Psychopharmacology
DOI 10.1007/s00213-005-0108-2

ORIGINAL INVESTIGATION

**Antonio Pedro de Mello Cruz · Gilson Pinheiro
Sérgio Henrique Alves · Graziela Ferreira
Marília Mendes · Letícia Faria
Carlos Eduardo Macedo · Vitor Motta
J. Landeira-Fernandez**

Behavioral effects of systemically administered MK-212 are prevented by ritanserin microinfusion into the basolateral amygdala of rats exposed to the elevated plus-maze

Received: 6 November 2004 / Accepted: 18 June 2005

Abstract *Rationale:* Although 5-HT_2 receptors seem to play an important role in anxiety, results from numerous studies are still highly variable. Moreover, little is known about the behavioral effects of centrally administered 5-HT_2 compounds in animal models of anxiety. *Objective:* The current study was performed to: (1) further investigate the effects of 5-HT_2 receptor activation in rats exposed to the elevated plus-maze (EPM) and the open-field arena, two widely used animal models for studying anxiety and locomotor activity; and (2) evaluate the involvement of the 5-HT_2 receptors within the basolateral nucleus of the amygdala (BLA) in the modulation of such effects. *Methods:* In the first experiment, male Wistar rats were exposed for 5 min to the EPM 27 min following intraperitoneal (i.p.) (1.0 ml/kg) injections of the preferential 5-HT_{2C} receptor agonist 6-chloro-2[1-piperazinyl]pyrazine (MK-212) at doses of 1.0, 2.0, or 4.0 mg/kg. Control animals were injected with saline. The percentage of open-arm entries and the percentage of time spent in these arms were employed as anxiety indexes, whereas the number of closed-arm entries was calculated as indicative of locomotor activity. In the second experiment, rats were exposed for 10 min in an open-field arena to further assess the interference of the same MK-212 doses upon locomotor activity. In Experiment 3, rats were microinjected (0.2 μl) either with the mixed 5-$HT_{2A/2C}$ receptor antagonist ritanserin (0.5, 1.25, 2.5, and 5.0 μg) or its vehicle into the BLA 12 min following i.p. injections of saline or the intermediate dose of MK-212 (2.0 mg/kg). Fifteen minutes later, each animal was exposed to the EPM as before. *Results:* Whereas the highest dose of MK-212 (4.0 mg/kg) induced motor-suppressant effects in both EPM and open-field arena, the intermediate dose of the drug (2.0 mg/kg) reduced open-arm exploration without significantly affecting the number of closed-arm entries. This behavioral profile, consistent with selective anxiogenic effect in the EPM, was dose-dependently prevented by ritanserin microinfusion into the BLA. In saline-pretreated animals, however, ritanserin (all doses) was ineffective. *Conclusions:* MK-212 increases anxiety and decreases locomotor activity. The anxiogenic-like profile of 5-HT_2 receptor activation is prevented by the blockade of 5-HT_2 receptors within the BLA, which does not have an effect by itself upon basal anxiety levels triggered by the EPM.

Keywords Elevated plus-maze · Open-field arena · 5-HT_2 receptors · Basolateral amygdala · MK-212 · Ritanserin · Anxiety · Locomotor activity

A. P. de Mello Cruz (✉) · G. Pinheiro · S. H. Alves · G. Ferreira · M. Mendes · L. Faria · V. Motta
Laboratório de Psicobiologia, Departamento de Processos Psicológicos Básicos, Instituto de Psicologia, Universidade de Brasília,
Brasília, DF, Brazil
e-mail: apmcruz@unb.br
Tel.: +55-61-3072625
Fax: +55-61-3072625

C. E. Macedo
Laboratório de Psicobiologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo,
Ribeirão Preto, SP, Brazil

J. Landeira-Fernandez
Departamento de Psicologia, Pontifícia Universidade Católica do Rio de Janeiro,
Rio de Janeiro, RJ, Brazil

J. Landeira-Fernandez
Curso de Psicologia, Universidade Estácio de Sá,
Rio de Janeiro, Brazil

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) 2 receptors (5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}) have been critically implicated in the modulation of anxiety states (Naughton et al. 2000; Graeff 2002; Wood 2003; Gordon and Hen 2004). However, results from pharmacological studies investigat-

ing the behavioral effects of 5-HT_2-acting compounds are highly variable. For example, the widely used 5-HT_2 receptor blocker ritanserin (Leysen et al. 1986; Leysen 2004) has been found to produce results ranging from anxiolysis (Ceulemans et al. 1985; Griez et al. 1988; Hensman et al. 1991), through no effect (Den Boer and Westenberg 1990), to anxiogenesis (Guimarães et al. 1997) in experimental and/or clinical anxiety. Similarly, systemic administration of selective and nonselective 5-HT_2 receptor antagonists in animal models of anxiety has been reported to display anxiolytic-like effects (Critchley and Handley 1987; Tomkins et al. 1990; Stutzmann et al. 1991; Wright et al. 1992; Kennett et al. 1994; Cervo and Samanin 1995; Griebel et al. 1997a; Jones et al. 2002; Martin et al. 2002; Nic Dhonnchadha et al. 2003), no effect (Chaouloff et al. 1997; Griebel et al. 1997b; Setem et al. 1999; Martin et al. 2002), and even anxiogenic-like effects (Pellow et al. 1987). Although these behavioral findings may depend at least partially on the selectivity for 5-HT_2 receptor subtypes, variation in dose range, behavioral task, different types of anxiety disorder, and other procedural differences, the precise reasons for such discrepancies remain unclear.

The effects of systemically administered 5-HT_2 receptor agonists are relatively more consistent and tend to agree, albeit with some few exceptions (e.g., Nic Dhonnchadha et al. 2003), that activation of 5-HT_{2C} receptors increase anxiety. For example, 5-HT_{2C} receptor activation, either by nonselective 5-HT_{2C} receptor agonists such as *m*-chlorophenylpiperazine (*m*-CPP) and trifluoromethyl–phenylpiperazine (TFMPP) or the preferential 5-HT_{2C} receptor agonist 6-chloro-2[1-piperazinyl]pyrazine (MK-212), induces a profile consistent with anxiogenesis in humans (Charney et al. 1987; Lowy and Meltzer 1988; Bastani et al. 1990; Bourin et al. 1998) and increases anxiety-like behaviors in several animal models of anxiety, including the light–dark transition (Kshama et al. 1990), the social interaction (Kennett et al. 1989; Bagdy et al. 2001), the stress-induced ultrasound vocalization (Olivier et al. 1998), the "stretched attend posture" (Grewal et al. 1997), the elevated T maze (Mora et al. 1997; Zangrossi et al. 2001), and the elevated plus-maze (EPM; Benjamin et al. 1990; Kshama et al. 1990; Rodgers et al. 1992; Gibson et al. 1994; Griebel et al. 1994; Fone et al. 1996; Wallis and Lal 1998; Setem et al. 1999; Jones et al. 2002; Bull et al. 2003; Durand et al. 2003).

In comparison to the findings cited above, less is known about the effects of intracerebrally infused 5-HT_2 receptor agonists and antagonists (Menard and Treit 1999). This is an important issue especially because there are intriguing suggestions that different 5-HT_2 pathways might be involved in specific types of anxiety-like behavior and drug responses for 5-HT ligands (Deakin and Graeff 1991; Mora et al. 1997; Griebel et al. 1997a). Among postsynaptic sites containing 5-HT_2 receptors (Pompeiano et al. 1994; Barnes and Sharp 1999; Clemett et al. 2000; Leysen 2004), the amygdaloid complex has attracted attention on the basis of its well-documented involvement in anxiety (Blanchard and Blanchard 1972; Hitchcock and Davis 1986; LeDoux et al. 1988; Davis et al. 1994). The amygdaloid complex is one of the major forebrain structures innervated by 5-HT-containing fibers originating from the dorsal raphe nucleus (DRN; Azmitia and Segal 1978; Vertes 1991). Electrical or chemical stimulation of the DRN (Viana et al. 1997) and exposure to a variety of stressful situations (Fernandes et al. 1994; Kawahara et al. 1993; Adell et al. 1997) enhanced the amount of 5-HT in the amygdaloid complex. Additionally, it has been shown that the DRN–amygdala pathway establishes preferential contact with postsynaptic 5-HT_2 receptors (Mammounas et al. 1991), suggesting that 5-HT_{2C} receptors within the amygdaloid complex play an important role in the occurrence of anxiety states.

Many of the effects of 5-HT_2-acting compounds seem to be selectively mediated by the basolateral nucleus of the amygdaloid (BLA) complex (Rainnie 1999; Chen et al. 2003; McGaugh 2004). Accordingly, BLA infusion of 5-HT was found to increase anxiety-like behaviors in a modified version of the Geller–Seifter conflict test, while the nonselective 5-HT_2 receptor antagonist methysergide displayed anxiolytic-like properties (Hodges et al. 1987). Moreover, microinjections of the nonselective 5-HT_{2C} receptor agonist *m*-CPP and the selective 5-HT_{2C} receptor agonist interleukin (IL)-639 into the BLA increased anxiety-related behaviors of rats in an open field, an effect which was attenuated by systemic pretreatment with the selective 5-HT_{2C} receptor antagonist SB-242084 (Campbell and Merchant 2003). Nonetheless, it has been also found that BLA/basomedial amygdala infusion of the nonselective 5-HT_2 receptor antagonist ketanserin increases anxiety-like behaviors in the EPM (Zangrossi and Graeff 1994).

Taking into account the inconclusive results from systemically administered 5-HT_2-acting compounds and the scarce number of studies infusing such compounds into the BLA, the current study comprised three experiments. In the first one, the behavioral effects of systemically administered MK-212 were investigated in rats exposed to the EPM. Such a preferential 5-HT_{2C} receptor agonist has been found to increase physiological and behavioral measures of anxiety in man (Bastani et al. 1990; Lowy and Meltzer 1988) and in some animal models of anxiety (King et al. 1989; McKearney 1990), including the EPM (Kshama et al. 1990). Because 5-HT_2 receptor activation is also known to induce motor-suppressant effects (Kennett et al. 1989; Gibson et al. 1994; Durand et al. 2003), a second experiment tested this prediction by injecting the same MK-212 doses in rats exposed to an open-field arena. Finally, a third experiment evaluated the behavioral effects in the EPM of microinfusing the mixed 5-$HT_{2A/2C}$ receptor blocker ritanserin (Hoyer et al. 1994) into the BLA in rats previously injected either with saline or MK-212 systemically. Therefore, our study allowed to: (1) further investigate in rats exposed to the EPM and the open-field arena the behavioral effects of systemically administered MK-212, (2) examine whether MK-212-induced behavioral changes in the EPM are prevented by intra-BLA infusion

of ritanserin, and (3) evaluate the influence in the EPM of intra-BLA infusion of ritanserin in the absence of MK-212-induced 5-HT_2 receptor activation.

Material and methods

Subjects

Naive male Wistar rats weighing 200–290 g were employed as subjects. Animals were born and raised in the vivarium at the University of Brasilia. Two weeks before the experiment, animals were brought to the holding room of the laboratory facilities and housed in groups of two in polycarbonate cages measuring 30×30×50 cm. All the rats had free access to food and water. Room temperature was controlled (25±1°C), and a light–dark cycle was maintained on a 12-h on–off cycle (0700–1900 hours lights on). The experimental sessions were conducted during the light phase of the cycle. The experimental protocols employed in the present study were in conformity with the recommendations of the Brazilian Society of Neuroscience and Behavior (SBNeC), which are based on the US National Institutes of Health's *Guide for Care and Use of Laboratory Animals* (revised in 1996).

Surgery and microinjections

Animals were anaesthetized with sodium thiopental (45 mg/kg, i.p.) and fixed in a stereotaxic frame. A subcutaneous injection of 2% lidocaine with vasoconstrictor was made in the surgical area in a small volume to form a bubble. Each rat was implanted with a single stainless steel guide cannula made of an odontological needle (o.d., 0.7 mm) aimed at 1.0 mm above the BLA at the right hemisphere. Taking bregma as the reference for each plane according to the Paxinos and Watson (1986) atlas, the coordinates were 2.8 mm posterior to bregma, 5.0 mm lateral to the midline, and 6.3 mm ventral to the skull. Guide cannulae were fixed to the skull by means of dental acrylic and three stainless screws. After implantation, the guide cannula was sealed with a stainless steel wire to prevent eventual congestion. Four days after surgery, the animals were gently wrapped in a cloth and handled for 5 min during three consecutive days. EPM testing occurred on the 8th day postsurgery.

For intracerebral infusions, a thin (0.3 mm) o.d. needle was introduced through the guide cannula until its tip was 1 mm below the guide cannula end. The internal cannula was connected to a 10-μl Hamilton syringe via PE-10 tubing, which allowed the experimenter to deliver the drug during a 30-s period. Confirmation of successful infusion was obtained by monitoring the movement of a small air bubble in the PE-10 tubing. At the end of microinjection, the internal cannula was held inside the brain for 2 min to prevent drug back-up and allow absorption.

Histology

At the end of the behavioral testing, rats were killed with an overdose of thionembutal and perfused intracardially with saline followed by a 10% formalin solution. The brains were removed and stored in formalin (15%) for at least 2 weeks and then sectioned, using the cryostatic method, at 50–60 μm. Brain slices were stained with cresyl violet to localize the cannula position.

Drugs

MK-212 [6-chloro-2-(1-piperaziny)pyrazine hydrochloride; Tocris, Ballwin, MO, USA] was dissolved in sterile saline (0.9% NaCl) and injected intraperitoneally (1.0 ml/kg, i.p.) 27 min before testing. Ritanserin [6-(2-[4-[bis(4-fluorophenyl)methylene]-1-piperidinyl]7-methyl-5HT-thiazolo[3,2-a]pyrimidine-5-one; Jansen, Denmark] was suspended in a saline–Tween 80 2% solution, which served as vehicle control. Drug and vehicle were microinjected into the BLA in a volume of 0.2 μl 15 min before testing.

Apparatus

The EPM anxiety test employed in the present study has been described elsewhere (Cruz et al. 1994; Setem et al. 1999). Briefly, it consisted of two opposite arms (50×10 cm) enclosed by walls 40 cm high that were crossed at a right angle with two opposed open arms of the same size. The four arms, arranged in the shape of a plus sign, delimited a central area of 100 cm^2. The maze was elevated 50 cm above the ground. To minimize rats falling down, a rim of Plexiglas (1 cm high) surrounded the open arms.

The open-field arena consisted of a square box (60×60×40 cm) made of wood. Thin black lines divided the floor into nine equal squares (20 cm) to allow quantification of the rat's ambulatory activity. Arena and EPM illumination (55 lx) was provided by a dim light bulb (60 W) in the ceiling of the experimental room above the center of the apparatus. The experimental sessions were recorded by a vertically mounted video camera linked to a monitor and videocassette recorder (VCR) in an adjacent room.

Behavioral testing

Experiment 1

This experiment investigated in a dose–response manner the behavioral effects of i.p. injections with the preferential 5-HT_{2C} receptor agonist MK-212 among rats exposed to the EPM. Thirty two rats were randomly assigned to four groups (n=8 each) according to treatment with either saline

or the three different MK-212 doses (1.0, 2.0, or 4.0 mg/kg). These doses, and the latency for action of MK-212, were selected based on our preliminary pilot experiments. Control animals were injected with saline (1.0 ml/kg). Twenty seven minutes later, each rat was placed in the central platform of the maze facing a closed arm and allowed to freely explore the apparatus for 5 min. Animals from each group were tested in a counterbalanced manner. The experimental session was videotaped for later analysis by a highly trained observer who remained blind to treatment conditions. The number of entries and the time spent in the open and closed arms of the maze were recorded. From these measures, the percentage of open-arm entries (100× open-arm entries/total arm entries) and the percentage of time spent in the open arms (100×time open/time open + time closed) were calculated for each animal as anxiety indexes. The absolute number of closed-arm entries was interpreted as an index of motor activity (File 1992; Cruz et al. 1994).

Experiment 2

To further investigate the behavioral effects of MK-212 upon exploration levels of rats in an open-field arena, four different groups of animals (n=8 each) were injected (1.0 ml/kg, i.p.) either with saline or the same MK-212 doses employed in Experiment 1. Twenty seven minutes later, animals from each group were exposed for 10 min to the arena in a counterbalanced manner. The number of crossings of the black lines dividing adjacent floor areas and the number of rearings in the apparatus were blindly registered as motor activity indexes.

Experiment 3

This experiment investigated in the EPM whether the behavioral effects of systemically administered MK-212 could be attenuated by ritanserin microinfusion into the BLA. Animals with cannula implanted in the BLA were randomly assigned to two different treatment conditions and injected (1.0 ml/kg, i.p.) with saline or MK-212 at the intermediate dose (2.0 mg/kg) employed in Experiments 1 and 2. Twelve minutes later, each correspondent group received microinfusions (0.2 μl) of either ritanserin (0.5, 1.25, 2.5, or 5 μg) or its vehicle control into the BLA. Fifteen minutes latter, each rat was exposed for 5 min to the EPM as before. Ritanserin doses and the time of microinfusion before the EPM exposure were chosen on the basis of previously reported results (Audi et al. 1991; Nogueira and Graeff 1991).

Statistical analysis

Behavioral results from EPM and open-field arena were statistically analyzed by an analysis of variance (ANOVA) to detect overall differences. Whenever ANOVA was significant, Fisher's least significant difference (LSD) post hoc test was employed to determine specific differences between groups. Because a significant effect of MK-212 upon the absolute number of closed-arm entries was in the EPM results from Experiment 1, an analysis of covariance (ANCOVA), using this parameter as covariant, was additionally performed with all other variables to examine whether the closed-arm entries could account for the eventual differences in the percentage of open-arm entries and the time spent in the open arms (File 1992). The level of statistical significance was P<0.05.

Results

Experiment 1

Figure 1 presents the behavioral effects in the EPM of the four MK-212 doses administered systemically. One-way ANOVA indicated a main effect of the treatments [F(3,28) =3.93, P<0.05] in the percentage of open-arm entries (Fig. 1a). Post hoc comparison revealed that the doses of 2.0 and 4.0 mg/kg significantly decreased this measure as

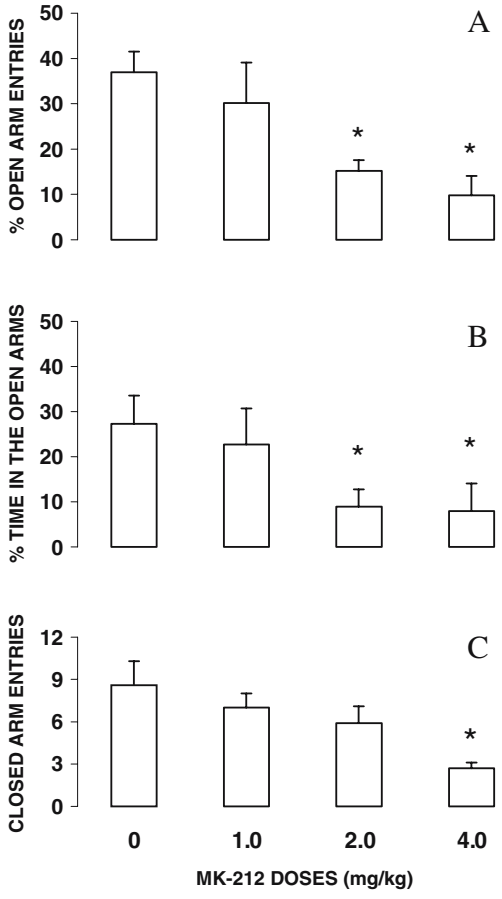

Fig. 1 Mean (+SEM) percent of open-arm entries (**a**), percent of time spent in the open arms (**b**), and closed-arm entries (**b**) in the EPM among groups injected either with saline or MK-212 systemically. * indicates P<0.05 compared with vehicle control (see text for details)

Table 1 Mean (±SEM) number of crossings and rearings in the open-field arena among groups injected either with saline or MK-212 systemically

Treatments	Crossings	Rearings
Saline control	78.13±7.04	18.63±4.12
MK-212, 1.0 mg/kg	81.04±9.42	17.25±6.33
MK-212, 2.0 mg/kg	72.03±8.81	14.01±3.12
MK-212, 4.0 mg/kg	17.48±2.98*	9.75±3.36*

*P<0.05 compared with vehicle control

compared to saline control animals (Ps<0.05). A similar pattern was observed in the percentage of time spent in the open arms (Fig. 1b). Therefore, ANOVA revealed a main effect of the treatments [F(3,28)=4.33, P<0.05]. Again, post hoc comparisons showed a significant decrease in the time spent in the open arms among animals injected with MK-212 at the doses of 2.0 and 4.0 mg/kg as compared to animals injected with saline. Figure 1c depicts the effect of MK-212 in the absolute number of closed-arm entries. As can be observed from Fig. 1c, MK-212-induced decrease in the percentages of entries and time spent in the open arms at a dose of 4.0 mg/kg was accompanied by a reduction in the absolute number of closed-arm entries. ANOVA confirmed this impression, as indicated by a significant effect of the treatments [F(3,28)=8.99, P<0.001]. Pairwise comparisons revealed that the dose of 4.0 mg/kg significantly decreased the number of closed-arm entries as compared to saline control animals (P<0.05).

Because MK-212-induced reduction in the open-arm exploration was accompanied by a significant decrease in the closed-arm entries, an additional ANCOVA using this parameter as covariant factor was performed. Results from this analysis confirmed a drug main effect for both the percentage of open-arm entries [F(3,27)=3.58, P<0.05] and the percentage of time spent in these arms [F(3,27)=2.97, P<0.05]. Post hoc comparisons showed that the doses of 2.0 and 4.0 mg/kg of MK-212 significantly decreased these two anxiety measures as compared to saline-treated animals (all Ps>0.05). Therefore, parameters from ANOVA and ANCOVA were similar even when the closed-arm entries were statistically controlled for.

Experiment 2

The behavioral effects of MK-212 in the open-field arena are shown in Table 1. One-way ANOVA revealed a main effect of drug treatment on crossings [F(3,28)=26.79, P<0.001] and rearings [F(3,28)=3.03, P<0.05]. Post hoc comparison indicated that the highest dose of MK-212 (4.0 mg/kg) significantly decreased such motor activity measures as compared to saline-treated animals (P<0.05). No other significant difference was found.

Experiment 3

As indicated by a representative photomicrograph of the guide cannula tip location (Fig. 2), histological examination of the brain slices showed that most of the cannulae were located approximately 1.0 mm above the BLA. Considering that the internal needle extended 1.0 mm beyond the tip of the guide cannula, the infusions were performed at sites corresponding to the BLA. A diagrammatic representation of coronal sections showing the target area of the BLA is shown in Fig. 3. Behavioral results from animals with cannula outside this area (n=26) were removed from each group and joined together in an additional control group for statistical analysis.

Figure 4 presents the behavioral effects in the EPM of BLA ritanserin infusion following i.p. injections of MK-212 or saline. Such data were analyzed via two-way ANOVA. The first factor was related to the BLA doses of ritanserin (0.5, 1.25, 2.5, or 5 μg) or its vehicle control. The other factor indicated whether the animal was previously treated either with MK-212 or saline. As can be observed from Fig. 4a, MK-212 reduced the percentage of open-arm entries, an effect seemingly prevented by intra-BLA infusion of ritanserin in a dose-dependent manner. Two-way ANOVA confirmed this impression, indicating a significant interaction between i.p. injections of MK-212 and the ritanserin doses microinjected into the BLA [F(5,99)=4.62, P<0.05]. Post hoc comparison showed that MK-212 significantly decreased the percentage of open-arm entries as compared to saline control animals when both groups had received infusions of either vehicle or the lowest dose

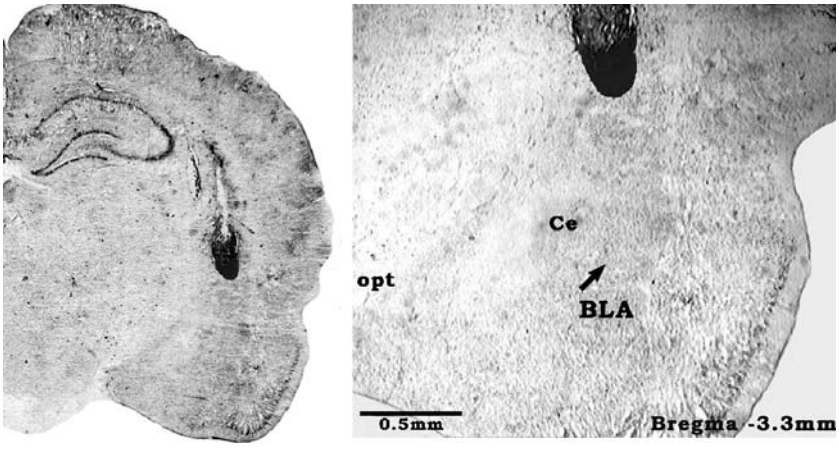

Fig. 2 Panoramic (*left*) and focal (*right*) photomicrographs (**a**) of a representative coronal brain section illustrating the cannula tip placement approximately 1 mm above the target area of the BLA. *Scale bar* is equal to 500 μm. *Black arrow* indicates the exact point reached by the tip of the internal needle. The adjacent areas corresponding to the central nucleus of the amygdala (*Ce*) and the optic tract are also illustrated

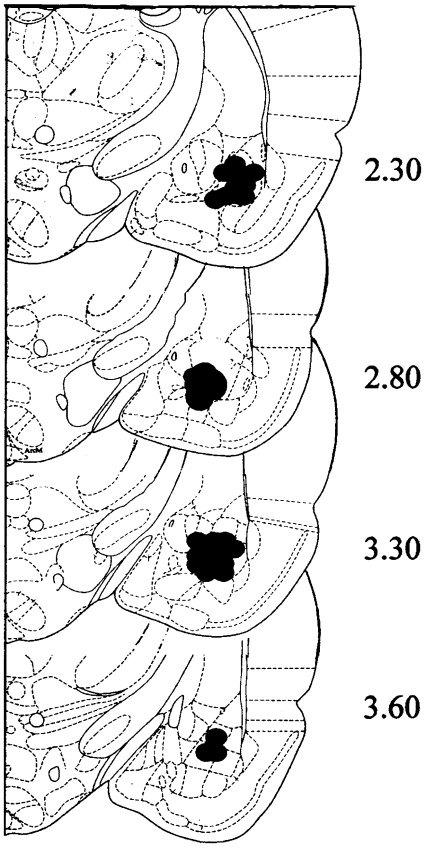

Fig. 3 Composite of internal cannula location aimed at the BLA. With the reference to the Paxinos and Watson (1986) atlas, the *numbers* on the *right side* of each plate indicate the distance in mm from bregma

of ritanserin (0.5 μg) into the BLA (all *P*s<0.05). No other differences in the percentage of open-arm entries were found (all *P*s>0.05).

A similar pattern of results is illustrated in Fig. 4b. Thus, two-way ANOVA outcomes from the percentage of time spent in the open arms revealed a significant interaction between i.p. injections of MK-212 and the ritanserin doses microinjected into the BLA [$F(5,99)=4.18$, $P<0.05$]. Again, pairwise comparisons found that MK-212 significantly decrease this anxiety index as compared to saline pretreated animals when both groups had received infusions of either vehicle or the lowest dose of ritanserin into the BLA (all *P*s<0.05).

Figure 4c presents the absolute number of closed-arm entries. Despite MK-212, pretreated animals showed a trend for a decrease in this measure of motor activity in the EPM; two-way ANOVA indicated the absence of interaction as well as no significant effect of both MK-212 injections or ritanserin microinfusions into the BLA.

Discussion

5-HT_{2C} receptor activation, either by nonselective, preferential, or selective 5-HT_{2C} receptor agonists, increases anxiety-like behaviors (Kennett et al. 1989; Kshama et al. 1990; Rodgers et al. 1992; Mora et al. 1997; Wallis and Lal 1998; Setem et al. 1999; Durand et al. 2003) and induces motor-suppressant effects in several animal models (Lucki et al. 1989; Kshama et al. 1990; Fone et al. 1998; Setem et al. 1999; Bull et al. 2003). Results from Experiments 1 and 2 corroborated these general findings in a dose-dependent manner. Whereas the lowest dose (1.0 mg/kg) of the

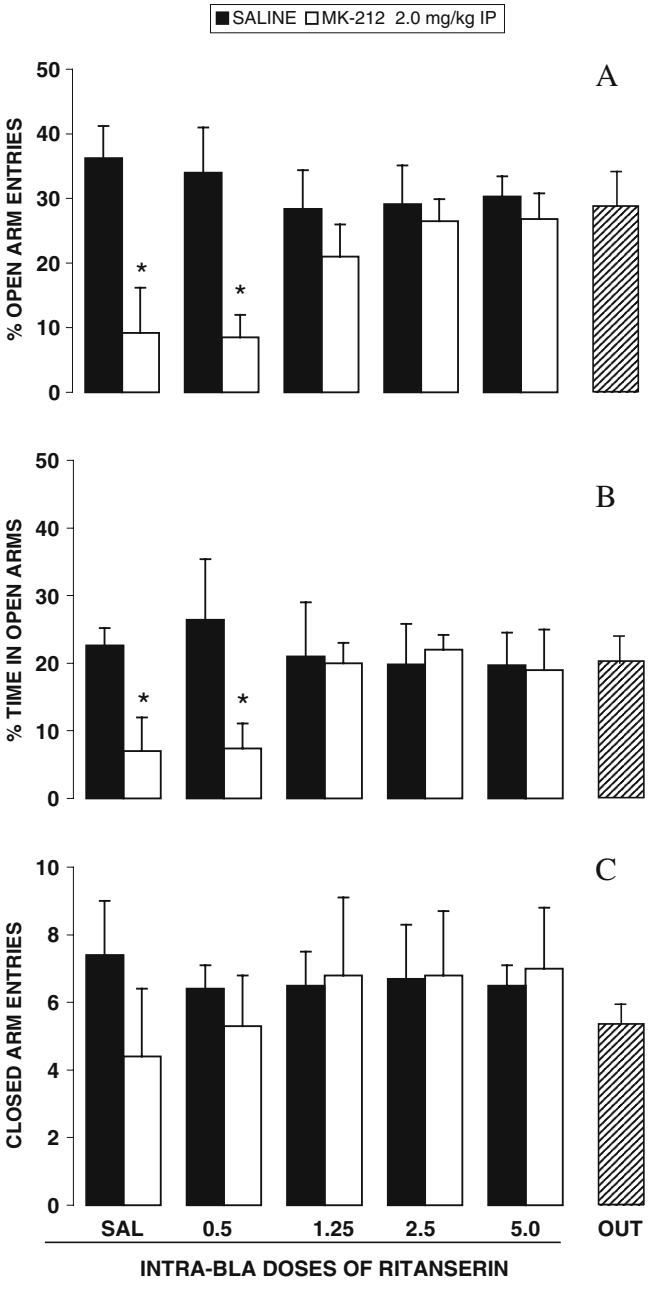

Fig. 4 Mean (+SEM) percent of open-arm entries (**a**), percent of time spent in the open arms (**b**), and closed-arm entries (**c**) in EPM among groups centrally injected with ritanserin into the BLA following i.p. injections of either saline [0.0 μg (n=10); 0.5 μg (n=8); 1.25 μg (n=8); 2.5 μg (n=9); and 5.0 μg (n=9)] or MK-212 [(0.0 μg (n=8); 0.5 μg (n=9); 1.25 μg (n=11); 2.5 μg (n=10); and 5.0 μg (n=12)]. *OUT* illustrates a representative group of rats (n=16) microinjected either with vehicle or different doses of ritanserin at sites localized outside the BLA. * indicates $P<0.05$ compared with vehicle control (see text for details)

preferential 5-HT_{2C} receptor agonist MK-212 was ineffective in both the EPM and the open-field arena, the intermediate dose of the drug (2.0 mg/kg) decreased open-arm exploration without significantly affecting the absolute number of closed-arm entries, a profile consistent with an anxiogenic-like action in the EPM (File 1992; Cruz et al. 1994). Correspondingly, this same intermediate dose of MK-212 did not affect motor activity in rats exposed to the open-field arena. At the dose of 4.0 mg/kg, however, MK-212-induced decrease in the open-arm exploration was accompanied by a significant reduction in the absolute number of closed-arm entries. This behavioral profile, indicative of motor-suppressant effect in the EPM, was also detected in the open-field arena by a significant decrease of crossings and rearings following i.p. injections of this highest dose of MK-212.

In spite of the motor-suppressant effects at the highest dose, such a behavioral pattern of MK-212 in the EPM did not account for the differences observed in the open-arm exploration, as indicated by significant ANCOVA outcomes performed from the two anxiety measures (percentage of open-arm entries and percentage of time spent in the open arms) using the closed-arm entry as covariant factor. Indeed, it must be acknowledged that although motor activity may affect the anxiety parameters in the EPM, such as open arm entries and time spent in the open arms, it is unclear how these performance variables may in fact interact in this anxiety animal test. For example, it has been shown that hypoactivity in the EPM can overcame the detection of anxiogenic-like effects in some experimental manipulations (e.g., Padovan and Guimarães 2000) but not in others (e.g., Maisonnette et al. 1993).

To the best of our knowledge, the only published study testing the behavioral effects of MK-212 in the rat EPM found that a single dose of the drug (0.5 mg/kg, i.p.) induced hypoactivity but no anxiety-like effect (Kshama et al. 1990). These findings are partially consistent with the present results since our lowest dose of MK-212 (1.0 mg/kg) was also ineffective to change anxiety levels in the EPM. The reason for the discrepancy in the MK-212 doses to detect hypoactivity in the EPM may be related to procedural differences. For example, Kshama et al. (1990) performed the EPM test during the dark cycle in a reversed light–dark cycle, whereas in the present procedure, the experimental sessions were conducted during the light phase of the cycle. Moreover, their animals were pre-exposed to a hole–board anxiety test before the EPM test. Finally, the authors employed preselected animals showing higher scores of exploration in an arena. Therefore, it is possible that a higher basal level of exploration together with methodological variations favored the detection of an MK-212-induced decrease in the number of closed-arm entries at lower doses of MK-212 injected systemically.

Central activation of 5-HT_{2C} receptors is generally accepted to mediate the anxiogenic-like properties of 5-HT_{2C} receptor agonists (Kennett and Curzon 1988; Gibson et al. 1994; Martin et al. 2002; Alves et al. 2004). In the present study, intra-BLA infusion of ritanserin dose-dependently prevented MK-212-induced anxiety-like behavior in the EPM, a result suggestive for a role of 5-$HT_{2A/2C}$ receptors. Despite showing affinity for both 5-HT_{2C} and 5-HT_{2A} receptors, MK-212 is a centrally acting 5-HT agonist that can be classified as a preferential 5-HT_{2C} receptor agonist on the basis of receptor binding (nM affinity for 5-HT_{2C} receptor and >16-fold lower for 5-HT_2 receptor subtypes; Porter et al. 1999) and behavioral studies (Clineschmidt 1979; Blackburn et al. 1984). Furthermore, the discriminative stimulus properties of MK-212 indicate the dependence of this behavioral action on selective stimulation of 5-HT_{2C} receptors (Cunningham et al. 1986).

Ritanserin is a mixed 5-HT_2 blocker that exhibits higher affinity at 5-HT_{2C} than 5-HT_{2A} receptors (Leysen et al. 1986; Leysen 2004). Actually, ritanserin has been able to antagonize the effects of several classes of 5-HT_{2C} agonists but not the behavioral effects of some preferential or selective 5-HT_{2A} agonists (Griebel et al. 1997a). Based upon such pharmacological and behavioral properties, our data support the view that MK-212-increased anxiety-like behaviors in the EPM via 5-HT_2 receptor activation might be at least partially mediated by 5-HT_{2C} receptors within the BLA, although the involvement of other 5-HT_2 receptor subfamilies cannot be discarded. Consistent with this view is the recent report that 5-HT_{2C} receptor activation, either by *m*-CPP or the selective 5-HT_{2C} agonist IL-639 microinjected directly in the BLA, produces ultrasound vocalization and increases the latency to investigate a new object in rats exposed to an open field, an anxiogenic-like profile that is attenuated by i.p. pretreatment with the selective 5-HT_{2C} receptor antagonist SB-24084 (Campbell and Merchant 2003).

The present study also found ritanserin (all doses) to be ineffective upon motor activity and anxiety-like behaviors in the EPM when the drug was microinjected in saline-pretreated animals. These null effects cannot be attributed to ritanserin doses considering that the same dose range of the drug was able to prevent an MK-212-induced decrease in open-arm exploration. Therefore, the blockade of BLA 5-HT_2 receptors by ritanserin seems to display antiaversive properties under MK-212-enhanced anxiety level via 5-HT_{2C} receptor activation but not upon basal anxiety levels triggered by the EPM. This lack of effect of ritanserin alone within the BLA is in agreement with a number of results showing the drug to cause little or no effect in the EPM following systemic administration (Audi et al. 1989; Graeff et al. 1990; Stutzmann et al.1991; Wright et al. 1992; Gatch et al. 2000).

In contrast to our ritanserin null effects upon basal anxiety-like levels in the EPM, it has been reported that intra-BLA infusion of other nonselective 5-HT_2 receptor antagonists can induce anxiolytic or even anxiogenic-like effects in different animal models of anxiety. For example, Hodges et al. (1987) found that methysergide increased the ratio of punished responses in the Geller–Seifter conflict test, whereas Zangrossi and Graeff (1994) found ketanserin to decrease open-arm exploration in the rat EPM following microinfusion directly into the BLA.

The reasons for such inconsistencies are unclear but can be related to procedural differences and/or affinity to displayed ritanserin, methysergide, and ketanserin for 5-HT_2 receptor subfamilies. For example, in the Geller–Seifter conflict tests, well-trained and water-deprived rats are tested on a multiple schedule consisting of rewarded, nonrewarded, and punished (reward + electric shock) responding. The conflict test is characterized by the simultaneous presentation of a reward and a foot-shock contingent to the animal response. On the other hand, the experimental protocol of the EPM does not involve any reward or punishment manipulations. Therefore, these animal models might have different sensitivities to detect different forms of anxiety processes. Indeed, it has been reported that 5-HT_{2C} receptor antagonists might have an anxiolytic-like effect in the conflict-based test (Kennett et al. 1997) but not in the EPM (Griebel et al. 1997a,b).

Additionally, ritanserin and methysergide bind importantly at 5-HT_{2C} receptors, while ketanserin is a nonselective 5-HT_2 receptor antagonist with higher affinity for 5-HT_{2A} than 5-HT_{2C} receptors (Kristiansen and Dahl 1996). In fact, ritanserin and methysergide, but not ketanserin, were also reported to antagonize the behavioral effects of selective and nonselective 5-HT_{2C} receptor agonists (McKearney 1990). In addition to these procedural and pharmacological variations, Zangrossi and Graeff (1994) did not differentiate BLA from basomedial amygdala sites, whereas in our study, ritanserin microinjections were restricted to the BLA. Nevertheless, further work is needed to establish the precise role of each 5-HT_2 receptor subtype in the different nuclei of the amygdaloid complex in such 5-HT-mediated anxiety.

Taken together, the present study extended previous results from our and other laboratories showing that 5-HT_2 receptor activation either by selective or nonselective 5-HT_{2C} receptor agonists induced anxiety-like behaviors and motor-suppressant effects in a dose-dependent manner (Benjamin et al. 1990; Rodgers et al. 1992; Gibson et al. 1994; Griebel et al. 1994; Fone et al. 1996; Wallis and Lal 1998; Setem et al. 1999; Jones et al. 2002; Bull et al. 2003). The blockade of BLA 5-HT_2 receptors by ritanserin was able to prevented MK-212-induced behavioral changes in the EPM while being ineffective upon basal anxiety levels triggered by this widely used animal model of anxiety. Although neither MK-212 nor ritanserin act selectively at 5-HT_{2C} receptors, it is possible that the present behavioral effects were mediated by these receptors located within the BLA. Further work with newly available selective 5-HT_{2C} receptor agonists (e.g., Ro-60-0175 or WAY-161503) and antagonists (e.g., SB-242084) microinjected into the BLA will provide novel insights into the 5-HT-mediated anxiety.

Acknowledgements A.P.M.C. and J.L.F. are supported by grants from the Brazilian National Research Council (CNPq, 350214/1998-0 and 522720/95-10). We thank Dr. Fernando Cardenas (Pontifícia Universidade Católica do Rio de Janeiro, Brazil) for his assistance in histology.

References

Adell A, Casanovas JM, Artigas F (1997) Comparative study in the rat of the actions of different types of stress on the release of 5-HT in raphe nuclei and forebrain areas. Neuropharmacology 36:735–741

Alves SH, Pinheiro G, Motta V, Landeira-Fernandez J, Cruz APM (2004) Anxiogenic effects in the rat elevated plus-maze of 5-HT2C agonists into ventral but not dorsal hippocampus. Behav Pharmacol 15:37–43

Audi EA, de-Oliveira CE, Graeff FG (1989) Serotonergic mediation of the anxiolytic effect of intracerebrally injected propranolol measured in the elevated plus-maze. Braz J Med Biol Res 22:699–701

Audi EA, de-Oliveira CE, Graeff FG (1991) Microinjection of propranolol into the dorsal periaqueductal gray causes an anxiolytic effect in the elevated plus-maze antagonized by ritanserin. Psychopharmacology 105:533–537

Azmitia EC, Segal M (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 179:641–667

Bagdy G, Graf M, Anheuer ZE, Modos EA, Kantor S (2001) Anxiety-like effects by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with 5-HT2C receptor antagonist SB-242084 but not the 5-HT1A antagonist WAY-100635. Int J Neuropsychopharmacol 4:399–408

Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38:1083–1152

Bastani B, Nash JF, Meltzer HY (1990) Prolactin and cortisol responses to MK-212, a serotonin agonist, in obsessive–compulsive disorder. Arch Gen Psychiatry 47:833–839

Benjamin D, Lal H, Meyerson LR (1990) The effects of 5-HT1B characterizing in the mouse elevated plus-maze. Life Sci 47:195–203

Blackburn TP, Kemp JD, Martin DA, Cox B (1984) Evidence that 5-HT agonist-induced rotational behavior in the rat is mediated via 5-HT_1 receptors. Psychopharmacology 83:163–165

Blanchard DC, Blanchard RJ (1972) Innate and conditioned reactions to threat in rats with amygdaloid lesions. J Comp Physiol Psychol 81:281–290

Bourin M, Baker GB, Bradwejn J (1998) Neurobiology of panic disorder. J Psychosom Res 1:163–180

Bull EJ, Huston PH, Fone KC (2003) Reduced social interaction following 3,4-methylenedioxymethamphetamine is not associated with enhanced 5-HT2C receptor responsivity. Neuropharmacology 44:439–448

Campbell BM, Merchant KM (2003) Serotonin 2C receptors within the basolateral amygdala induce acute fear-like responses in an open-field environment. Brain Res 993:1–9

Cervo L, Samanin R (1995) 5-HT1A receptor full and partial and 5-HT2C (but not 5-HT3) receptor antagonists increase rates of punished responding in rats. Pharmacol Biochem Behav 52:671–676

Ceulemans DLS, Hoppenbrouwers MLJA, Gelders YG, Reyntjens AJM (1985) The influence of ritanserin, a serotonin antagonist in anxiety disorders: a double bind placebo controlled study versus lorazepam. Pharmacopsychiatry 18:303–305

Chaouloff F, Kulikov A, Mormède P (1997) Repeated DOI and SR 46349B treatments do not affect elevated plus-maze anxiety despite opposite effects on cortical 5-HT_{2A} receptors. Eur J Pharmacol 334:25–29

Charney DS, Woods SW, Goodman WR, Heninger GR (1987) Serotonin function in anxiety. II. Effects of the serotonin agonist mCPP on panic disorder patients and healthy subjects. Psychopharmacology 92:14–21

Chen A, Hough CJ, Li H (2003) Serotonin type II receptor activation facilitates synaptic plasticity via *n*-methyl-D-aspartate-mediated mechanism in the rat basolateral amygdala. Neuroscience 119:53–63

Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KC (2000) Immunohistochemical localization of the 5-HT_{2C} receptor protein in the rat CNS. Neuropharmacology 39:123–132

Clineschmidt BV (1979) MK-212: a serotonin-like agonist in the CNS. Gen Pharmacol 10:287–290

Critchley MA, Handley SL (1987) Effects in the X-maze model of agents acting at 5-HT1 and 5-Ht2 receptors. Psychopharmacology 93:502–506

Cruz APM, Frei F, Graeff FG (1994) Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav 49:171–176

Cunningham KA, Callahan PM, Appel JB (1986) Discriminative stimulus properties of the serotonin agonist MK-212. Psychopharmacology 90:193–197

Davis M, Raiunnie D, Cassell M (1994) Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci 17:17–24

Deakin JFW, Graeff FG (1991) 5-HT and mechanisms of defense. J Psychopharmacol 5:305–315

Den Boer JA, Westenberg HGM (1990) Serotonin function in panic disorder: a double blind placebo controlled study with flovoxamine and ritanserin. Psychopharmacology 102:85–94

Durand M, Mormèd P, Chaouloff F (2003) Wistar–Kyoto rats are sensitive to the hypolocomotor and anxiogenic effects of mCPP. Behav Pharmacol 14:173–177

Fernandes C, Andrews N, File SE (1994) Diazepam withdrawal increases [3H]-5-HT release from rat amygdaloid slices. Pharmacol Biochem Behav 49:359–362

File SE (1992) Behavioural detection of anxiolytic actions. In: Elliot JM, Heal DJ, Marsden CA (eds) Experimental approaches to anxiety and depression. Wiley, London, pp 25–44

Fone KC, Shalders K, Fox ZD, Arthur R, Marsden CA (1996) Increased 5-HT2C receptor responsiveness occurs on rearing rats in social isolation. Psychopharmacology 123:346–352

Fone KC, Austin RH, Topham LA, Kennett GA, Punhani T (1998) Effect of chronic m-CPP on locomotion, hypophagia, plasma corticosterone and 5-HT_{2C}-receptor levels in the rat. Br J Pharmacol 123:1707–1715

Gatch MB, Wallis CJ, Lal H (2000) Effects of ritanserin on ethanol withdrawal-induced anxiety in rats. Alcohol 21:11–17

Gibson EL, Barnfield AM, Curzon G (1994) Evidence that mCPP-induced anxiety in the plus-maze is mediated by postsynaptic 5-HT_{2C} receptors but not by sympathomimetic effects. Neuropharmacology 33:457–465

Gordon JA, Hen R (2004) The serotonin system and anxiety. Neuromolecular Med 5:27–40

Graeff FG (2002) On serotonin and experimental anxiety. Psychopharmacology 163:467–476

Graeff FG, Audi EA, Almeida SS, Graeff EO, Hunziker MH (1990) Behavioral effects of 5-HT ligands in the aversive brain stimulation, elevated plus-maze and learned helplessness tests. Neurosci Biobehav Rev 14:501–506

Grewal SS, Shepherd JK, Bill DJ, Fletcher A, Dourich CT (1997) Behavioural and pharmacological characterization of the canopy stretched attend posture test as a model of anxiety in mice and rats. Psychopharmacology 133:29–38

Griebel G, Moreau JL, Jenck F, Mutel V, Martin JR, Misslin R (1994) Evidence that tolerance to the anxiogenic-like effects of mCPP does not involve alteration in the function of 5-$HT_{(2C)}$ receptors in the rat choroid plexus. Behav Pharmacol 5:642–645

Griebel G, Perrault G, Sanger DJ (1997a) A comparative study of the effects of serotonin of selective and non-selective 5-HT_2 receptor antagonists in rat and mouse models of anxiety. Neuropharmacology 36:793–802

Griebel G, Rodgers RJ, Perrault G, Sanger DJ (1997b) Risk-assessment behaviour: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. Pharmacol Biochem Behav 53:817–827

Griez E, Pols H, Lousberg H (1988) Serotonin antagonism in panic disorder. Acta Psychiatr Belg 88:372–377

Guimarães FS, Mbaya PS, Deakin JFW (1997) Ritanserin facilitates anxiety in a stimulated public-speaking paradigm. J Psychopharmacol 11:225–231

Hensman H, Guimarães FS, Wang M, Deakin JFW (1991) Effects of ritanserin on aversive classical conditioning in humans. Psychopharmacology 104:220–224

Hitchcock JM, Davis M (1986) Lesion of the amygdala, but not of the cerebellum or red nucleus, block conditioned fear as measured with the potentiated startle paradigm. Behav Neurosci 100:11–22

Hodges H, Green S, Glenn B (1987) Evidence that the amygdala is involved in benzodiazepine and serotonergic effects on punished responding but not on discrimination. Psychopharmacology 92:491–504

Hoyer D, Clarke DE, Fozard JR, Hartin PR, Martin GR, Mylercharane EJ, Saxena PR, Humphrey PPA (1994) VII. International Union of Pharmacology Classification of Receptors for 5-hydroxytryptamine (serotonin). Pharmacol Rev 46:157–203

Jones N, Duxon MS, King SM (2002) 5-HT_{2C} receptor mediation of unconditioned escape behaviour in the unstable elevated exposed plus maze. Psychopharmacology 164:214–220

Kawahara H, Yoshida H, Yokoo H, Nishi M, Tanaka M (1993) Psychological stress increases serotonin release in the amygdala and prefrontal cortex assess by in vivo microdialysis. Neurosci Lett 162:81–84

Kennett GA, Curzon G (1988) Evidence that mCPP may have behavioral effects mediated by 5-HT1C receptors. Br J Pharmacol 94:137–147

Kennett GA, Whitton P, Shah K, Curzon G (1989) Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT1C receptor antagonists. Eur J Pharmacol 164:445–454

Kennett GA, Pittaway K, Blackburn TP (1994) Evidence that 5-HT2c receptor antagonists are anxiolytic in the Geller–Seifter model of anxiety. Psychopharmacology 114:90–96

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes I, Brown AM, Middlemiss DN, Blackburn TP (1997) SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. Neuropharmacology 36:609–620

King BH, Brazzel C, Dourish CT, Middlemiss DN (1989) MK-212 increases rat plasma ACTH concentration by activation of the 5-HT_{1C} receptor subtype. Neurosci Lett 105:174–176

Kristiansen K, Dahl SG (1996) Molecular modeling of serotonin, ketanserin, ritanserin and their 5-HT2C receptor interactions. Eur J Pharmacol 306:195–210

Kshama D, Hrishikeshavan HJ, Shanbhogue R, Munonyedi US (1990) Modulation of baseline behavior in rats by putative serotonergic agents in three ethoexperimental paradigms. Behav Neural Biol 54:234–253

LeDoux JE, Iwata J, Cichetti P, Reis DJ (1988) Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. J Neurosci 8:2517–2519

Leysen JE (2004) 5-HT_2 receptors. Curr Drug Target CNS Neurol Disord 3(1):11–26

Leysen JE, Van Gompel P, Gommeren W, Weestenborghs R, Jansen PAJ (1986) Down regulation of serotonin S_2 receptor sites in rat brain by chronic treatment with the serotonin-S_2 antagonists: ritanserin and setoperone. Psychopharmacology 88:434–444

Lowy MT, Meltzer HY (1988) Stimulation of cortisol and prolactin secretion in humans by MK-212, a centrally active serotonin agonist. Biol Psychiatr 23:818–828

Lucki J, Ward HR, Frazer A (1989) Effect of 1-(m-chlorophenyl)-piperazine and 1-(m-trifluoromethylphenil)piperazine on locomotor activity. J Pharmacol Exp Ther 249:155–164

Maisonnette S, Morato S, Brandao ML (1993) Role of resocialization and of 5-HT1A receptor activation on the anxiogenic effects induced by isolation in the elevated plus-maze test. Physiol Behav 54:753–758

Mammounas LA, Mullen CA, O'Hearn E, Molivier ME (1991) Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. J Comp Neurol 314:558–586

Martin JR, Ballard TM, Higgins GA (2002) Influence of the 5-HT2C receptor antagonist, SB-242084, in tests of anxiety. Pharmacol Biochem Behav 71:615–625

McGaugh JL (2004) The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 27:1–28

McKearney JW (1990) Effects of serotonin agonists on operant behavior in the squirrel monkey: quizapine, MK-212, trifluoromethylphenilpiperazine, and chlorophenylpiperazine. Pharmacol Biochem Behav 35:181–185

Menard J, Treit D (1999) Effects of centrally administered anxiolytic compounds in animal models of anxiety. Neurosci Biobehav Rev 23:591–613

Mora PO, Ferreira Neto C, Graeff FG (1997) Role of 5-HT2A and 5-HT2C receptor subtypes in two types of fear generated by the elevated T maze. Pharmacol Biochem Behav 58:1051–1057

Naughton M, Mulrooney JB, Leonard BE (2000) A review of the role of serotonin receptors in psychiatric disorders. Hum Psychopharmacol 15:397–415

Nic Dhonnchadha BA, Bourin M, Hascoet M (2003) Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. Behav Brain Res 140:203–214

Nogueira RL, Graeff FG (1991) 5-HT mediation of the antiaversive effect of isamoltane injected into the dorsal periaqueductal grey. Behav Pharmacol 2:73–77

Olivier B, Molewijk HE, van der Heyden JA, van Oorschot R, Ronken E, Mos J, Miczek KA (1998) Ultrasonic vocalizations in rat pups: effects of serotonergic ligands. Neurosci Biobehav Rev 23:215–227

Padovan CM, Guimarães FS (2000) Restraint-induced hipoactivity in an elevated plus-maze. Braz J Med Biol Res 33:79–83

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic, New York

Pellow S, Johnston AL, File SE (1987) Selective agonists and antagonists for 5-hydroxytryptamine subtypes and interaction with yohimbine and FG 7142 using the elevated plus-maze test in rat. J Pharm Pharmacol 39:917–928

Pompeiano M, Palacios JM, Mengod G (1994) Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT_{2C} receptors. Brain Res Mol Brain Res 23:163–178

Porter RHP, Benwell KR, Lamb H, Malcolm CS, Allen NH, Revell DF, Adams DR, Sheardown MJ (1999) Functional characterization of agonists at recombinant human 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors in CHO-K1 cells. Br J Pharmacol 128:13–20

Rainnie DG (1999) Serotonergic modulation in the rat basolateral amygdala. J Neurophysiol 82(1):69–85

Rodgers RJ, Cole JC, Cobain MR, Daly P, Doran PJ, Eells JR, Wallis P (1992) Anxiogenic-like effects of fluprazine and eltoprazine in the mouse elevated plus-maze: profile comparisons with 8-OH-DPAT, CG 12066b, TFMPP and mCPP. Behav Pharmacol 3:621–634

Setem J, Pinheiro AP, Motta VA, Morato S, Cruz APM (1999) Ethopharmacological analysis of 5-HT ligands on the rat elevated plus-maze. Pharmacol Biochem Behav 62:515–521

Stutzmann JM, Bon B, Darch F, Lucas M, Rataud J, Piot O, Blan JC, Laduron PM (1991) Are 5-HT2 antagonists endowed with anxiolytic properties in rodents. Neurosci Lett 128:4–8

Tomkins DM, Costall B, Kelly ME (1990) Action of ritanserin and DOI on the elevated X-maze. Psychopharmacology 101:S57

Vertes RP (1991) A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. J Comp Neurol 313:643–668

Viana MB, Graeff FG, Loschmann PA (1997) Kainate microinjection into the dorsal raphe nucleus induces 5-HT release in the amygdala and periaqueductal gray. Pharmacol Biochem Behav 58:167–172

Wallis CJ, Lal H (1998) A discriminative stimulus produced by 1-(3-chlorophenyl)-piperazine (mCPP) as a putative animal model of anxiety. Prog Neuropsychopharmacol Biol Psychiatry 22:547–565

Wood MD (2003) Therapeutic potential of 5-HT2C receptor antagonists in the treatment of anxiety disorders. Curr Drug Target CNS Neurol Disord 2:383–387

Wright IK, Heaton M, Upton N, Marsden CA (1992) Comparisons of acute and chronic treatment of various serotonergic agents with those of diazepam and idazoxam in the rat elevated plus-maze. Psychopharmacology 107:405–414

Zangrossi H, Graeff FG (1994) Behavioral effects of intra-amygdala injections of GABA and 5-HT acting drugs in the elevated plus-maze. Braz J Med Biol Res 27:2453–2456

Zangrossi H, Vianna MB, Zanoveli J, Bueno C, Nogueira R, Graeff FG (2001) Serotonergic regulation of inhibitory avoidance and one-way scape in the rat elevated T-maze. Neurosci Biobehav Rev 25:637–645