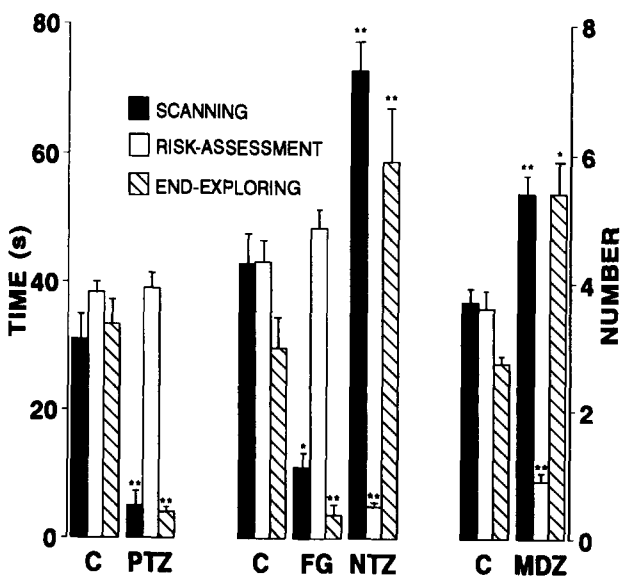

FIG. 3. Effect of anxiolytic and anxiogenic drugs on behavioral categories that loaded highly on the anxiety-related Factor 1 (see Table 1). Other specifications in Fig. 1 legend.

circumventing the influence of motor activity may be the use of the percentage of open arm entries, since this parameter also loaded only on Factor 1.

The percentage of time on open arms also appeared as a clean index of anxiety in both the present and File's (11) results. However, this variable is biased because the time spent on the enclosed arms is in the denominator of the equation:

$$\% \text{ time open} = 100 \times \text{time open}/(\text{time open} + \text{time enclosed}).$$

As a consequence, any increase in session length decreases the index, since rats tend to remain on one of the enclosed arms, after an initial period of intense exploration. For example, a reported study using a 10-min session has shown that this index was not significantly affected by intracerebrally injected propranolol, in contrast to the percentage of open arm entries (4).

Therefore, among the traditional indexes of anxiety the percentage (or ratio) of open/total arm entries and the time spent on the open arms seem to reflect anxiety better than the absolute number of open arm entries. The time spent on the enclosed arms is almost a mirror image of the time on open arms (thus reflecting anxiety quite well) because the time spent at the central square is relatively small.

In agreement with previously reported results (11), the present observations indicate that the total number of arm entries is not a reliable index of motor activity. In both studies this variable was contaminated by anxiety, since it loaded importantly on Factor 1, as well as on Factor 2. In turn, the number of enclosed arm entries loaded very highly and exclusively on the latter factor. Therefore, the absolute number of enclosed arm entries seems to reflect general motor activity very well.

In the present results, time on the central square loaded only on Factor 3. Also, the drug results showed that this parameter was significantly reduced by NTZ, and a nonsignificant trend towards reduction was also observed following MDZ. Furthermore, a similar study by Trullas et al. (26) has shown that another benzodiazepine anxiolytic, chlordiazepoxide, decreased the time spent on the center of the elevated plus-maze. In contrast, a ligand of the glycine site associated with NMDA receptors of excitatory aminoacids (ACPC) increased central time, despite having an anxiolytic effect in the elevated plus-maze. Since reported results indicate that benzodiazepines reduce waiting capacity (25), and decision-making (16), Trullas et al. (26) suggested that time on the central square of the elevated plus-maze may reflect one or both these processes.

Regarding the parameters from the ethogram, the present factor analysis showed that scanning and end-exploring loaded highly and only on Factor 1 and, therefore, they may be viewed as valid indexes of anxiety. In contrast, risk-assessment (often regarded to reflect anxiety [5,21]) proved ambiguous, since it loaded simultaneously on two other factors (Factor 3 and 4). In turn, rearing loaded markedly on Factor 2 alone and, thus, seems to reflect motor activity quite well. Finally, self-grooming loaded highly and negatively on Factor 4 only. It is difficult to figure out the meaning of this factor, since it was not significantly affected by any of the drugs used in the present study. Nevertheless, grooming has been used as an index of displacement behavior in conflict situations (3). This view does not seem incompatible with the significant loading (also with a negative sign) of risk-

assessment on Factor 4, since this behavior typically occurs as a result of approach-avoidance conflict (5,6).

The above factor analysis leads to the prediction that variables loading highly on Factor 1 should be changed to one direction by the two anxiolytics and to the opposite direction by the two anxiogenic drugs used. Moreover, this pattern should not appear for measures loading highly on the remaining factors. The results of the second experiment fulfilled both predictions. Indeed, absolute and relative number of entries onto and of time spent on open arms, as well as scanning and end-exploring—all being measures that loaded highly and positively on Factor 1—were increased by NTZ and MDZ while being decreased by FG and PTZ. Conversely, time on enclosed arms, a parameter that loaded negatively on the same factor, underwent opposite changes following administration of the same drugs. Seemingly, risk-assessment did not comply with the rule, since it was not affected by either PTZ or FG, although it was decreased by both NTZ and MDZ. However, risk-assessment also loaded on Factors 3 and 4 and, therefore, is not a clean measure of anxiety.

Also, according to the above predictions none of the variables that loaded predominantly on factors other than Factor 1 was affected in opposite ways by the two classes of drugs used. Enclosed arm entries and rearing loaded highly on Factor 2 and were significantly decreased by PTZ, only. Rearing was also significantly decreased by FG. However, these measures were not affected by the anxiolytics. An exception may be total arm entries, since this measure was significantly decreased by both PTZ and FG while being significantly enhanced by NTZ. However, although total arm entries loaded mainly on Factor 2, it also loaded rather markedly on Factor 1 and, as discussed above, is likely to reflect both anxiety and motor activity.

Overall, it may be concluded that both the factor analysis and the pharmacological results of the present study give strong support to the hypothesis that Factor 1 reflects the reverse of anxiety or tranquility. The addition of behavioral categories from the ethogram strengthens this conclusion. Nevertheless, neither scanning nor end-exploring, and even less risk-assessment, proved superior to traditional indexes, like the percentage of open/total arm entries or the time spent on open arms. Since ethological analysis is time consuming and requires considerable amount of work, its standard use may not be practical. However, the present results alone are not enough to dismiss systematic observation completely, since all the drugs used act through GABA-mediated neurotransmission (28). Indeed, there are indications that ethological analysis may be useful for compounds primarily acting on 5-HT neurotransmission, that do not consistently change the traditional indexes of elevated plus-maze exploration (8,13). For example, Rodgers et al. (22) have shown that anxiogenic compounds, such as mCPP and TFMPP, enhanced risk-assessment in mice on the elevated plus-maze, without significantly affecting open-arm entries and time. In the same way, buspirone has been shown to selectively increase closed arm returns, in the rat (18). Another possibility is that sensitivity to drugs may depend on the precise definition of the behavioral items, which varies to some extent among different studies.

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REFERENCES

1. Adamec, R. E.; Shallow, T. Lasting effects on rodent anxiety of a single exposure to a cat. Physiol. Behav. 54:101–109; 1993.
2. Almeida, S. S.; De Oliveira, L. M.; Graeff, F. G. Early life protein malnutrition changes exploration of the elevated plus-maze and reactivity to anxiolytics. Psychopharmacology 103:513–518; 1991.
3. Archer, J. Tests for emotionality in rats and mice: A review. Anim. Behav. 21:205–235; 1973.
4. Audi, E. A.; de Oliveira, R. M. W.; Graeff, F. G. Microinjection of propranolol into the dorsal periaqueductal gray causes an anxiolytic effect in the elevated plus-maze antagonized by ritanserin. Psychopharmacology 105:553–557; 1991.
5. Blanchard, D. C.; Blanchard, R. J.; Tom, P.; Rodgers, R. J. Diazepam changes risk assessment in an anxiety/defense test battery. Psychopharmacology 101:511–518; 1990.
6. Blanchard, R. J.; Brain, P. F.; Blanchard, D. C.; Parmigiani, S. Ethoexperimental approaches to the study of behavior. Dordrecht: Kluwer; 1989.
7. Briley, M.; Chopin, P.; Moret, C. Effects of serotonergic lesions on "anxious" behavior measured in the elevated plus-maze in the rat. Psychopharmacology 101:187–189; 1990.
8. Chopin, P.; Briley, M. Animal models of anxiety: The effect of compounds that modify 5-HT neurotransmission. Trends Pharmacol. Sci. 8:383–388; 1987.
9. Critchley, M. A. E.; Njung'e, K.; Handley, S. L. Actions and some interactions of 5-HT_{1a} ligands in the elevated X-maze and effects of dorsal raphe lesions. Psychopharmacology 106:484–490; 1992.
10. Dorow, R.; Horowski, R.; Paschelke, G.; Amin, M.; Braestrup, C. Severe anxiety induced by FG 7142, a β-carboline for benzodiazepine receptors. Lancet 9:98–99; 1983.
11. 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. Chichester: John Wiley & Sons Ltd.; 1992:25–44.
12. Guimarães, F. S.; Carobrez, A. P.; de Aguiar, J. C.; Graeff, F. G. Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. Psychopharmacology 103:91–94; 1991.
13. Handley, S. L.; McBlane, J. W. 5-HT drugs in animal models of anxiety. Psychopharmacology 112:13–20; 1993.
14. Handley, S. L.; Mithani, S. Effects of alpha-adrenoceptor agonists in a maze-exploration model of "fear"—motivated behaviour. Naunyn-Schmiedeberg's Arch. Pharmacol. 327:1–5; 1984.
15. Kostowski, W.; Plaznik, A.; Stefanski, R. Intra-hippocampal buspirone in animal models of anxiety. Eur. J. Pharmacol. 186: 393–396; 1989.
16. Ljungberg, T.; Lidfors, L.; Enquist, M.; Ungerstedt, U. Impairment of decision making in rats by diazepam: Implications for the "anticonflict" effects of benzodiazepines. Psychopharmacology 92:416–423.
17. Montgomery, K. C. The relationship between fear induced by novel stimulation and exploratory behaviour. J. Comp. Physiol. Psychol. 48:254–260; 1955.
18. Moser, P. C. An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. Psychopharmacology 99:48–53; 1989.
19. Pellow, S.; File, S. E. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24:525–529; 1986.
20. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open: Closed arm entries in the elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Meth. 14:149–167; 1985.

21. Rodgers, R. J.; Cole, J. C. Anxiety enhancement in the elevated plus-maze by immediate prior exposure to social stressors. Physiol. Behav. 53:383–388; 1993.
22. Rodgers, R. J.; Cole, J. C.; Cobain, M. R.; Daly, P.; Doran, P. J.; Eells, J. R.; Wallis, P. Anxiogenic-like effects of fluprazine and eltoprazine in the elevated plus-maze; Profile comparisons with 8-OH-DPAT, CGS 12066B, TFMPP and mCPP. Behav. Pharmacol. 3:621–634; 1992.
23. Rodin, E. A.; Calhoun, H. D. Metrazol tolerance in a 'normal' volunteer population. J. Nerv. Ment. Dis. 150:438–450; 1970.
24. Russo, A. S.; Guimarães, F. S.; de Aguiar, J. C.; Graeff, F. G. Role of benzodiazepine receptors located in the dorsal periaqueductal grey in anxiety. Psychopharmacology 110:198–202; 1993.
25. Thiébot, M. H.; Bizot, J. C.; Soubrié, P. Waiting capacity in animals. A behavioral component crossing nosologic boundaries of anxiety and depression? In: Soubrié, P. ed. Animal models in psychiatric disorders, vol. 3: Anxiety, depression and mania. Basel: Karger; 1991:48–67.
26. 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. London: Macmillan; 1991:382–394.
27. Treit, D.; Menard, J.; Royan, C. Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44:463–469; 1993.
28. Zorumski, C. F.; Isenberg, K. E. Insights into the structure and function of GABA-benzodiazepine receptors: Ion channels and psychiatry. Am. J. Psychiat. 148:162–173; 1991.