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Risk Assessment Behaviour: Evaluation of Utility in the Study of 5-HT-Related Drugs in the Rat Elevated Plus-Maze Test

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GRIEBEL, G., R. J. RODGERS, G. PERRAULT AND D. J. SANGER. *Risk assessment behaviour: Evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test.* PHARMACOL BIOCHEM BEHAV **57**(4) 817–827, 1997.—The present study compared the effects of a wide range of 5-hydroxytryptamine (5-HT)-modulating and potential anxiolytic agents in the rat elevated plus-maze using spatiotemporal (i.e., open arm time and entries) and ethologically derived measures (i.e., risk assessment activities and directed exploration). The drugs used were 5-HT_{1A} receptor partial (buspirone and ipsapirone) and full (8-OH-DPAT and flesinoxan) agonists, mixed 5-$HT_{2A/2C}$ receptor antagonists (ritanserin, ketanserin, mianserin, and pirenperone), selective 5-HT_3 receptor antagonists (ICS 205-930, MDL 72222, ondansetron, and (*R*S)-zacopride), and selective (fluoxetine, fluvoxamine, and zimelidine) and nonselective (imipramine) 5-HT reuptake inhibitors. Only buspirone and mianserin produced effects indicative of an anxiolytic-like action on the spatiotemporal measures. However, all 5-HT_{1A} receptor ligands, as well as mianserin, ketanserin, ondansetron, and zacopride, decreased the number of aborted attempts at entry into open arms (risk assessment). In addition, buspirone, mianserin, and zacopride increased head-dipping (directed exploration). Among the 5-HT reuptake inhibitors, zimelidine reduced head-dipping and total entries. The present findings demonstrate that risk assessment responses are sensitive to the action of 5-HT_{1A} receptor ligands, but their modulation by drugs targetting 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptors was not convincingly established. © 1997 Elsevier Science Inc.

5-HT-interacting drugs Anxiety Elevated plus-maze Rat Risk assessment

THE discovery of buspirone's efficacy in treating generalized anxiety disorder (10), together with the subsequent finding that it alters 5-hydroxytryptamine (5-HT) neurotransmission (20) through an action at 5-HT_{1A} receptors (12), rekindled interest in the role of the 5-HT system in anxiety. The identification and characterization of multiple 5-HT binding sites in brain tissues (21) and the synthesis of selective ligands for these receptors were the starting points in the mid-1980s for numerous studies investigating the behavioural effects of 5-HT-related drugs in animal models of anxiety (15). Overall, these studies showed that the anxiolytic-like effects of 5-HT drugs are weaker and considerably more variable than those of the benzodiazepines (BZs) (13), with some models frequently yielding paradoxical effects (15).

Among these models, the elevated plus-maze is particularly remarkable for the variability in the pattern of results that has been reported for 5-HT agents. The effects of buspirone and related compounds have been extensively assessed in this test [for recent reviews, see (15,32)], which is based upon the high thigmotactic response of rodents in novel environments (47). A number of research groups have found that these drugs display anxiolytic-like effects in this test [e.g.,

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(41)], whereas others have reported a lack of activity or even an anxiogenic-like profile [e.g., (26)]. Variable effects have also been reported for drugs targetting other 5-HT receptors. For example, although the 5-$HT_{2A/2C}$ receptor antagonist ritanserin was initially found to have anxiogenic-like activity (28), a subsequent study has reported anxiolytic-like effects of the drug (45). Despite these findings, however, the majority of reports indicate that ritanserin fails to modify the behaviour of rodents exposed to the plus-maze test (11,24,37,42,50). Similarly, although several laboratories have not been able to detect anxiolytic-like effects of 5-HT_3 receptor antagonists, other reports have suggested that these agents reduce open arm avoidance [for review, see (15)].

The reasons for these variable and sometimes paradoxical responses have been discussed extensively. For instance, it has been suggested that they may at least partially be due to variation in dose ranges or routes of administration (41,46). Others have suggested that the inconsistencies may be explained on the basis that the elevated plus-maze detects multiple effects of drugs interacting with the 5-HT system (19). Finally, it has been proposed that a more detailed analysis of behaviour may yield a clearer picture of the profile displayed by 5-HT drugs in the elevated plus-maze (30). On the basis of extensive observations on mice, Rodgers and coworkers designed a plus-maze ethogram based upon a range of behavioural acts and postures displayed in this test, including both conventional and novel measures (33). A recent factor analytic study (36) demonstrated that a subset of these behaviours appears to reflect risk assessment (RA), a concept that has emerged from parallel work on antipredator defense in rodents. RA refers to a pattern of responses (scanning, stretch attend, flat back approach) invariably observed in potentially dangerous situations (1,16). In the murine plus-maze, the most prominent RA measure is the stretched attend posture (SAP), a behaviour that has been of particular interest because it has been shown to be more sensitive to the effects of classical (i.e., BZ receptor ligands) and atypical (i.e., 5-HT_{1A} receptor ligands) anxiolytics than are the traditional indices of anxiety (6,7,31,33,34). Although two recent studies on the effects of BZ receptor ligands in rats have generally failed to confirm that the SAP is superior to traditional indices of anxiety (8,18), Shepherd and colleagues (40) have provided some evidence from the rat "zero-maze" that, as for mice, this measure may be more sensitive (than open arm avoidance parameters) to the anxiety-modulating effects of 5-HT receptor ligands.

In an attempt to explore further the possibility that a more detailed analysis of behaviour may yield a clearer picture of the profile displayed by 5-HT drugs in the elevated plus-maze, as was shown in mice (6,7,31,33,34), the present study used an ethologically oriented scoring method to investigate the effects of a wide range of 5-HT-modulating agents in rats. The focus was on RA responses (aborted attempts at entry into open arms, which included SAPs) and on directed exploration (head-dipping). The drugs used include 5-HT_{1A} receptor partial and full agonists, mixed 5-$HT_{2A/2C}$ receptor antagonists, selective 5-HT_3 receptor antagonists, and selective and nonselective 5-HT reuptake inhibitors (Table 1). The specificity of drug response was additionally examined by measuring spontaneous locomotion in activity cages in separate groups of animals.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats weighing 180–220 g at the time of testing were used. All animals were housed in groups of five and maintained under standard laboratory conditions with free access to food and water. They were kept on a 12 L:12 D cycle with light onset at 0600 h. Animals were bred and provided by Charles River France (Saint-Aubin-les-Elbeuf, France) and Iffa Credo (L'Arbresle, France).

TABLE 1

5-HT-INTERACTING COMPOUNDS AND DOSE RANGES (mg/kg) USED IN THE PRESENT STUDY

	Activity on 5-HT neurotransmission	Elevated plus-maze	Actimeter
8-OH-DPAT	5-HT_{1A} receptor full agonist	0.1–1	0.03–3
Flesinoxan	5-HT_{1A} receptor full agonist	0.1–1	0.03–3
Buspirone	5-HT_{1A} receptor partial agonist	0.1–1	0.03–3
Ipsapirone	5-HT_{1A} receptor partial agonist	1–10	0.3–30
Ritanserin	5-$HT_{2A/2C}$ receptor antagonist	1–10	0.1–10
Mianserin	5-$HT_{2A/2C}$ receptor antagonist	0.3–3	0.1–10
Ketanserin	5-$HT_{2A/2C}$ receptor antagonist	0.1–1	0.03–3
Pirenperone	5-$HT_{2A/2C}$ receptor antagonist	0.01–0.1	0.01–1
Zacopride	5-HT_3 receptor antagonist	0.01–0.1	0.003–0.3
Ondansetron	5-HT_3 receptor antagonist	0.01–0.1	0.003–0.3
ICS 205-930	5-HT_3 receptor antagonist	0.01–0.1	0.003–0.3
MDL 72222	5-HT_3 receptor antagonist	0.01–0.1	0.003–0.3
Imipramine	5-HT/noradrenaline reuptake inhibitor	1–10	0.3–30
Fluoxetine	5-HT reuptake inhibitor	1–10	0.3–30
Fluvoxamine	5-HT reuptake inhibitor	1–10	0.1–10
Zimelidine	5-HT reuptake inhibitor	1–10	0.3–30

In the elevated plus-maze, n = 6–9 in each group, with the exception of the 5-HT reuptake inhibitors, 8-OH-DPAT, and buspirone, for which n = 11 or 12. In the actimer, n = 8 in each group.

Drugs

All drugs were prepared as solutions or suspensions in physiological saline containing one or two drops of Tween 80. They were given in a constant volume of 2 ml/kg. The drugs used were 8-OH-DPAT, buspirone, ipsapirone, mianserin, (*RS*)-zacopride, ondansetron, fluoxetine, zimelidine (synthesized by the Department of Chemistry, Synthélabo Recherche, Bagneux, France), ritanserin, ketanserin, pirenperone (gifts from Janssen, Beerse, Belgium), flesinoxan, fluvoxamine (both gifts from Solvay Duphar, Weesp, The Netherlands), ICS 205-930, MDL 72222 (purchased from RBI, Natick, MA, USA), and imipramine (purchased from Sigma, Saint Quentin Fallavier, France). Drugs were injected subcutaneously (SC) 30 min before experiments were carried out. Doses are expressed as the bases.

Behavior on the Elevated Plus-Maze

All parts of the apparatus were made of dark polyvinyl plastic with a black rubber floor. It consisted of a maze elevated to a height of 50 cm with two open (50 × 10 cm) and two enclosed arms (50 × 10 × 50 cm), arranged so that arms of the same type were opposite each other, connected by an open central area (10 × 10 cm). To avoid rats falling off, a rim of Plexiglas (0.5 cm high) surrounded the perimeter of the open arms. The illumination in the experimental room consisted of one red neon tube fixed on the ceiling, so that experiments were performed under dim light conditions. At the beginning of the experiment, rats were placed in the centre of the maze, facing one of the enclosed arms, and observed for 4 min. The apparatus was equipped with infrared beams and sensors capable of measuring time spent in open arms, number of open arm entries, and number of closed arm entries (entry defined as entry of all four limbs into an arm of the maze). In addition, rats were observed via video link by an observer located in an adjacent room. This permitted the recording of the more ethologically oriented measures: (a) attempt: attempt at entry into open arms followed by avoidance responses, including SAP (the rat stretches forward and retracts to original position); (b) head-dipping: protruding the head over the ledge of an open arm and down towards the floor (this response can occur while the animal's body is in the closed arms, central square, or open arms). The results were expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms, mean total number of open arm entries, mean total number of entries in both

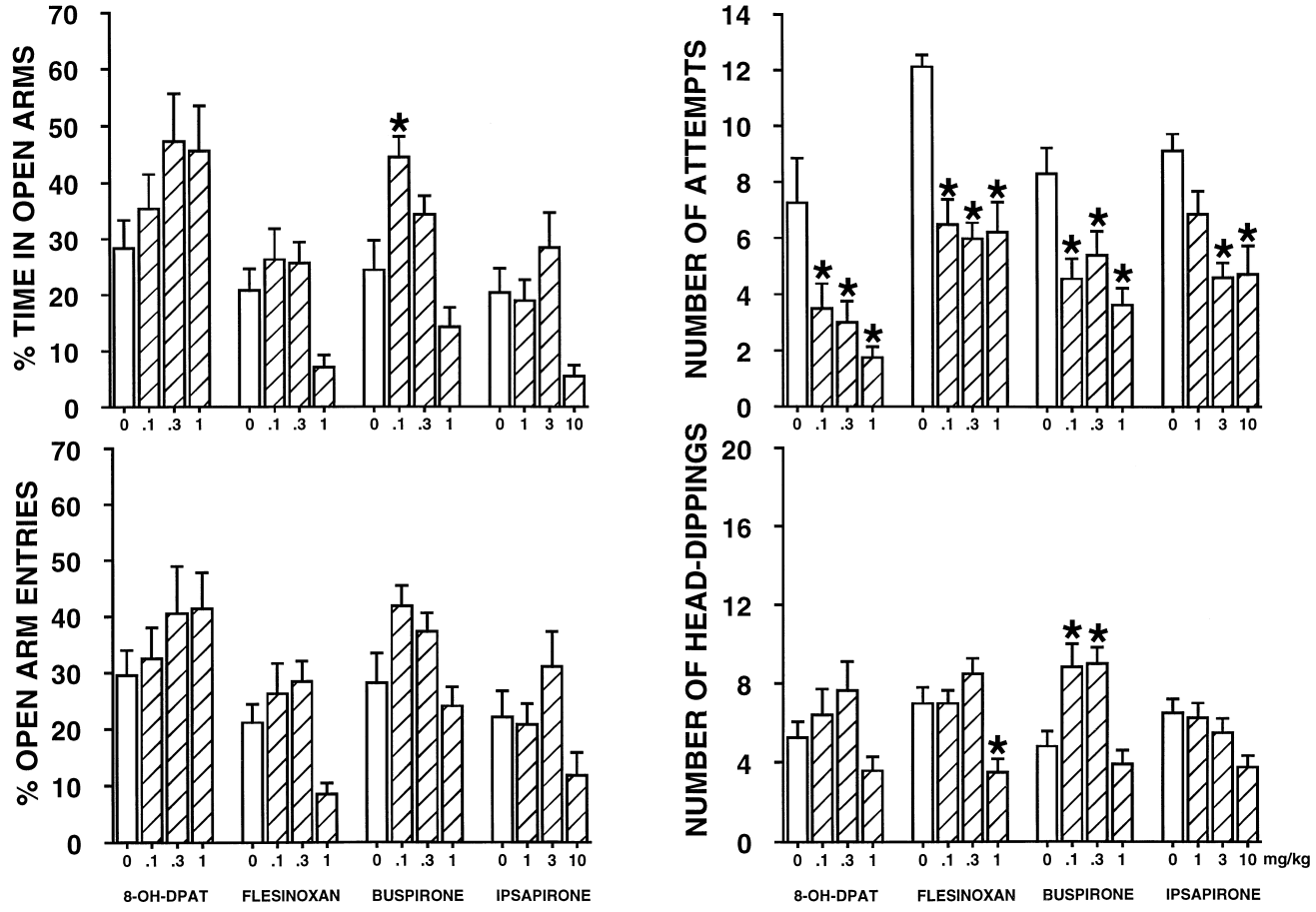

FIG. 1. Effects of four 5-HT$_{1A}$ receptor ligands on the behavior of rats exposed to the elevated plus-maze test for traditional indices of anxiety (% time in open arms, number of open arm entries) and ethologically derived measures (number of attempts, number of head-dippings). Drugs were administered SC 30 min before testing. Data represent means ± SEM. *$p <$; 0.05 (Dunnett's test).

open and closed arms, mean total number of attempts, and mean total number of head-dippings. Testing was performed between 0830 and 1300 h.

Effects on Spontaneous Locomotor Activity: The Actimeter

Testing was conducted in clear Plexiglas boxes (40 × 40 × 15 cm) equipped with infrared beams and sensors and housed in sound-attenuated cupboards. Horizontal locomotor activity was expressed as total number of beams crossed during a 5-min period. Thirty minutes after the injection, rats were placed in the centre of the apparatus. Testing was performed between 0830 and 1300 h.

Statistical Analysis

Data were analyzed by a one-way analysis of variance (ANOVA) followed by a Dunnett's a posteriori *t*-test.

RESULTS

For purposes of comparing effects of 5-HT compounds on anxiety-related responses, composite profiles for 5-HT_{1A}, 5-$HT_{2A/2C}$, 5-HT_3, and reuptake blockers are shown in Figs. 1, 3, 5, and 7. Corresponding data on total arm entries are displayed with actimeter data in Figs. 2, 4, 6, and 8. Because no significant drug effects were observed for closed arm entries, data for this measure are not shown.

5-HT_{1A} Receptor Agonists

Elevated plus-maze. Figure 1 shows that only buspirone significantly increased percentage of time spent by rats on open arms [$F(3, 42) = 7.64$, $p < 0.001$]. The percentage of open arm entries remained unaffected by all drugs. By contrast, drug treatment significantly decreased the number of attempts [8-OH-DPAT: $F(3, 43) = 5.57$, $p < 0.01$; flesinoxan: $F(3, 27) = 3.28$, $p < 0.001$; buspirone: $F(3, 43) = 6.46$, $p < 0.001$; and ipsapirone: $F(3, 27) = 5.22$, $p < 0.01$]. Buspirone [$F(3, 43) = 8.58$, $p < 0.001$] and flesinoxan [$F(3, 27) = 5.48$, $p < 0.01$], but not the other compounds, significantly affected head-dipping. The former increased the response at 0.1 and 0.3 mg/kg, whereas flesinoxan decreased head-dipping at 1 mg/kg. Figure 2 shows that the total number of arm entries was reduced by 8-OH-DPAT [$F(3, 43) = 6.40$, $p < 0.01$], bus-

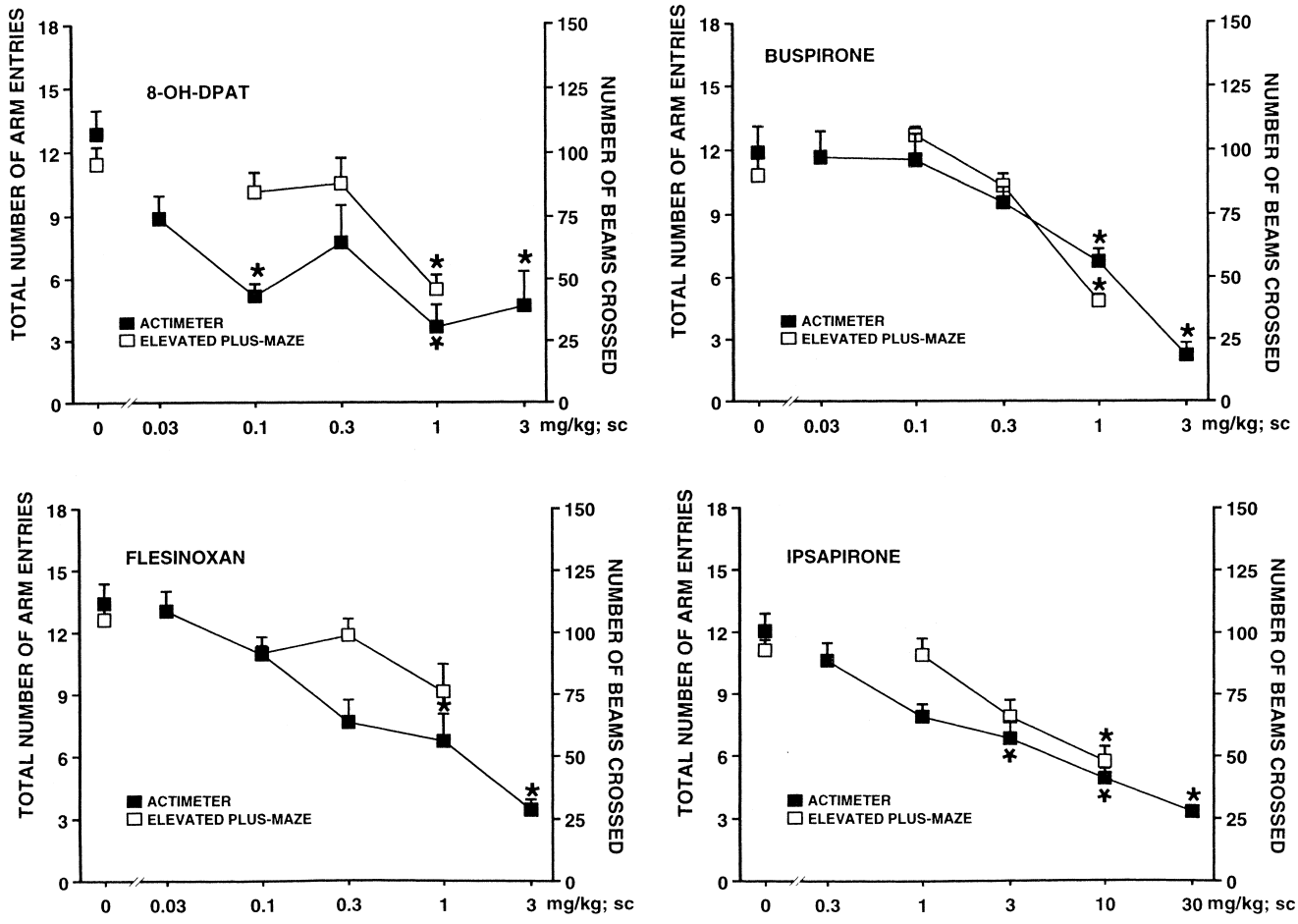

FIG. 2. Effects of four 5-HT_{1A} receptor ligands on horizontal locomotor activity in an actimeter (solid symbols) and on total number of arm entries in the elevated plus-maze test (open symbols). Drugs were administered SC 30 min before testing. Data represent means ± SEM. *$p <$ 0.05 (Dunnett's test).

pirone [$F(3, 42) = 19.81$, $p < 0.01$], and ipsapirone [$F(3, 27)$ 6.43, $p < 0.01$], but not flesinoxan.

Spontaneous locomotor activity (Fig. 2). Activity was significantly affected by all compounds [8-OH-DPAT: $F(4, 41) = 6.75$, $p < 0.001$; flesinoxan: $F(4, 41) = 16.07$, $p < 0.001$; buspirone: $F(5, 42) = 16.29$, $p < 0.001$; and ipsapirone: $F(4, 41) = 24.54$, $p < 0.001$]. Dunnett comparisons indicated a dose-dependent decrease in the number of beams crossed for flesinoxan, buspirone, and ipsapirone. 8-OH-DPAT decreased the activity at all doses, but this effect was not significant at 0.03 and 0.3 mg/kg.

Mixed 5-HT$_{2A/2C}$ Receptor Antagonists

Elevated plus-maze. Figure 3 shows that mianserin significantly increased the percentage of time spent on open arms [$F(3, 27) = 2.64$, $p < 0.001$], whereas pirenperone produced the opposite effect [$F(3, 24) = 4.17$, $p < 0.05$]. Ritanserin and ketanserin did not affect this measure. Unlike the other compounds in this series, pirenperone significantly affected the percentage of open arm entries [$F(3, 24) = 3.04$, $p < 0.05$]; the drug reduced this response at the lowest dose (0.01 mg/kg). Mianserin and ketanserin, but not the other drugs, significantly reduced the number of attempts [mianserin: $F(3, 27) = 7.84$, $p < 0.001$; and ketanserin: $F(3, 26) = 11.6$, $p < 0.001$]. Mianserin also significantly increased the frequency of head-dipping [$F(3, 27) = 7.42$, $p < 0.001$] and the total number of arm entries [$F(3, 27) = 7.99$, $p < 0.001$]. The latter responses were decreased by pirenperone [$F(4, 38) = 18.69$, $p < 0.001$] (Fig. 4).

Spontaneous locomotor activity (Fig. 4). Only ketanserin and pirenperone significantly affected locomotor activity [$F(4, 41) = 3.67$, $p < 0.01$; and $F(4, 41) = 11.66$, $p < 0.001$, respectively]. Subsequent post-test comparisons revealed that the drugs decreased this response in a significant manner at 3 and 1 mg/kg, respectively.

Selective 5-HT$_3$ Receptor Antagonists

Elevated plus-maze. Figure 5 shows that none of the compounds significantly modified the percentage of time spent on open arms and the proportion of entries made into open arms. (*RS*)-Zacopride [$F(3, 27) = 2.92$, $p < 0.05$] and ondansetron [$F(3, 26) = 4.25$, $p < 0.05$] significantly reduced the number of attempts, whereas ICS 205-930 and MDL 72222 did not modify this response. Head-dipping was significantly increased by (*RS*)-zacopride, whereas it remained unaffected by all other drugs. The total number of arm entries was not modified by any of these compounds (Fig. 6).

Spontaneous locomotor activity (Fig. 6). All four drugs belonging to this group failed to modify locomotor activity.

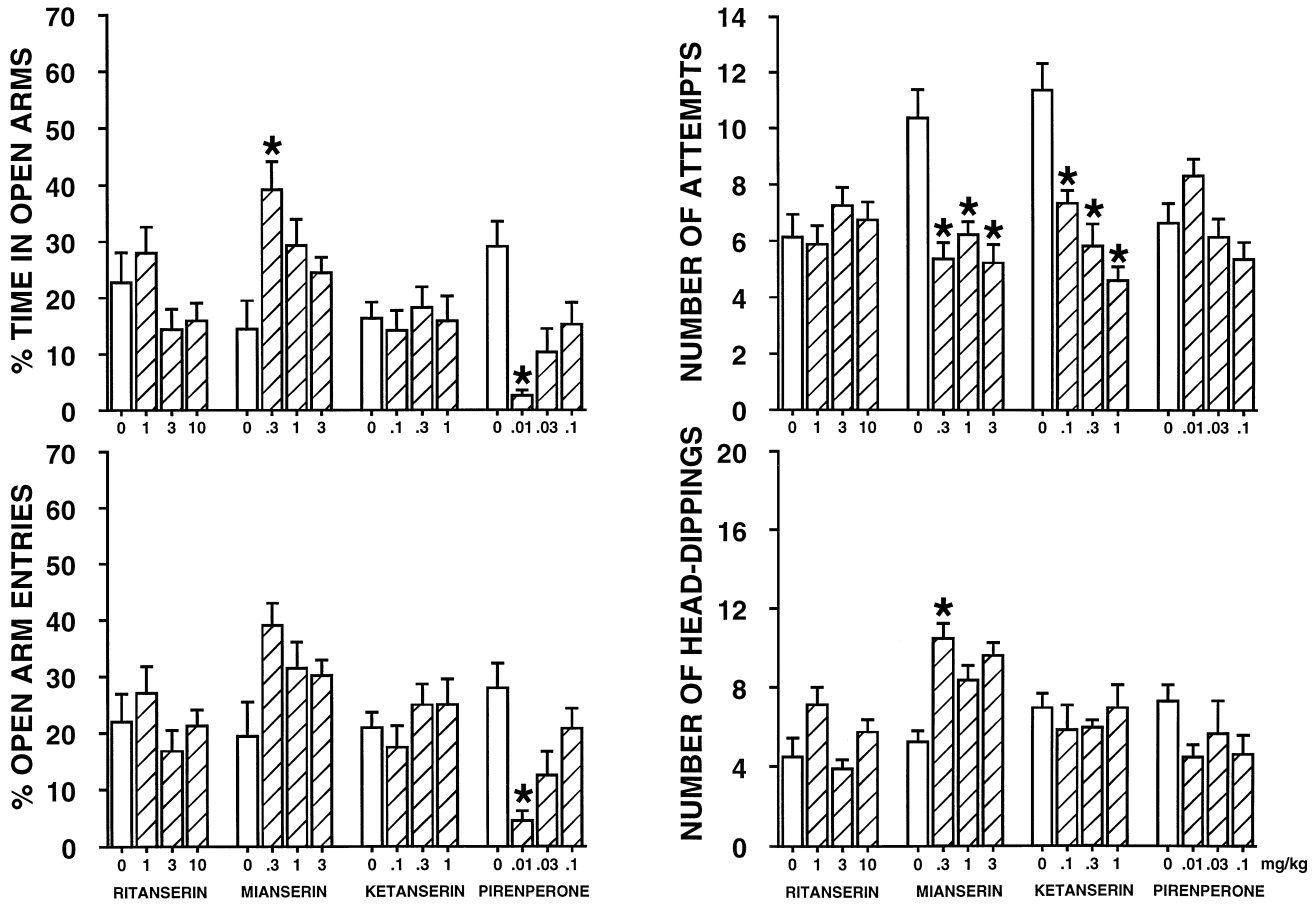

FIG. 3. Effects of four mixed 5-HT$_{2A/2C}$ receptor antagonists on the behavior of rats exposed to the elevated plus-maze test. Drugs were administered SC 30 min before testing. Data represent means ± SEM. *$p < 0.05$ (Dunnett's test).

Selective and Nonselective 5-HT Reuptake Inhibitors

Elevated plus-maze. Figure 7 shows that the proportion of time and entries in open arms were not affected by these agents. Unlike the other drugs, imipramine significantly reduced the number of attempts [$F(3, 42) = 2.86, p < 0.05$] at a single dose (1 mg/kg). Zimelidine significantly decreased head-dipping [$F(3, 43) = 3.38, p < 0.05$] and total number of arm entries [$F(3, 43) = 7.29, p < 0.001$] (Fig. 8). All other drugs failed to modify these two measures.

Spontaneous locomotor activity (Fig. 8). With the exception of zimelidine [$F(4, 41) = 7.62, p < 0.001$], the drugs did not affect baseline levels. Post-test comparisons indicated that zimelidine significantly reduced locomotor activity at 30 mg/kg.

DISCUSSION

The results of the present study agree with numerous previous reports showing that behaviour of the rat on the elevated plus-maze is sensitive to the effects of drugs affecting 5-HT neurotransmission. They also show that the inclusion of RA measures in the scoring of the plus-maze can reveal changes in behaviour in the absence of significant effects on the more conventional spatiotemporal indices.

Among the 5-HT_{1A} receptor ligands, only the partial agonist buspirone at a single low dose displayed anxiolytic-like effects on both conventional and ethological parameters. The drug increased the percentage of time spent in open arms and head-dipping, while simultaneously reducing RA. When compared with the effects obtained recently with classical BZs, such as diazepam, clorazepate, or chlordiazepoxide (18), the action of buspirone was smaller for both the conventional anxiety measures and head-dipping. Buspirone reduced attempts at all dose levels [see also (7)], whereas the BZs displayed a similar action at a single dose. In addition, differences between these drugs were apparent for total arm entries, which were increased by the BZs but not by buspirone.

The three other 5-HT_{1A} receptor ligands were devoid of clear effects on both open arm measures and on head-dipping. Flesinoxan significantly reduced head-dipping at 1 mg/kg, but this effect was accompanied by a reduction in spontaneous locomotor activity as revealed by the actimeter data. Like buspirone, all three 5-HT_{1A} receptor ligands markedly decreased attempts over the entire dose ranges. In the case of 8-OH-DPAT and ipsapirone, the reduction in attempts may have been contaminated by depressant effects. Indeed, data from the actimeter revealed that both drugs reduced locomotor activity at the doses affecting attempts, thereby suggesting that

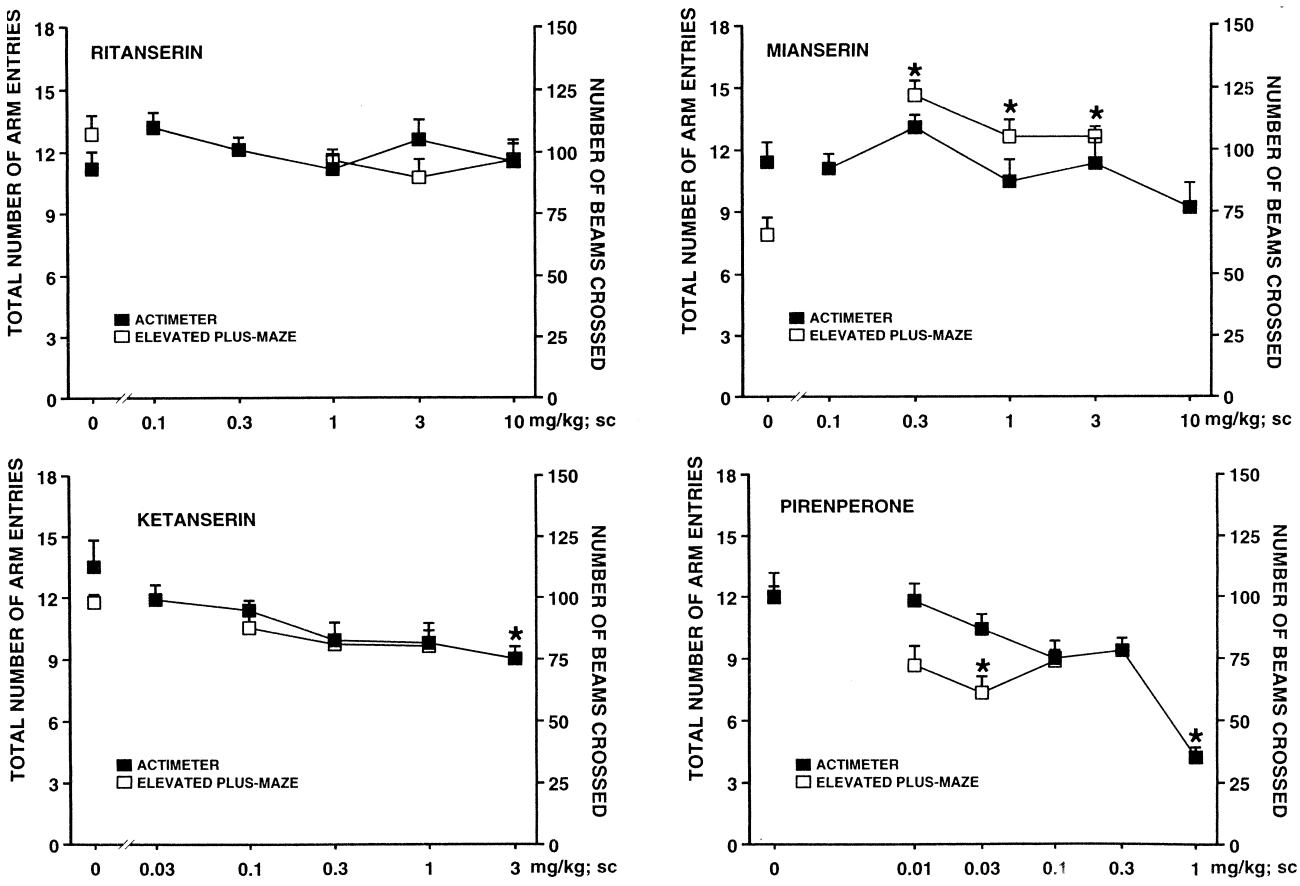

FIG. 4. Effects of four mixed 5-$HT_{2A/2C}$ receptor antagonists on horizontal locomotor activity in an actimeter (solid symbols) and on total number of arm entries in the elevated plus-maze test (open symbols). Drugs were administered SC 30 min before testing. Data represent means ± SEM. *$p < 0.05$ (Dunnett's test).

their action may be nonspecific. At least in the case of 8-OH-DPAT, these motoric effects may have been a consequence of the 5-HT syndrome (48). Overall, however, the results for attempts indicate that this RA response is particularly sensitive to the action of 5-HT_{1A} receptor ligands. Similar drug profiles have recently been obtained by Rodgers and colleagues in the murine plus-maze. These authors demonstrated that acute treatment of 5-HT_{1A} receptor ligands, including 8-OH-DPAT, buspirone, and flesinoxan, primarily affected the SAP, a behaviour included in the "attempts" parameter in the present study (7,33,34). Other studies have also reported that measures that incorporate the SAP are very sensitive to the action of 5-HT_{1A} receptor ligands (3,17,25,40). The differences in behavioural profiles between buspirone and the other 5-HT_{1A} receptor ligands are somewhat enigmatic. In view of the low effective doses, the possibility that buspirone's action involves non-5-HT mechanisms [e.g., an antagonist action on dopaminergic neurotransmission (3,44)] seems unlikely. It is possible that the differences observed among the agonists used in this study concern the relative balance of activity at pre- and postsynaptic 5-HT_{1A} receptors (38,39).

Of the mixed 5-$HT_{2A/2C}$ receptor antagonists, only mianserin showed an effect on both conventional and ethological measures. The drug increased percentage of time spent in the open arms and head-dipping at 0.3 mg/kg, and decreased attempts at all doses. In addition, it increased the total number of arm entries. In contrast, pirenperone produced an enhancement of open arm avoidance (entries and time) in the absence of an effect on RA. The drug also reduced total arm entries, an effect that may also reflect an increase in the level of anxiety (5,9). Whereas ketanserin decreased attempts at all doses, ritanserin failed to affect any behavioural measure over the dose range tested.

The profile obtained with mianserin was indicative of an anxiolytic-like action of the drug. However, this result is somewhat at variance with those reported in two studies using the elevated plus-maze in rats, in which mianserin was found to be either inactive (27) or anxiogenic (14). The doses used in those studies were higher (<10 mg/kg) than those employed in the present experiment (0.3–3 mg/kg), thereby raising the possibility that dose may be a significant factor in these discrepant findings. This view is supported by the observation that, at doses below 10 mg/kg, mianserin also produces clear anxiolytic-like effects in conflict (4,23,43) and social interaction (22) paradigms.

Because pirenperone has previously been found inactive in two conflict tests [rats: (49); monkeys: (4)], yet to have anxiolytic-like effects in a murine model of antipredator defense

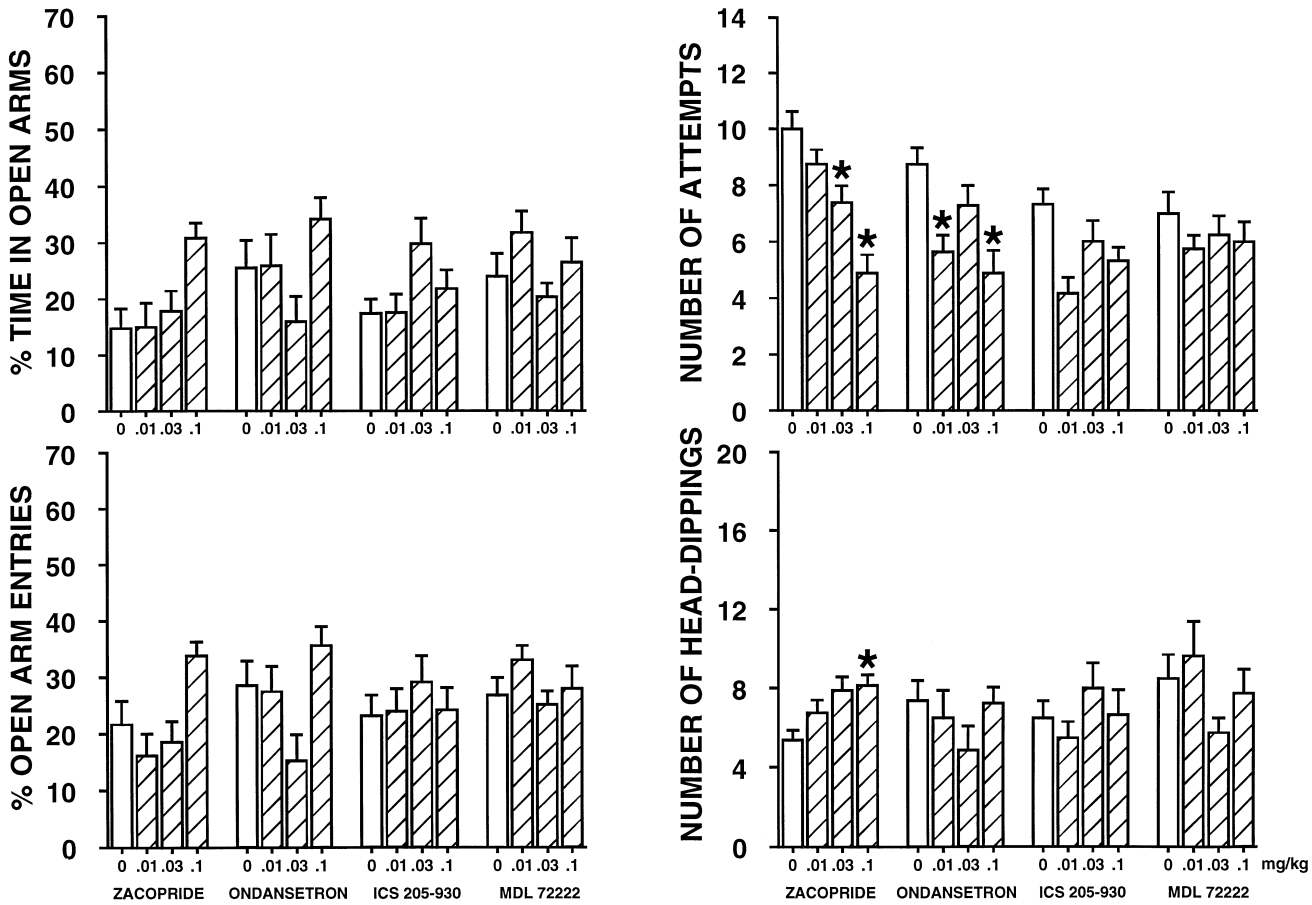

FIG. 5. Effects of four selective 5-HT_3 receptor antagonists on the behavior of rats exposed to the elevated plus-maze test. Drugs were administered SC 30 min before testing. Data represent means ±SEM. *$p < 0.05$ (Dunnett's test).

(17), the anxiogenic-like activity observed in the present study was somewhat surprising. These discrepancies cannot readily be attributed to differences in dose range (similar in all studies) or to behavioural suppression (anxiogenesis was not associated with a significant modification locomotor activity in the actimeter up to 1 mg/kg). Thus, the variable effects of this compound in animal models of anxiety warrant further investigation and may eventually be attributable to intermodel differences in the type of anxiety studied.

The present negative findings with ritanserin agree well with previous plus-maze work in rats and mice (11,24,42,50) and with a recent study using an ethological version of the murine plus-maze showing that the drug failed to alter either spatiotemporal or RA measures (37). Overall, the results with the mixed 5-$HT_{2A/2C}$ receptor antagonists revealed weak (if any) effects on the spatiotemporal measures and on head-dipping. With respect to attempts, it must be noted that control values for attempts in the mianserin and ketanserin groups were much higher than those observed in the experiments with ritanserin and pirenperone, thereby compromising a direct comparison among these four drugs. It is therefore not clear whether the profile displayed by mianserin and ketanserin can be attributed to rate-dependency factors or to a specific action of the drug on attempts.

Among the 5-HT_3 receptor antagonists, only (*RS*)-zacopride and, to a lesser extent, ondansetron, modified behaviour in the plus-maze. Both compounds affected RA responses while leaving spatiotemporal measures unchanged [see also (37,40)]. The lack of significant effects of ICS 205-930 and MDL 72222 may be due to differences in baselines, because control values of attempts for (*RS*)-zacopride and ondansetron (10.0 and 8.75, respectively) were higher than those for ICS 205-930 and MDL 72222 (7.3 and 7.0, respectively). However, in view of the effects on attempts observed with other drugs, such as the 5-HT_{1A} receptor ligands, which decreased the responses from baseline levels comparable to those of ICS 205-930 and MDL 72222, this explanation seems unlikely. Furthermore, the failure of ICS 205-930 and MDL 72222 to modify RA responses cannot be attributed to dose range, because doses employed overlapped with those reported to produce effects in other anxiety models [for review, see (15)].

The selective and nonselective 5-HT reuptake inhibitors had no effect on conventional indices of anxiety and little or no effect on RA and head-dipping. Only a single dose of imipramine (1 mg/kg) significantly reduced the number of attempts, and zimelidine decreased both head-dipping and total arm entries. Several reports have suggested that these antidepressants may potentiate fear-related responses after a single

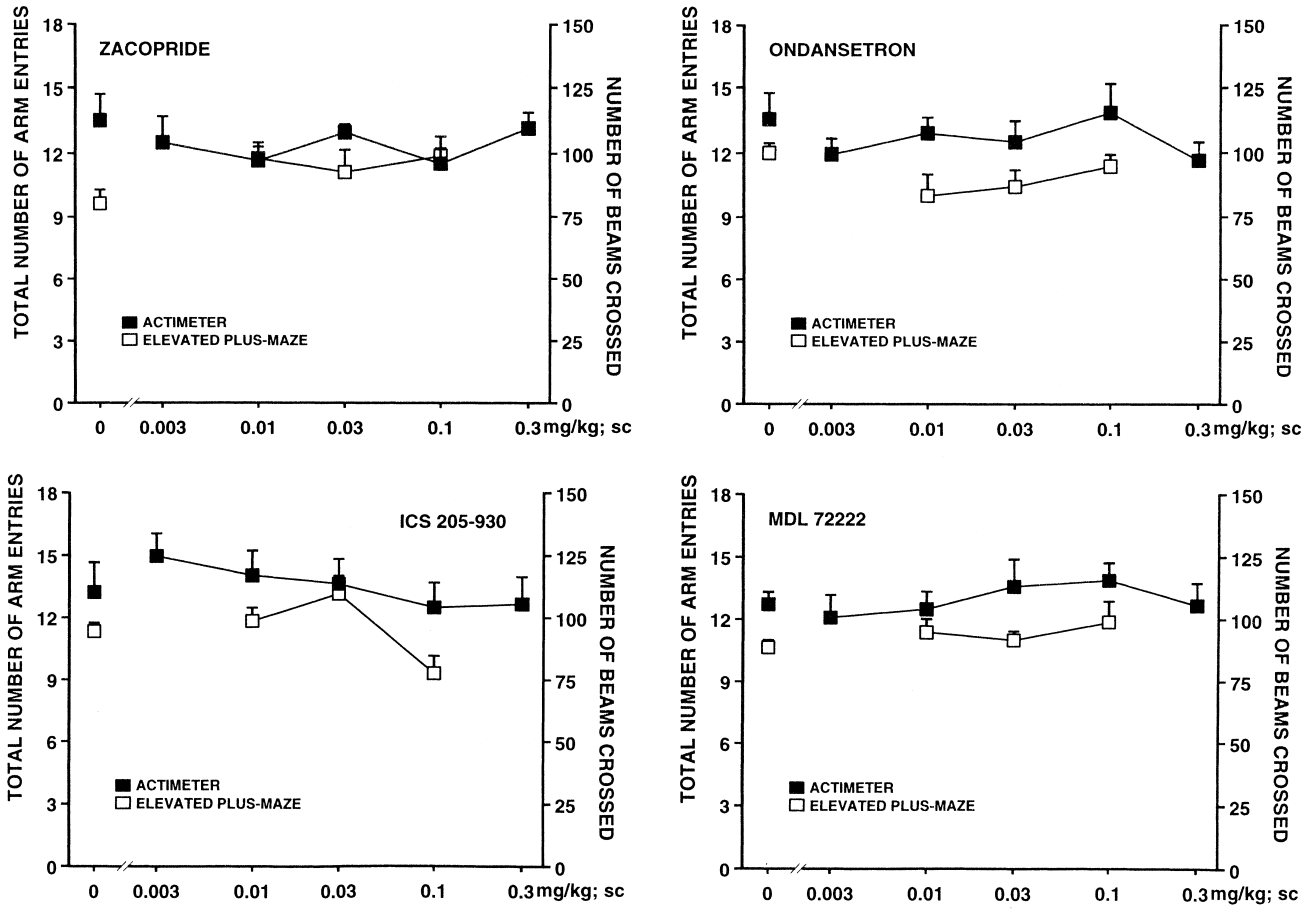

FIG. 6. Effects of four selective 5-HT_3 receptor antagonists on horizontal locomotor activity in an actimeter (solid symbols) and on total number of arm entries in the elevated plus-maze test (open symbols). Drugs were administered SC 30 min before testing. Data represent means ± SEM. No significant effects were noted.

dose [for review, see (15)]. Such a pattern was not evident in the present study for fluoxetine, imipramine, and fluvoxamine. However, in the case of zimelidine, the decrease of head-dipping observed in the absence of depressant effects on locomotor activity might indicate that the drug produced a mild anxiogenic effect. This interpretation is supported by the observation that, although not statistically significant, percentage of time spent in open arms was reduced by 50% at 3 and 10 mg/kg.

In conclusion, the present results indicate that the incorporation of RA measures into the scoring of the rat plus-maze may be useful, at least with drugs acting at 5-HT_{1A} receptors. Importantly, although the same experimental conditions were used for all compounds, variation in baseline levels somewhat compromised the interpretation of several results. The reasons for this behavioural variability remain unknown, but this result indicates that rats exposed to this test are sensitive to factors under poor experimental control. Results obtained in other ethologically based models of anxiety, including the murine plus-maze [e.g., (7)], the rat "zero-maze" [e.g., (40)], the rodent defense test battery [e.g., (2)], and a modified defensive burying paradigm (25), also indicate the utility of RA measures. Importantly, the present results suggest that a decrease in attempts (the measure of RA used in the current study) does not necessarily imply increased exploration of the open arms, as indicated by the lack of statistically significant effects on the spatiotemporal indices of most compounds affecting attempts. Thus, it is not clear yet whether an effect on RA only (e.g., 5-HT_{1A} receptor ligands) is indicative of a weak anxiolytic-like action or a behavioural disruption unrelated to anxiety. A recent factor analysis of spatiotemporal and ethological measures in the rat plus-maze showed that although RA (i.e., the stretch attend behaviour) and conventional anxiety measures loaded on the same factor, the former also loaded on a separate factor thought to be related to more cognitively oriented (i.e., decision-making) aspects of anxiety (8). This suggests that the marked effects of the 5-HT_{1A} receptor ligands on RA measures (e.g., attempts) may reflect modulation of a specific, perhaps more cognitively related, aspect of anxiety responses. There is suggestive clinical evidence that the 5-HT_{1A} agent buspirone may more effectively treat the cognitive aspects of anxiety (29). Whether or not this idea may be extended to other 5-HT receptor ligands remains to be determined.

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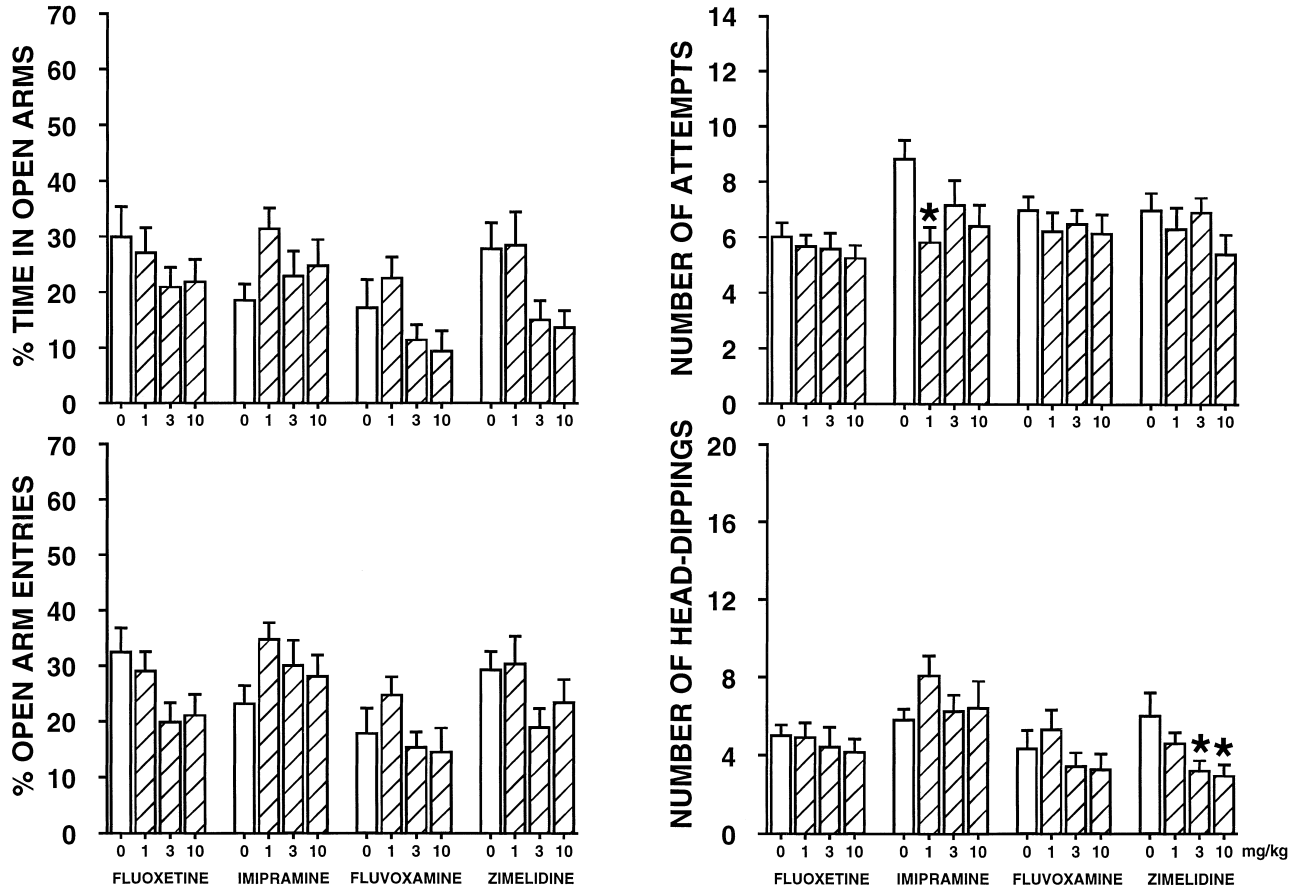

FIG. 7. Effects of three selective (fluoxetine, fluvoxamine, and zimelidine) and one nonselective (imipramine) 5-HT reuptake inhibitors on the behavior of rats exposed to the elevated plus-maze test. Drugs were administered SC 30 min before testing. Data represent means ± SEM. *$p < 0.05$ (Dunnett's test).

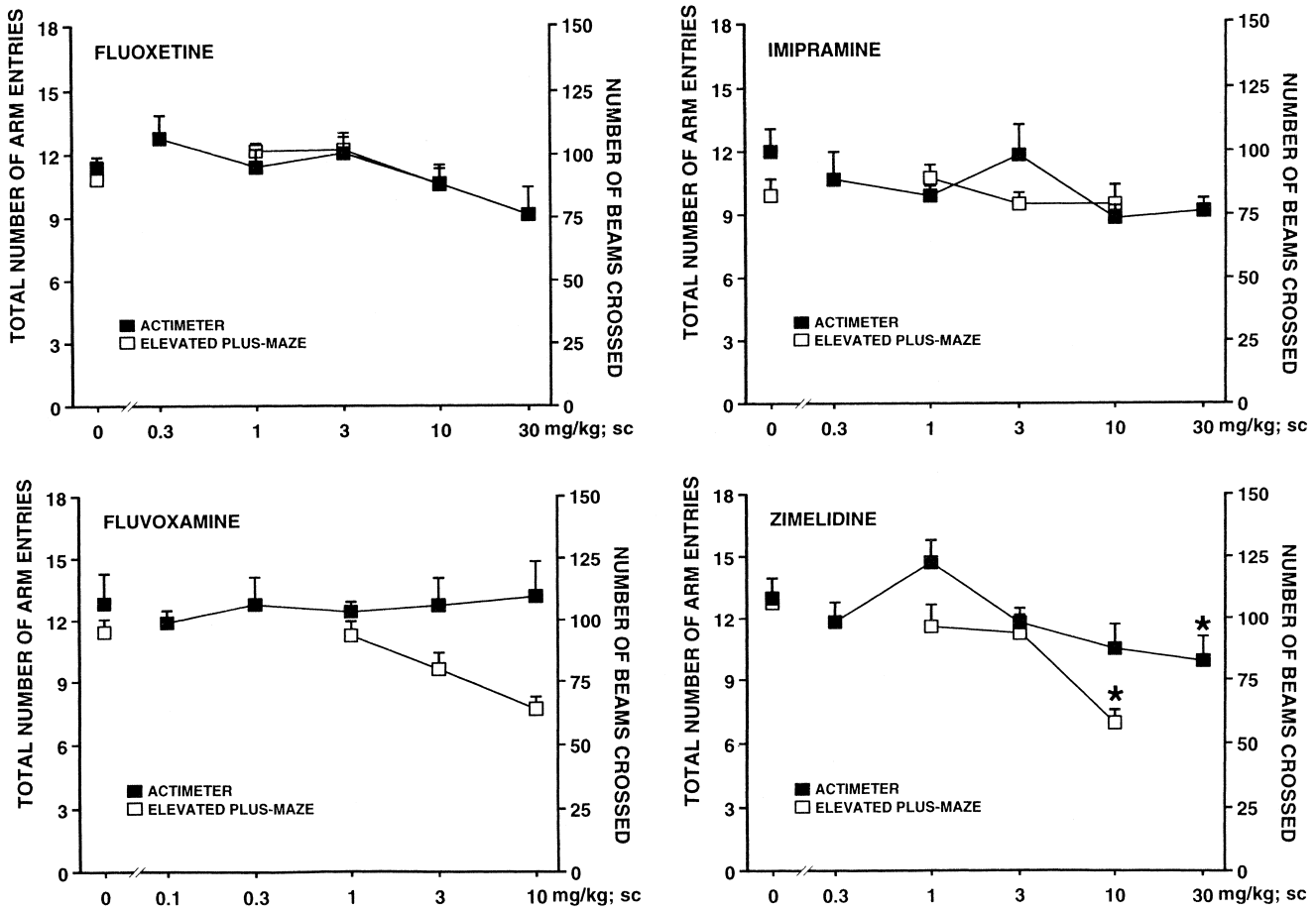

FIG. 8. Effects of three selective (fluoxetine, fluvoxamine, and zimelidine) and one nonselective (imipramine) 5-HT reuptake inhibitors on horizontal locomotor activity in an actimeter (solid symbols) and on total number of arm entries in the elevated plus-maze test (open symbols). Drugs were administered SC 30 min before testing. Data represent means ± SEM. $*p < 0.05$ (Dunnett' s test).

REFERENCES

1. 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.
2. Blanchard, D. C.; Shepherd, J. K.; Rodgers, R. J.; Blanchard, R. J.: Evidence for differential effects of 8-OH-DPAT on male and female rats in the anxiety/defense test battery. Psychopharmacology 106:531–539; 1992.
3. Bogdanov, N. N.; Bogdanov, M. B.: The role of 5-HT_{1A} serotonin and D_2 dopamine receptors in buspirone effects on cortical electrical activity in rats. Neurosci. Lett. 177:1–4; 1994.
4. Brady, L. S.; Barrett, J. E.: Effects of serotonin receptor antagonists on punished responding maintained by stimulus-shock termination or food presentation in squirrel monkeys. J. Pharmacol. Exp. Ther. 234:106–112; 1985.
5. Cole, B. J.; Hillmann, M.; Seidelmann, D.; Klewer, M.; Jones, G. H.: Effects of benzodiazepine receptor partial inverse agonists in the elevated plus maze test of anxiety in the rat. Psychopharmacology 121:118–126; 1995.
6. Cole, J. C.; Rodgers, R. J.: An ethological analysis of the effects of chlordiazepoxide and bretazenil (Ro 16-6028) in the murine elevated plus-maze. Behav. Pharmacol. 4:573–580; 1993.
7. Cole, J. C.; Rodgers, R. J.: Ethological evaluation of the effects of acute and chronic buspirone treatment in the murine elevated plus-maze test: Comparison with haloperidol. Psychopharmacology 114:288–296; 1994.
8. 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.
9. Dawson, G. R.; Tricklebank, M. D.: Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol. Sci. 16:33–36; 1995.
10. Goldberg, H. L.: Buspirone—A new antianxiety agent not chemically related to any presently marketed drugs [proceedings]. Psychopharmacol. Bull. 15:90–92; 1979.
11. Gonzalez, M. I.; Farabollini, F.; Albonetti, E.; Wilson, C. A.: Interactions between 5-hydroxytryptamine (5-HT) and testosterone in the control of sexual and nonsexual behaviour in male and female rats. Pharmacol. Biochem. Behav. 47:591–601; 1994.
12. Gozlan, H.; el Mestikawy, S.; Pichat, L.; Glowinski, J.; Hamon, M.: Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature 305:140–142; 1983.
13. Green, S.: Benzodiazepines, putative anxiolytics and animal models of anxiety. Trends Neurosci. 14:101–104; 1991.
14. Griebel, G.: Système sérotoninergique et réactivité émotionnelle chez le rat et chez la souris. Approche pharmacologique. Strasbourg, France: Université Louis Pasteur; 1993.
15. Griebel, G.: 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: More than 30 years of research. Pharmacol. Ther. 65:319–395; 1995.
16. Griebel, G.; Blanchard, D. C.; Jung, A.; Blanchard, R. J.: A

model of 'antipredator' defense in Swiss–Webster mice: Effects of benzodiazepine receptor ligands with different intrinsic activities. Behav. Pharmacol. 6:732–745; 1995.
17. Griebel, G.; Blanchard, D. C.; Jung, A.; Masuda, C. K.; Blanchard, R. J.: 5-HT_{1A} agonists modulate mouse antipredator defensive behavior differently from the 5-HT_{2A} antagonist pirenperone. Pharmacol. Biochem. Behav. 51:235–244; 1995.
18. Griebel, G.; Sanger, D. J.; Perrault, G.: The use of the rat elevated plus-maze to discriminate between non-selective and BZ-1 (ω1) selective, benzodiazepine receptor ligands. Psychopharmacology 124:245–254; 1996.
19. Handley, S. L.: 5-Hydroxytryptamine pathways in anxiety and its treatment. Pharmacol. Ther. 66:103–148; 1995.
20. Hjorth, S.; Carlsson, A.: Buspirone: Effects on central monoaminergic transmission—Possible relevance to animal experimental and clinical findings. Eur. J. Pharmacol. 83:299–303; 1982.
21. Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. A., VII: International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev. 46:157–204; 1994.
22. Kennett, G. A.: 5-HT_{1C} receptor antagonists have anxiolytic-like actions in the rat social interaction model. Psychopharmacology 107:379–384; 1992.
23. Mason, P.; Skinner, J.; Luttinger, D.: Two tests in rats for antianxiety effect of clinically anxiety attenuating antidepressants. Psychopharmacology 92:30–34; 1987.
24. Millan, M. J.; Brocco, M.: Serotonin and anxiety: Mixed 5-HT_{1A} agonists—5-$HT_{1C/2}$ antagonists, potential anxiolytic agents. In: Hamon, M.; Ollat, H.; Thiebot, M. H., eds. Anxiety: Neurobiology, clinic and therapeutic perspectives. Paris: Les Editions INSERM/John Libbey Eurotext; 1993:153–164.
25. Molewijk, H. E.; Vanderpoel, A. M.; Olivier, B.: The ambivalent behaviour "stretched approach posture" in the rat as a paradigm to characterize anxiolytic drugs. Psychopharmacology 121:81–90; 1995.
26. Moser, P. C.: An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. Psychopharmacology 99:48–53; 1989.
27. 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.
28. Pellow, S.; Johnston, A. L.; File, S. E.: Selective agonists and antagonists for 5-hydroxytryptamine receptor subtypes, and interactions with yohimbine and FG 7142 using the elevated plus-maze test in the rat. J. Pharm. Pharmacol. 39:917–928; 1987.
29. Rickels, K.; Weisman, K.; Norstad, N.; Singer, M.; Stoltz, D.; Brown, A.; Danton, J.: Buspirone and diazepam in anxiety: A controlled study. J. Clin. Psychiatry 43:81–86; 1982.
30. Rodgers, R. J.: A step in the right direction: Comment on "5-HT and mechanisms of defence." J. Psychopharmacol. 5:316–319; 1991.
31. Rodgers, R. J.; Cole, J. C.: Anxiolytic-like effect of (*S*)-WAY 100135, a 5-HT_{1A} receptor antagonist, in the murine elevated plus-maze test. Eur. J. Pharmacol. 261:321–325; 1994.
32. Rodgers, R. J.; Cole, J. C.: The elevated plus-maze: Pharmacology, methodology and ethology. In: Cooper, S. J.; Hendrie, C. A., eds. Ethology and psychopharmacology. Chichester, UK: John Wiley & Sons Ltd.; 1994:9–44.
33. Rodgers, R. J.; Cole, J. C.; Cobain, M. R.; Daly, P.: Anxiogenic-like effects of fluprazine and eltoprazine in the mouse elevated plus-maze: Profile comparisons with 8-OH-DPAT, CGS 12066B, TFMPP and mCPP. Behav. Pharmacol. 3:621–634; 1992.
34. Rodgers, R. J.; Cole, J. C.; Davies, A.: Antianxiety and behavioral suppressant actions of the novel 5-HT_{1A} receptor agonist, flesinoxan. Pharmacol. Biochem. Behav. 48:959–963; 1994.
35. Rodgers, R. J.; Cole, J. C.; Tredwell, J. M.: Profile of action of 5-HT_3 receptor antagonists, ondansetron and WAY 100289, in the elevated plus-maze test of anxiety of mice. Psychopharmacology 117: 306–312; 1995.
36. 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.
37. Rodgers, R. J.; Johnson, N. J. T.; Norton, S. J.; Cole, J. C.: Effects of ritanserin and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) in the murine elevated plus-maze test of anxiety: An ethopharmacological study. J. Psychopharmacol. 9:38–42; 1995.
38. Schreiber, R.; De Vry, J.: 5-HT_{1A} receptor ligands in animal models of anxiety, impulsivity and depression: Multiple mechanisms of action? Prog. Neuropsychopharmacol. Biol. Psychiatry 17:87–104; 1993.
39. Schreiber, R.; De Vry, J.: Neuronal circuits involved in the anxiolytic effects of the 5-HT_{1A} receptor agonists 8-OH-DPAT, ipsapirone and buspirone in the rat. Eur. J. Pharmacol. 249:341–351; 1993.
40. Shepherd, J. K.; Grewal, S. S.; Fletcher, A.; Bill, D. J.; Dourish, C. T.: Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. Psycho-pharmacology 116:56–64; 1994.
41. Soderpalm, B.; Hjorth, S.; Engel, J. A.: Effects of 5-HT_{1A} receptor agonists and L-5-HTP in Montgomery's conflict test. Pharmacol. Biochem. Behav. 32:259–265; 1989.
42. Stutzmann, J. M.; Eon, B.; Darche, F.; Lucas, M.; Rataud, J.; Piot, O.; Blanchard, J. C.; Laduron, P. M.: Are 5-HT2 antagonists endowed with anxiolytic properties in rodents? Neurosci. Lett. 128:4–8; 1991.
43. Sullivan, J. W.; Gold, L.; Sepinwall, J.: Anticonflict effects of three compounds having antiserotonergic activity: Metergoline, minaserin, and clozapine. Soc. Neurosci. Abstr. 11:1187; 1985.
44. Taylor, D. P.; Riblet, L. A.; Stanton, H. C.; Eison, A. S.; Eison, M. S.; Temple, D. L.: Dopamine and antianxiety activity. Pharmacol. Biochem. Behav. 17(suppl. 1):25–35; 1982.
45. Tomkins, D. M.; Costall, B.; Kelly, M. E.: Action of ritanserin and DOI on the elevated X-maze. Psychopharmacology 101:S57; 1990.
46. Treit, D.: Anxiolytic effects of benzodiazepines and 5-HT_{1A} agonists: Animal models. In: Rodgers, R. J.; Cooper, S. J., eds. 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines. Their comparative behavioural pharmacology. Chichester, UK: John Wiley & Sons; 1991:107–131.
47. Treit, D.; Menard, J.; Royan, C.: Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44:463–469; 1993.
48. Tricklebank, M. D.; Forler, C.; Fozard, J. R.: The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-*n*-propylamino)tetralin in the rat. Eur. J. Pharmacol. 106:271–282; 1984.
49. Witkin, J. M.; Perez, L. A.: Comparison of effects of buspirone and gepirone with benzodiazepines and antagonists of dopamine and serotonin receptors on punished behavior of rats. Behav. Pharmacol. 1:247–254; 1990.
50. Wright, I. K.; Heaton, M.; Upton, N.; Marsden, C. A.: Comparison of acute and chronic treatment of various serotonergic agents with those of diazepam and idazoxan in the rat elevated X-maze. Psychopharmacology 107:405–414; 1992.