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Ethopharmacological Analysis of 5-HT Ligands on the Rat Elevated Plus-Maze

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SETEM, J., A. P. PINHEIRO, V. A. MOTTA, S. MORATO AND A. P. M. CRUZ. *Ethopharmacological analysis of 5-HT ligands on the rat elevated plus-maze.* PHARMACOL BIOCHEM BEHAV **62**(3) 515–521, 1999.—The present study investigated the behavioral effects of five 5-HT agonists and antagonists in the rat elevated-plus-maze using conventional and ethologically derived measures. An anxiolytic effect of the 5-HT_{1A} agonist ipsapirone (0.25, 0.75, and 2.25 mg/kg) was detected by risk-assessment and scanning but not by percentage of open-arm entries and time spent on open arms. Anxiogenic effects of the 5-HT_{2C} agonist TFMPP (0.1, 0.2, and 0.4 mg/kg) and 5-HT_{2A} antagonist SR 46349B (1, 3, and 10 mg/kg) were detected by percentage of open-arm entries, time spent on open arms, scanning, end exploring, but not by risk assessment. Finally, the effects of the 5-HT_3 antagonist BRL 46470 A (0.001, 0.01, and 0.1 mg/kg) and 5-$HT_{2A/2C}$ antagonist RP 62203 (0.25, 1, and 4 mg/kg) were scarce in both conventional and ethologically derived measures. These results are indicative that ethological measures may sometimes be more sensitive than the standard ones, and should be used together with them when assessing serotonergic or any other novel drugs in the elevated plus-maze. © 1999 Elsevier Science Inc.

5-HT ligands 5-HT_{1A} agonists–5-HT_{2C} agonists 5-$HT_{2A/2C}$ Antagonists 5-HT_3 Antagonists Anxiety Elevated plus-maze Ethopharmacological analysis Rats

THE elevated plus-maze, introduced in 1994 (26), have been widely used for studying anxiolytic drugs and neurobiological anxiety mechanisms. Inspired in an earlier elevated Y-maze (36) and based on the natural fear of rodents for open space (56), this test has been validated for use with both rats (38) and mice (34). The ratios of entries and time spent on the open arms have been traditionally used as anxiety indexes. Truly, these indexes are negatively related to anxiety, because they are usually increased by classical anxiolytics while being decreased by anxiogenic drugs (1,4,19,24,25,42,44,46,57).

In fact, the traditional anxiety indexes in the elevated plus-maze show a good sensitivity to both anxiolytic and anxiogenic drugs acting at the GABAA/benzodiazepine receptor complex [for review, see (48)]. However, serotonergic drugs have produced inconsistent results in this test. For example, anxiolytic (1,14,33,52), anxiogenic (13,32,40,43,45), and even absence of effects (3,13,14,18,35,44) have been reported for treatments with 5-HT_{1A} agonists. Similar inconsistencies have been reported for 5-HT_2 (22,45,53,58) and 5-HT_3 (3,5,20,58) receptor antagonists.

In addition to the traditional measures, other behavioral categories have been more recently investigated in the elevated plus-maze. Some results showed that benzodiazepine and 5-HT_{1A} agonists reduced risk-assessment patterns in mice (9,10) and rats (23) exposed to this test. This reduction, interpreted as an anxiolytic effect, was not convincingly established for 5-HT_2 and 5-HT_3 ligands (23). It has also been demonstrated (15) that some other ethological categories (which showed high loadings in a factor related to anxiety from a factor analysis of the rat elevated plus-maze) 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). In the present article we investigated the effects of serotonergic drugs in rats exposed to the elevated plus-maze using the same indexes as reported elsewhere (15), considering that

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they might yield more consistent results than the traditional parameters.

METHOD

Animals

Male Wistar rats weighing 190–240 g with free access to food and water were housed in groups of six on a 12 L:12 D cycle. The experimental sessions were conducted during the light phase of the cycle, between 0800 and 1200 h. Rats were daily handled for 5 min during the last 3 days before testing.

Apparatus

The elevated plus-maze was made of wood, according to specifications described elsewhere (38). The apparatus consisted of two opposed open arms (50 × 10 cm) crossed at right angles, 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 100 cm^2. The whole apparatus was elevated 50 cm above the floor. To avoid the rats falling down, a rim of Plexiglas (1 cm high) surrounded the perimeter of the open arms. Illumination was provided by a 60-W light bulb suspended 175 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 analyzed by highly trained observers who remained blind to treatment conditions. Samples of animal behavior were correlated and the coefficient rate was greater than 0.9. This reliability has been repeated in our laboratory setting.

Drugs

The following drugs were used: the 5-HT_{1A} partial agonist ipsapirone (Bayer, Germany), the 5-HT_3 antagonist BRL 46470 A (Beecham, UK), the 5-HT_{2C} agonist TFMPP (RBI, USA), the 5-$HT_{2A/2C}$ antagonist RP 62203 (Rhône-Poulenc, France), and the 5-HT_{2A} antagonist SR 46349 B (Sanofi, France). Before injections, TFMPP, SR 46349B, and BRL 46470A were dissolved in sterile saline. Ipsapirone was dissolved in distilled water, while RP 62203 was dissolved in a 10% ethanol, 40% propylene glycol, and 50% distilled water solution. SR46349B and RP62203 were sonicated for 10 min. All drugs were injected (1.0 ml/kg IP) 30 min before testing.

Procedure

The procedure was described elsewhere (15). Briefly, rats were placed in the central square facing a closed arm, and allowed to 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 conventional measures (number of entries into open and closed arms, and the time spent on open and closed arms) were recorded. In addition, the time displaying the following behavioral categories was measured—scanning: protruding the head over the edge of an open arm and scrutinizing in any direction (it includes head dipping); risk assessment: exiting a closed arm with the forepaws and head only, and investigating the surroundings (this behavior was often, but not necessarily, accompanied by body stretching); rearing: rising on the hind limbs; grooming: cleaning any part of the body surface with the tongue, teeth, and/or forepaws. Finally, the number of entries at the distal (end) part of the open arms (end exploring) was also recorded. All groups studied had 12 rats, but animals that fell off the maze during the sessions were discarded. Each drug was studied with its own control group.

Data Analysis

For each animal, the total number of entries (open + closed arms), the percentage of open-arm entries (100 × open/total), and the time spent in the open arms were calculated and analyzed by one-way analysis of variance (ANOVA). Whenever ANOVA was significant, the Dunnett's test for multiple comparisons of individual groups with a control was performed. The level of statistical significance was $p < 0.05$. Thus, ipsapirone was compared to distilled water control, TFMPP, SR 46349B, and BRL 46470A to saline control, and RP 62203 to ethanol–propylene glycol solution control.

RESULTS

Figure 1 shows the effect of drugs on conventional anxiety indexes in the elevated plus-maze. ANOVA (Fig. 1A) indicated significant difference among TFMPP, SR 46349 B, BRL 46470 A, and saline control, $F(9, 112) = 1.915$, $p < 0.05$. Post hoc comparisons showed a significant decrease in the percentage of open-arm entries for TFMPP (0.4 mg/kg, $p < 0.05$) and SR 46349 B (10 mg/kg, $p < 0.05$), but not for BRL 46470 A ($ps > 0.05$). Neither ipsapirone, $F(3, 42) = 0.226$, $p > 0.05$, nor RP 62203, $F(3, 41) = 0.629$, $p > 0.05$, significantly changed this measure. A similar pattern was observed on the time spent in open arms (Fig. 1B). Thus, ipsapirone, $F(3, 42) = 0.067$, $p > 0.05$, and RP, $F(3, 41) = 0.467$, $p > 0.05$, failed to alter the time spent in the open arms, whereas TFMPP (0.1 and 0.4 mg/kg) and SR 46349 B (at all doses) significantly decreased this measure, $F(9, 112) = 4.760$, $p < 0.05$; Dunnett's test, $ps < 0.05$. Control injection with the ethanol–propylene glycol solution also decreased the time spent in the open arms, $F(2, 32) = 5.915$, $p < 0.05$, when compared to saline controls.

Figure 2 shows the effects of drugs on risk-assessment and scanning. Ipsapirone (0.75 and 2.25 mg/kg) significantly decreased risk assessment, $F(3, 42) = 19.872$, $p < 0.05$; Dunnett's test, $ps < 0.05$, and increased (at all doses) scanning, $F(3, 42) = 4.541$, $p < 0.05$; Dunnett's test, $ps < 0.05$. Conversely, SR 46349 B (at all doses) significantly decreased scanning, $F(9, 112) = 4.358$, $p < 0.05$; Dunnett's test, $ps < 0.05$, and showed a tendency to increase risk assessment. TFMPP significantly decreased scanning (0.1 and 0.4 mg/kg; $ps < 0.05$) and risk assessment (0.2 mg/kg), $F(9, 112) = 3.099$, $p < 0.05$; Dunnett's test, $p < 0.05$. Both risk assessment, $F(3, 41) = 1.289$, $p > 0.05$, and scanning, $F(3, 41) = 0.120$, $p > 0.05$, were not affected by RP 62203. Similar pattern was found for BRL 46470 A (Dunnett's test, $ps > 0.05$).

Figure 3 illustrates the effect of drugs on end-exploring and rearing. In spite of a trend to increasing (Fig. 3A), end exploring was not altered by ipsapirone, $F(3, 42) = 1.955$, $p > 0.05$, while being significantly decreased by TFMPP (0.1 and 0.4 mg/kg), SR 46349 B (at all doses), and BRL 46470 A (0.001 mg/kg), $F(9, 112) = 2.433$, $p < 0.05$; Dunnett's test, $ps < 0.05$, and increased by RP 62203 (0.25 mg/kg), $F(3, 41) = 4.906$, $p < 0.05$; Dunnett's test, $p < 0.05$. Rearing (Fig. 3B) was not altered by ipsapirone, $F(3, 42) = 0.263$, $p > 0.05$, and RP 62203, $F(3, 41) = 0.183$, $p > 0.05$, but was decreased by TFMPP (all doses), SR 46349 B (3 and 10 mg/kg) and BRL 46470 A (0.001 mg/kg), $F(9, 112) = 11.022$, $p < 0.05$; Dunnett's test, $ps < 0.05$.

Finally, none of drug treatments had a significant effect (data not shown) on both closed-arm entries [ipsapirone, $F(3,$

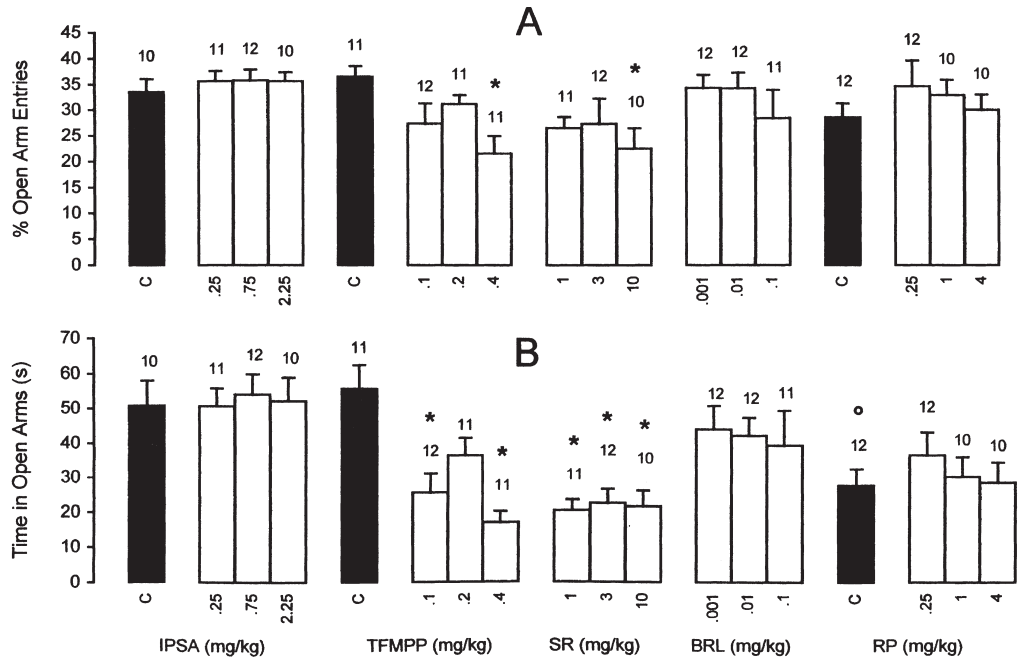

FIG. 1. Effect of the drugs on the percentage of entries (A) and on the time spent in open arms (B). Bars represent the mean and the vertical lines the SEM. Number of subjects are indicated above the bars. *Indicates a significant difference from the respective control (first control bar to the left of the dose bars) and ○ indicates a significant difference from the saline control (Dunnett's test for multiple comparisons with a control group, $p < 0.05$).

39) = 0.787, $p > 0.05$; TFMPP, SR 46349 B, and BRL 46470 A, $F(9, 103) = 0.086$, $p > 0.05$; RP 62203, $F(3, 38) = 2.262$, $p > 0.05$] and time spent grooming [ipsapirone, $F(3, 42) = 1.130$, $p > 0.05$; TFMPP, SR 46349 B, and BRL 46470 A, $F(9, 112) = 1.194$, $p < 0.05$; RP 62203, $F(3, 41) = 0.456$, $p > 0.05$].

DISCUSSION

5-HT$_{1A}$ Agonist

In agreement with previously reported results (3,12–14, 18,41,44,45,49), the 5-HT$_{1A}$ agonist ipsapirone had no effect on the conventional anxiety parameters in the elevated plus-maze. In contrast, both anxiolytic (1,14,52) and anxiogenic (12,13,32,40,43,45) effects of 5-HT$_{1A}$ agonists have also been reported in the elevated plus-maze. Such inconsistencies have led some authors (40,41) to suggest that the plus-maze test is either insensitive to 5-HT ligands or is measuring a mechanism in rodents that is unrelated to human anxiety.

Although ipsapirone failed to influence the conventional measures in the elevated plus-maze, this drug decreased risk assessment and increased scanning. As scanning is negatively correlated, and risk assessment positively correlated with anxiety (15), our ethological data suggest an anxiolytic profile for this 5-HT$_{1A}$ antagonist. Similar anxiolytic effects have been reported for buspirone in the murine elevated plus-maze (10).

Despite a clear trend, none of the doses of ipsapirone significantly increased end exploring. In contrast, other results (58) showed that ipsapirone decreased the time spent on the distal (extremity) portions of the open arms. This effect, however, was observed at one dose only (0.1 mg/kg) that was much smaller than those used in the present study, ranging from 0.25 to 2.25 mg/kg.

The number of closed-arm entries and the time spent rearing were not changed by ipsapirone, indicating that the drug did not affect behaviors related to locomotor activity. Regarding rearing, there are reports showing a decrease in the frequency of this activity after administration of the 5-HT$_{1A}$ agonist flesinoxan (49). However, it is difficult to compare the data because the authors scored the frequency instead of the time spent rearing measured in the present experiment.

5-HT$_{2C}$ Agonist

TFMPP presented an anxiogenic profile on both conventional and ethological parameters. As this compound did not affect closed-arm entries, despite a reduction in the time spent rearing, the decrease in open-arm entries at a dose of 0.4 mg/kg was not due to locomotor impairments but to increases in the open-arm aversion. In addition, TFMPP at doses of 0.1 and 0.4 mg/kg, but curiously not at a dose of 0.2 mg/kg, reduced the time spent in the open arms. Similar anxiogenic effects were observed in the elevated plus-maze for TFMPP (27) and 1-(3-Chlorophenyl)piperazine (mCPP) (21). Besides, there are results (37) showing TFMPP to exhibit both anxiolytic (impairing escape responses) and anxiogenic (facilitation of inhibitory avoidance) effects in an elevated T-maze.

TFMPP reduced the time spent scanning, but this anxiogenic effect was not accompanied by changes in risk assessment (except a curious reduction in the middle of the dose range tested for which we have no explanation). In a previous study (15), anxiogenic drugs (pentylenetIetrazol and FG 7142) also decreased scanning without producing symmetrical changes in risk assessment. Conversely, benzodiazepines (nitrazepam and midazolam), like ipsapirone in the present exper-

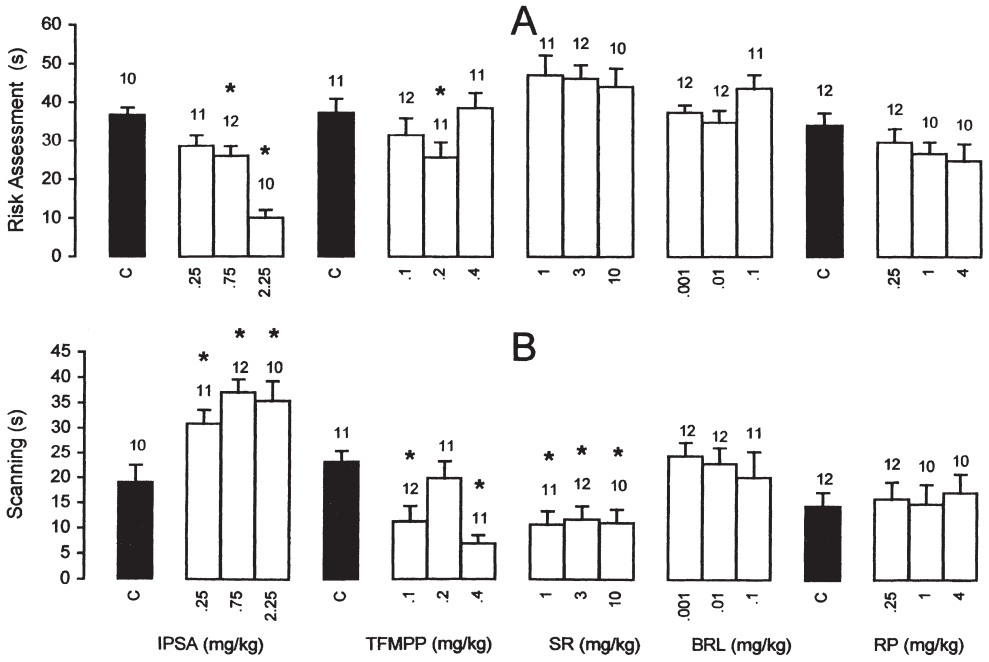

FIG. 2. Effect of the drugs on risk assessment (A) and scanning (B). Bars represent the mean and the vertical lines the SEM. Number of subjects are indicated above the bars. *Indicates a significant difference from the respective control (first control bar to the left of the dose bars) (Dunnett's test for multiple comparisons with a control group, $p < 0.05$).

iment, reduced risk assessment while increasing scanning. It seems, therefore, that risk assessment is less sensitive than scanning for detecting anxiogenic effects in the elevated plus-maze.

Finally, TFMPP decreased end exploring, thereby confirming its anxiogenic effect.

5-HT$_{2A}$ Antagonist

SR 46349 B significantly reduced scanning, end exploring, and the time spent in open arms. This anxiogenic effect, which was observed in the absence of locomotor impairments, was not again detected by the risk assessment. Similar anxiogenic effects have been reported for 5-HT$_{2A}$ antagonists (5,45), whereas other studies have found a lack of activity (22,51,53,58) and even anxiolytic effects (1,13,54). The reason for this discrepancy remains unclear.

5-HT$_{2A/2C}$ Antagonist

In the present study, RP 62203 had no effect whatever on the percentage of open-arm entries, time spent in the open arms, risk assessment, scanning, and rearing. The effect of this compound was limited to a single increase on end exploring at a dose of 0.25 mg/kg, a result that seems insufficient to suggest an anxiolytic-like profile.

5-HT$_3$ Antagonist

BRL 46470 A had no effect on both conventional and ethological parameters. The only observed effect of this compound was a reduction on end exploring (and even so at one dose only, 0.1 mg/kg). Like RP 62203, this modest effect seems insufficient to attribute any selective effect for this compound. Similar negative findings have been reported for 5-HT$_3$ antagonists in the elevated plus-maze (3,11,20,29–31,47), whereas other studies have shown anxiolytic-like effects (2,3,5,11,16,27).

DISCUSSION

The conventional plus-maze is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABA$_A$–benzodiazepine complex [for review, see (48)]. However, this model produces contradictory data when evaluating serotonergic drugs. The present results indicated that some ethologically derived parameters were more sensitive than the conventional indexes for the study of 5-HT ligands in the rat elevated plus-maze. This result is consistent not only with findings in the murine elevated plus-maze (9,10,51), but also with several other ethopharmacological procedures [for review, see (50)].

Risk assessment and scanning were particularly useful in detecting anxiolytic effects of the 5-HT$_{1A}$ agonist ipsapirone. Similar results were recently reported in another ethological version of the rat elevated plus-maze test (23).

The anxiogenic effects of both TFMPP and SR 46349 B were detected by time spent on open arms, end exploring, and scanning, but not by risk assessment. This observation for TFMPP and SR 46349 B is similar to those of Cruz and colleagues (15). They reported that pentilenetetrazol and FG 7142 also failed to change risk assessment in the rat elevated plus-maze, whereas benzodiazepines (like ipsapirone in the present experiment) markedly reduced this measure and increased scanning. It seems, therefore, that anxiogenic compounds decrease scanning without necessarily producing a symmetrical increase in risk assessment. The present findings seem to support this conclusion based on the effects of 5-HT ligands.

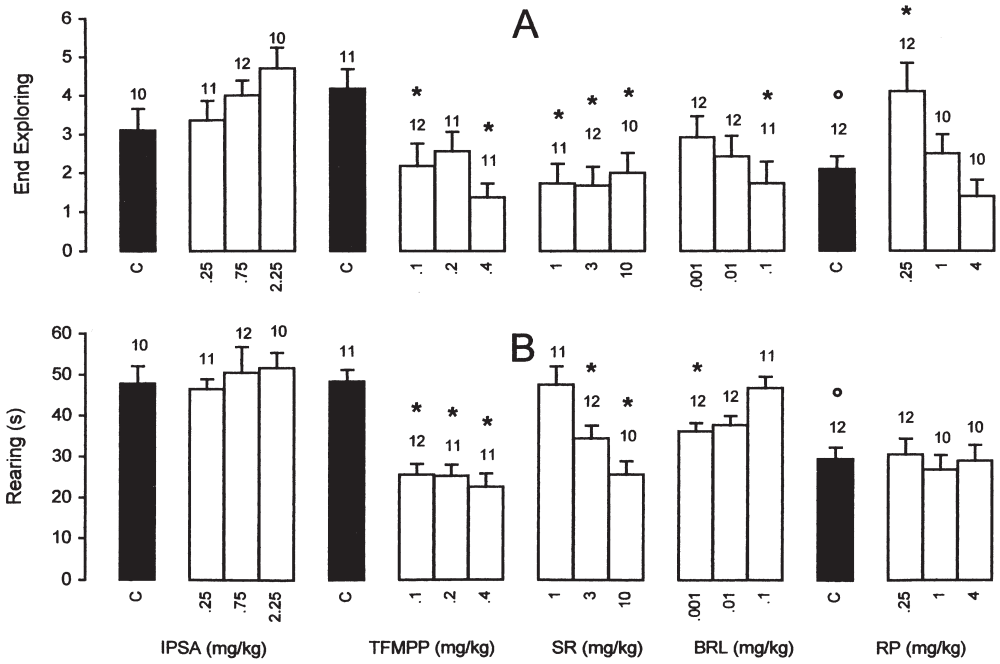

FIG. 3. Effect of the drugs on end exploring (A) and rearing (B). Bars represent the mean and the vertical lines the SEM. Number of subjects are indicated above the bars. *Indicates a significant difference from the respective control (first control bar to the left of the dose bars) and ○ indicates a significant difference from the saline control (Dunnett's test for multiple comparisons with a control group, $p < 0.05$).

BRL 46470 A and RP 62203 showed no effects on the anxiety measures (except for very little changes in end exploring). Similarly, the effects of some drugs interacting with 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptor subtypes were not convincingly established in a recent study using both conventional and ethological measures of the rat elevated plus-maze (23). Also, none of the drug treatments had a significant effect in grooming. In a previous study (15), grooming (a variable that loaded on a factor other than anxiety) was not modified by both anxiolytic and anxiogenic drugs acting at different sites on the $GABA_A$–benzodiazepine receptor complex.

In fact, 5-HT ligands have been found to produce effects ranging from anxiolysis, through no effect, to anxiogenesis in several animal models [for reviews, see (25,50,55)]. In the elevated plus-maze, such inconsistencies may be a consequence of interlaboratory differences in the use of the test including: the way of scoring behaviors, the amount and type of previous manipulation and handling, presence or not of edges along the open arms, maze experience, level of illumination, duration of sessions, animal species, strains, number of animals kept per cage, time of the day in which the sessions occur, and other interference sources [(17); for reviews, see (28,48)]. The reasons for the larger vulnerability of 5-HT ligands to these methodological factors remain unclear.

In addition to these methodological issues, there is the intriguing possibility that the same levels of anxiety may produce different results in the elevated plus-maze. It has recently been shown (38,39) that different aversive stimuli (like being isolated for 24 h, transported in metal carts, or spending 24 h in different vivaria) reduce exploratory behaviors of rats in the open arms. Paradoxically, when two of these aversive stimuli are combined (for instance, isolation and novelty), the decrease in exploratory behavior caused by either of two aversive manipulations alone is reverted and the open-arm entries increase up to the level of control rats. Therefore, different methodological conditions may produce different levels of anxiety that may distort drug effects. For example, risk-assessment behavior may be reduced by benzodiazepine-like anxiolytics, as would be expected because this measure correlates positively with anxiety. However, experimental procedures that produce high levels of anxiety could also decrease or even prevent risk assessment patterns in the elevated plus-maze (e.g., inducing immobility in the closed arms). To this respect, Blanchard's group has shown that under a baseline of freezing (high fear), anxiolytics will increase risk assessment, but under a baseline characterized by high levels of risk assessment (lower fear), they will reduce such responses (6–8). Therefore, high levels of anxiety can also block the expression of risk-assessment patterns in the elevated plus-maze. If this is the case, then other anxiety indexes (conventional and/or ethological) should be used to such effects.

Despite such observations, the present results suggest that ethological measures may sometimes be more sensitive than the standard ones. It is suggested that these measures be used along with classic parameters in measuring serotonergic manipulations in the elevated plus-maze.

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