Psychology & Neuroscience

REFERÊNCIA

SCARPELLI, Graziela *et al*. Effects of two selective 5-HT2C receptor-acting compounds into the ventral hippocampus of rats exposed to the elevated plus-maze. **Psychology & Neuroscience**, v. 1, n. 1, pp. 87-96, 2008. DOI: https://doi.org/10.1590/S1983-32882008000100014. Disponível em: https://www.scielo.br/j/pn/a/jjkZ57tRryQhr7Y3DhmLV4p/?lang=en#. Acesso em: 10 set. 2021.

PSYCHOLOGY & NEUROSCIENCE

Psychology & Neuroscience, 2008, 1, 1, 87 - 96
DOI:10.3922/j.psns.2008.1.014

Effects of two selective 5-HT_{2C} receptor-acting compounds into the ventral hippocampus of rats exposed to the elevated plus-maze

Graziela Scarpelli[1], Sergio Henrique Alves[2], J. Landeira-Fernandez[3, 4] and Antonio Pedro de Mello Cruz[2]

1 Instituo de Ensino Superior de Brasília, Brazil

2 Universidade de Brasília, Brazil

3 Pontifícia Universidade Católica, Rio de Janeiro, Brazil

4 Universidade Estácio de Sá, Rio de Janeiro, Brazil

Abstract

This study investigated the effects of two selective serotonin$_{2C}$ (5-hydroxytryptamine, 5-HT_{2C}) receptor-acting compounds into the ventral hippocampus (VH) of rats exposed to the elevated plus-maze (EPM). In the first experiment, rats were exposed to the EPM 10 min following VH infusions of either vehicle or the selective 5-HT_{2C}-receptor agonist RO-60-0175 (0.3, 1.0, 3.0 and 10.0µg). In addition to conventional parameters of open arm exploration (i.e. percentages of open arm entries and of time spent in these arms), risk assessment-related behaviors were recorded as anxiety-like measures in EPM scoring. RO-60-0175 selectively decreased open arm exploration at the dose of 1.0 µg, while inducing locomotor-suppressant effects at the two highest doses. In the second experiment, VH infusions of the selective 5-HT_{2C} antagonist RS 102221 (0.75, 1.25 and 2.5 µg) did not affect open arm exploration, while reducing risk assessment in the closed ones. This behavioral profile of risk assessment is suggestive of an anxiolytic-like action. These results further corroborate our previous findings showing that VH 5-HT_{2C} receptor activation elicits anxiogenic-like and locomotor-suppressant effects, and suggest that the selective blockade of this receptor is accompanied by an anxiolytic-like action as detected by ethologically derived measures in the EPM. **Keywords:** anxiety, 5-HT_{2C} receptors, RO-60-0175, RS 102221, ventral hippocampus, elevated plus-maze, risk assessment.

Received 2 February 2008; received in revised form 2 June 2008; accepted 2 June 2008; Available online 10 June 2008.

Introduction

Serotonin$_{2C}$ (5-hydroxytryptamine, 5-HT_{2C}) receptor activation, either by nonselective 5-HT_{2C} agonists such as m-chlorophenylpiperazine (m-CPP) and trifluoromethyl-phenylpiperazine (TFMPP) or the preferential 5-HT_{2C} agonist 6-chloro-2[1-piperazinyl]pyrazine (MK-212), has long been associated with anxiogenic-like profiles in a variety of animal models of anxiety, including the elevated plus-maze (EPM; Benjamin, Lal, & Meyerson, 1990; Kshama, Hrishikeshavan, Shanbhogue, & Munonyedi, 1990; Rodgers et al., 1992; Gibson et al., 1994; Griebel, Moreau, Jenck, Mutel, Martin, & Misslin, 1994; Fone, Shalders, Fox, Arthur, & Marsden, 1996; Wallis and Lal, 1998; Setem, Pinheiro, Motta, Morato, & Cruz, 1999; Jones, Duxon, & King, 2002; Bull, Huston, & Fone , 2003; Durand, Mormèd, & Chaouloff, 2003). In fact, newly selective 5-HT_{2C} agonists (e.g. RO-60-0175) have been found to increase anxiety-related behaviors (Griebel et al., 1997; Kennett, et al., 1997, Kennett, Lightowler, S., Trail, Bright, & Bromidge, 2000; Martin, Ballard, & Higgins, 2002; Millan, Brocco, Gobert, & Dekeyne , 2005), although null and even anxiolytic-like effects have also been reported (Nic Dhonnchadha, Bourin, & Hascoet, 2003; Rippol, Hascoet, & Bourin, 2006).

Despite growing insights into the neural mechanisms through which 5-HT systems might influence defensive behavior, the circuits responsible for the above findings as well as the exact role of the 5-HT_{2C} receptor in specific types of anxiety remain unclear. For example, 5-HT_{2C} agonists increase anxiety-related behaviors in the basolateral nucleus of the amygdala (Campbell & Merchant, 2003) but decrease panic-related behaviors in the dorsal periaqueductal gray (Jenck, Bos, Wichmann, Stadler, Martin, & Moreau, 1998; Graeff, 2002; Jacob et al., 2002; Zanoveli, Nogueira, & Zangrossi, 2003). Therefore, different brain structures that receive direct 5-HT projections from the dorsal raphe nucleus might have a distinct contribution to anxiety mediation.

Besides the amygdala and the periaqueductal gray, the ventral portion of the hippocampus (VH) is another important postsynaptic 5-HT site whose cell bodies are located in the dorsal raphe nucleus (Azmitia & Segal, 1978; Vertes, 1991). It appears that 5-HT receptors present

Graziela Scarpelli, Curso de Psicologia, Instituo de Ensino Superior de Brasília, DF, Brasil. Sergio Henrique Alves and Antonio Pedro de Mello Cruz, Departamento de Processos Psicológicos Básicos, Instituto de Psicologia, Universidade de Brasília, Brasilia, DF, Brasil. J. Landeira-Fernandez, Departamento de Psicologia, Pontifícia Universidade Católica do Rio de Janeiro, Brasil and Curso de Psicologia, Universidade Estácio de Sá, Campus Akxe, Rio de Janeiro, Brasil. Correspondences regarding this articles should be directed to Antonio Pedro Mello Cruz, Ph.D. University of Brasília, Institute of Psychology, Asa Norte, Brasilia DF, 70910-900, Brazil. Tel/fax: +55 61 3307 2625 ext. 502. E-mail: apmcruz@unb.br

in the VH are involved in defensive behavior. For example, it has been found that electric stimulation of the dorsal raphe nucleus (McQuade & Sharp, 1997) or potentially dangerous situations such as a context previously associated to a footshock (Hajos-Korcsok, 2003) and acute EPM exposure (Wright, Upton, & Marsden, 1992; Voigt, Rex, Sohr, & Fink, 1999; Rex, Voigt, & Fink, 2005) enhance postsynaptic 5-HT levels in the VH, which may suggest an anxiogenic-like role for 5-HT within this forebrain site. In agreement with this view, selective VH lesions are associated with anxiolytic-like effects in contextual fear conditioning (Bannerman, Grubb, Deacon, Yee, Feldon, & Rawlins , 2003, Bannerman et al., 2004), light-dark transition (Kjelstrup, Tuvnes, Steffenach, Murison, Moser, & Moser, 2002; McHugh, Deacon, Rawlins, & Bannermen, 2004), social interaction test in rats (McHugh et al, 2004) and in the EPM (Bannerman et al., 2002; Kjelstrup et al., 2002; Degroot & Treit, 2004).

Interestingly, the fibers originating from the dorsal raphe nucleus establish preferential contact with postsynaptic 5-HT_2 receptors (Mammounas Mullen, O'Hearn, & Molivier, 1991). Based upon results showing the presence of the 5-HT_{2C} receptor subtype at a very high density in the VH (Pompeiano, Palácios, & Mengod, 1994; Fone, Shalders, Fox, Arthur., & Marsden 1996, Clemett, Punhani, Duxon, Blackburn, & Fone, 2000; Garcia-Alcover, Segura, Garcia Pena, Martinez-Torres, & Miledi, 2006), however being more abundant in the choroid plexus (Leysen, Van Gompel, Gommeren, Weestenborghs, & Jansen, 1986; Backstrom, Westphal, Canton, & Sanders-Bush, 1995; Leysen, 2004), it is possible that the anxiogenic-like role of 5-HT in the VH might be, at least in part, mediated via 5-HT_{2C} receptor activation. Accordingly, in another study, we found the preferential 5-HT_{2C} agonist MK-212 to elicit anxiogenic-like effects when infused directly into the ventral but not dorsal hippocampus of rats exposed to the EPM (Alves, Pinheiro, Motta, Landeira-Fernandez, & Cruz 2004).

It is of note that although MK-212 does not act selectively at 5-HT_{2C} receptors, its effects have been usually attributed to a 5-HT_{2C}-receptor activation 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 findings showing a clear dependence of discriminative action on selective stimulation of 5-HT_{2C} receptors (Clineschmidt, 1979; Blackburn, Kemp, Martin, & Cox, 1984; Cunningham, Callahan, & Appel, 1986). Therefore, such an MK-212-induced anxiogenic-like effect in the VH is likely to be due to a 5-HT_{2C}-receptor activation, although the participation of other 5-HT_2 receptors cannot be totally discounted.

The present study employed two newly selective 5-HT_{2C}-acting compounds in order to further investigate the role of VH 5-HT_{2C} receptors in mediating anxiety-like behaviors triggered by the EPM. In Experiment 1, rats were exposed to the EPM under the effects of intra-VH infusion with the selective 5-HT2C-receptor agonist RO-60-0175, a centrally acting compound that exhibits high affinity for the 5-HT_{2C}-receptor (nM affinity for 5-HT_{2C} and > 25 – 100-fold lower for other receptors; Boes et al., 1997; Porter et al., 1999). Although RO-60-0175 presents considerable selectivity for 5-HT_{2A} and 5-HT_{2B} receptors (Vickers et al., 2001; Knight et al., 2004), this compound seems to produce predominantly 5-HT_{2C}-receptor mediated behavior (Martin et al., 1998). This conclusion is supported by results in which highly selective 5-HT_{2C} antagonists, such as SB-242084 (Martin, Ballard, & Higgins, 2002), but not 5-HT_{2A} or 5-HT_{2B} antagonists (Dekeyne, Girardon, & Milan, 1999) prevent changes in behavior induced by R0-60-0175. These patterns of results support the use of RO-60-0175 in several laboratories as a reliable pharmacological tool for activating 5-HT_{2C} receptors.

Considering that VH 5-HT_{2C}-receptor activation might elicit an anxiogenic-like effect, it is reasonable to assume that the selective blockade of this receptor subtype might be accompanied by an anxiolytic-like action. Experiment 2 tested this hypothesis by infusing the selective 5-HT_{2C}-receptor antagonist RS 102221 directly into the VH. RS 102221 is a centrally acting antagonist that binds with high affinity to 5-HT_{2C} receptors (nM affinity for 5-HT_{2C} and > 35-fold lower for other 5-HT_2 receptor subfamilies; Bonhaus et al., 1997). To the best of our knowledge, the effects of RS 102221 on anxiety-like behaviors in the EPM have not yet been described.

Methods

Subjects

Experimentally naive male Wistar rats weighing 190-250 g were employed as subjects. The animals were born and raised in the vivarium at the University of Brasilia. One week before the study they 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 light-dark cycle was maintained on a 12-h on-off cycle (07:00-19:00h lights on). The experimental sessions were carried out during the light phase of the cycle. The experimental protocols were conducted in conformity with the recommendations of the Brazilian Society of Neuroscience and Behavior (SBNeC), which are based on the US National Institutes of Health Guide for the Care and Use of Laboratory Animals (revised in 1996).

Surgery

Animals were anaesthetized with sodium thiopental (45 mg/kg IP) and placed in a stereotaxic frame with the head level between bregma and lambda. A subcutaneous injection of 2% lidocaine with vasoconstrictor was administered in the surgical area until a small bubble was formed. Each rat was bilaterally implanted with a stainless steel guide cannula (o.d. 0.7 mm) aimed at 0.5 mm above the target area. Taking bregma as the reference for each plane according to the Paxinos and Watson atlas (1986), the coordinates were 4.8 mm posterior to bregma, 5.0 mm lateral to the midline

for each hemisphere, and 5.5 mm ventral to skull. Guide cannulae were anchored to the skull by means of dental acrylic and four stainless screws. After implantation, the guide cannulae were sealed with a stainless steel wire to prevent eventual congestion. Four days after surgery the animals were wrapped in a cloth and handled for 3 min for three consecutive days. Behavioral testing was performed on the 8th day post-surgery.

Apparatus

The EPM, elevated 50 cm above the ground, consisted of two open arms (50 × 10 cm) perpendicular to two other arms of the same size enclosed by 40 cm-high walls. These four arms delimited a central area of 10 × 10 cm. A rim of Plexiglas (1 cm high) surrounded the perimeter of the open arms to minimize rats falling off the maze. Illumination was provided by a dim light bulb (60 W) in the ceiling of the experimental room and the light intensity in the center of the maze was adjusted to 55 lux. A video camera linked to a monitor and VCR in an adjacent room videotaped the experimental sessions.

Drugs

RS 102221 [8-5[5-(2,4-dimethoxy-5-(4-trifluoromethyl-phenyl-sulphoamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro-[4.5]decane-2,4-dione HCl] and RO-60-0175 [(S)-2-(6-chloro-5-floro-indol-l-yl)-1-methyl-amine fumarate] (Tocris, Ballwin, MO, USA) were dissolved in sterile saline (0.9% NaCl) and infused 10 min before testing.

Procedure

Experiment 1. The animals were randomly assigned to five groups and infused into the VH either with vehicle or RO-60-0175 at the doses of 0.3, 1.0, 3.0 and 10 µg. Infusion was achieved by an internal cannula (o.d. 0.3 mm) that extended 0.5 mm beyond the guide cannula tip, attached to a 10 µl Hamilton syringe via PE-10 tubing. Confirmation of successful infusion was obtained by monitoring the movement of a small air bubble inside the PE-10 tubing. A volume of 0.2 µl/side was delivered over approximately 30 s with the needle left in place for a further 2 min to minimize reflux up to the cannula shaft.

Ten minutes after infusion, the animals from each group were exposed for 5 min to the maze in a counterbalanced manner. A highly trained observer who remained blind to treatment conditions later analyzed the videotapes. The number of entries and the time spent in the open and closed arms were recorded. From these measures, the percentage of open arm entries (100 x open arm entries/total arm entries) and the percentage of time spent in the open arms (100 x time open/time open + time closed) were calculated for each animal as anxiety-like indexes. In addition to these conventional measures, the time displaying risk assessment-related behaviors from a closed arm (exiting a closed arm with the forepaws and head only, and investigating the surroundings with horizontal movements of head) was recorded as ethologically derived measures of anxiety as described elsewhere (Cruz, Frei, & Graeff, 1994). The absolute number of closed arm entries was interpreted as a reliable index of locomotor activity (File, 1992; Cruz et al., 1994).

Experiment 2. In this experiment, four groups of rats were exposed to the same EPM procedure described in Experiment 1, 10 min after VH infusion either with vehicle or the selective 5-HT$_{2C}$-receptor antagonist RS 102221 at the doses of 0.75, 1.25 or 2.5 µg. These doses were chosen on the basis of previous studies in which RS 102221 was administered intracerebrally (McMahon, Filip, & Cunningham, 2001; Filip & Cunningham, 2002, 2003; Body et al., 2006).

Histology

At the end of behavioral testing, the rats were injected with sodium thiopental overdoses and a volume of 0.2 µl fast-green dye was infused into each brain site to aid visualize actual injection sites. They were then transcardially perfused with physiological saline followed by 10% formol-saline solution as fixative. The brains were removed, stored in 5% formol-saline for two weeks, sectioned horizontally by cryostatic method at 50-60, and stained with Cresyl violet. Drawings from the infusion locations were superimposed on the appropriate pages of the stereotaxic atlas of Paxinos and Watson (1986).

Statistical analysis

Results from the two experiments were statistically analyzed by a one-way analysis of variance (ANOVA) to detect overall differences. Fisher's least significant difference (LSD) post hoc test was employed to determine specific differences between groups. Because the absolute number of closed arm entries in Experiment 1 was significantly decreased by RO-60-0175, an analysis of covariance (ANCOVA) using this parameter as covariant (File, 1992) was additionally performed in order to examine whether locomotor activity could account for the eventual differences in the anxiety-like parameters. The level of statistical significance was $p < .05$.

Results

Histology

As illustrated by a diagrammatic representation of coronal sections showing the injection sites from Experiments 1 and 2 (Figure 1), most of the injections were distributed throughout the entire rostral-caudal extent of the target area within the VH. Behavioral results from animals with injection sites outside the VH (n = 12, *Experiment 1*; n = 7, Experiment 2) were removed from their respective groups and assigned to additional control groups in each experiment for statistical analysis.

Behavioral testing

Experiment 1. Effects of RO-60-0175 on conventional measures of exploration in the EPM are shown in Figure 2.

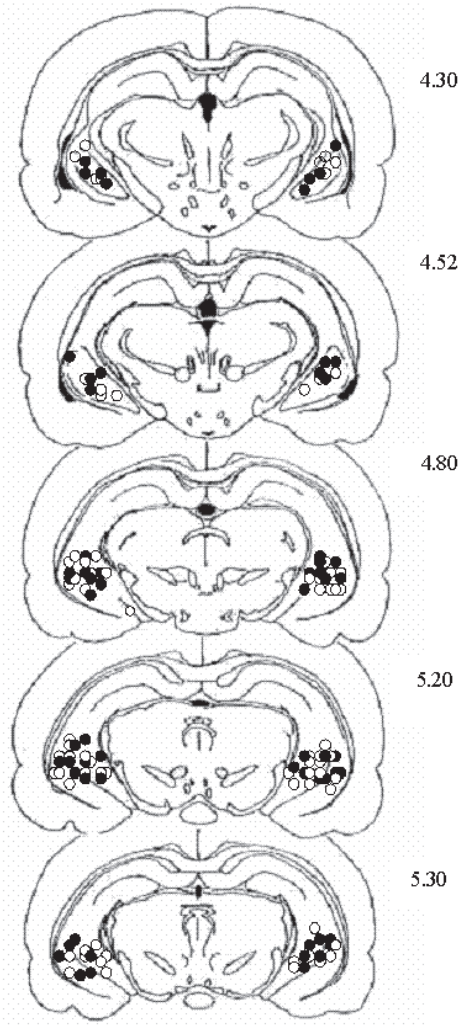

Figure 1. Composite of infusion sites aimed at the VH from Experiment 1 (white circles) and Experiment 2 (black circles). With the reference to the Paxinos and Watson (1986) atlas, the numbers on the right side of each plate indicate the distance in mm from bregma.

As suggested from the upper panel of this figure, the ANOVA confirmed a main effect of treatment in both the percentage of open arm entries, $F(5, 43) = 4.54$, $p < .05$, and the percentage of time spent in the open arms, $F(5, 43) = 6.42$, $p < .05$. Post hoc comparisons showed that the doses of 1.0, 3.0 and 10 µg to significantly decreased these two parameters of open arm exploration as compared to vehicle-infused animals ($p < .05$). The ANOVA also indicated a significant effect of treatment, $F(5, 43) = 9.71$, $p < .05$, on closed arm entries. Post hoc comparisons revealed that the doses of 3.0 and 10.0 µg significantly decreased this locomotor activity index as compared to vehicle-infused animals (ps < .05).

To dissociate anxiogenic-like effects from nonspecific locomotor impairments induced by the two highest RO-60-0175 doses, an additional ANCOVA using the closed arm entries as covariant factor was conducted in both the percentages of open arm entries and of time spent in these arms. In these two cases, the ANCOVA revealed no significant effects of treatment when the closed arm entries were statistically controlled for ($p > .05$). Therefore, the decrease in open arm exploration observed at the doses of 3.0 and 10.0 µg was probably due to a locomotor impairment.

Figure 2 (lower panel) illustrates the effects of RO-60-0175 microinjections on risk assessment. The ANOVA indicated a main effect of treatment,

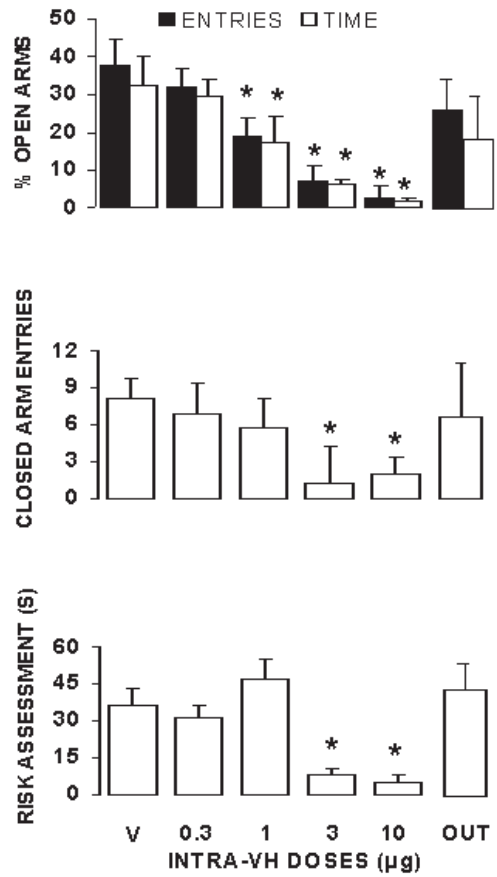

Figure 2. Mean (+SEM) percentage of open arm entries and time (upper panel), closed arm entries (middle panel) and risk assessment (lower panel) among groups microinjected either with vehicle (n = 7) or RO-60-0175 at the doses of 0.3 µg (n = 8), 1 µg (n = 7), 3 µg (n = 9) and 10 µg (n = 7) into the VH. OUT (n = 12) illustrates a representative group of rats infused with vehicle or different doses of RO-60-0175 at sites localized outside the VH. *indicates $p < .05$ as compared to vehicle control.

$F(5, 43) = 7.23$, $p < .05$, and post hoc comparisons revealed a significant reduction of risk assessment at the doses of 3.0 and 10.0 µg ($p < .05$ and .01, respectively). No other significant differences were found, despite a trend to increased risk-assessment at the dose of 1.0 µg. Again, the ANCOVA failed to detect significant differences on risk assessment parameters when the closed arm entries were used as covariate factor ($p > .05$).

Experiment 2. Figure 3 shows the effects in the EPM of infusing the 5-HT_{2C}-receptor antagonist RS 102221 into

the VH. None of the doses tested significantly affected open arm exploration (upper panel) or absolute number of closed arm entries. ANOVA outcomes from these anxiety and locomotor parameters confirmed a lack of effect of RS 102221 (F values not shown). Risk assessment (lower panel), however, was significantly changed by treatment, $F(4, 33) = 5.49$, $p < .05$. Post hoc comparisons revealed this effect to be restricted to a single RS 102221 dose (2.5 µg), which significantly decreased risk assessment-related behaviors from the closed arms as compared to vehicle-infused animals ($p < .05$).

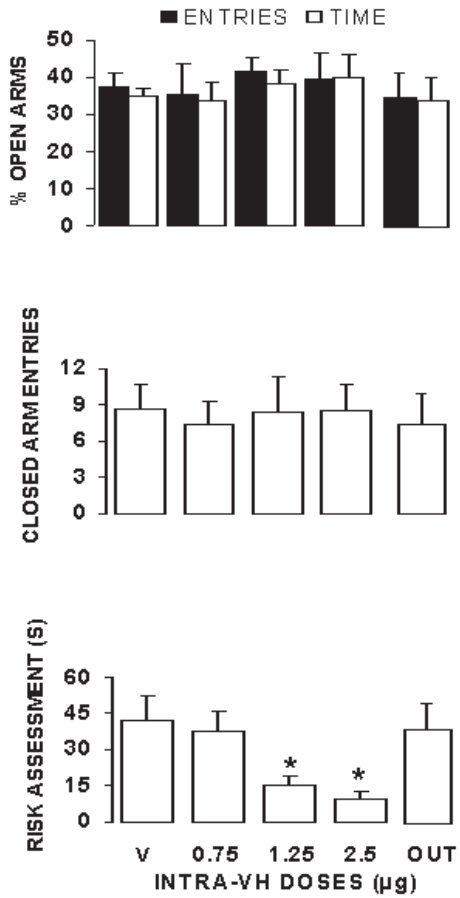

Figure 3. Mean (+SEM) percentage of open arm entries and time (upper panel), closed arm entries (middle panel) and risk assessment (lower panel) among groups microinjected either with vehicle (n = 7) or RS-102221 at the doses of 0.75 (n = 8), 1.25 (n = 7) and 2.5 µg (n = 10) into the VH. OUT (n = 7) illustrates a representative group of rats infused with vehicle or different doses of RS-102221 at sites localized outside the VH. *indicates $p < .05$ as compared to vehicle control.

Discussion

Pharmacological activation at 5-HT2C receptors is associated with anxiety states and locomotor suppressant effects (Kennett, Whitton, Shah, & Curzon, 1989; Rodgers et al., 1992; Griebel et al., 1994; Wallis & Lal, 1998; Setem, Pinheiro, Motta, Morato, & Cruz, 1999; Durand, Mormèd, & Chaouloff, 2003), whereas its blockage by selective 5-HT$_{2C}$ antagonists has been appraised as a potential target for anxiolytic compounds (Kennett et al., 1997; Bagdy, Graf, Anheuer, Modos, & Kantor, 2001; Andres et al., 2002; Jones, Duxon, & King, 2002; Martin, Ballard, & Higgins, 2002; Jones & Blackburn, 2002; Wood, 2003; Gordon, 2004; Millan, 2005). However, intriguing results ranging from anxiolytic-like effects of 5-HT$_{2C}$ receptor agonists (Nic Dhonnchadha et al., 2003) to little or null effects of 5-HT$_{2C}$ receptor antagonists (Griebel, Perrault, & Sanger, 1997, Griebel, Rodgers, Ghislaine, & Sanger, 1997; Nic Dhonnchadha et al., 2003) have also been reported in some animal models of anxiety. Moreover, many of these effects vary considerably in different postsynaptic 5-HT sites in the brain (Jenck, Bos, Wichmann, Stadler, Martin, & Moreau, 1998; Graeff, Guimaraes, De Andraede, & Deakin, 1996; Graeff, 2002; Zanoveli et al., 2003).

In the present study we investigated the effects of two selective 5-HT$_{2C}$-acting compounds microinjected into the VH of rats exposed to the EPM. In Experiment 1, the selective 5-HT$_{2C}$ agonist RO-60-0175 dose-dependently decreased both the percentage of open arm entries and the percentage of time spent in the open arms. At the dose of 1.0 µg, the RO-60-0175-induced decrease in open-arm exploration was devoid of a significant locomotor interference, despite a clear trend to reduce the absolute number of closed arm entries. This anxiogenic-like effect is in accordance with the behavioral profile of the preferential 5-HT$_{2C}$ receptor agonist MK-212 into this same brain site (Alves et al., 2004). Taking into account the higher selectivity of RO-60-0175 for 5-HT$_{2C}$ receptors, this result further corroborates the suggestion that enhanced VH 5-HT$_{2C}$-receptor responsiveness is associated with anxiety-like states.

Because the VH is a postsynaptic 5-HT site notably implicated in anxiety (Gray & McNaughton, 2000; Degroot & Treit, 2004; Rex et al., 2005), it is possible that the present RO-60-0175 effects in the VH might involve 5-HT projections from the dorsal raphe nucleus (Azmitia & Segal, 1978; Vertes, 1991). In this respect, it has been found that potentially dangerous situations such as a context previously associated to an aversive stimulus (Hajos-Korcsok, 2003) or acute EPM exposure (Wright et al., 1992; Voigt et al., 1999; Rex et al., 2005) markedly enhance postsynaptic 5-HT levels in the VH. Conversely, selective VH lesions attenuate anxiety-related behaviors in these animal models of anxiety (Kjelstrup et al., 2002; Bannerman et al., 2003). Taking into account the presence of the 5-HT$_{2C}$ receptor subtype at a very high density in the VH (Pompeiano et al., 1994; Backstrom et al., 1995; Fone et al., 1996, Clemett et al., 2000; Garcia-Alcover et al., 2006), it seems reasonable to assume that 5-HT-induced anxiety in the VH might be at least in part mediated via 5-HT$_{2C}$-receptor activation.

It must be noted that the behavioral effects of RO-60-0175 in the VH resemble those induced by 5-HT$_{2C}$ agonists in the amygdala, another important anxiety-related structure

(LeDoux, Iwata, & Cichetti, 1988; Davis, Raiunnie, & Cassell, 1994; Davis & Shi, 1999) innervated by 5-HT fibers from the dorsal raphe nucleus (Vertes, 1991; Rainnie, 1999). For example, infusions of the nonselective 5-HT2C agonist mCPP and the selective 5-HT2C agonist IL-639 into the basolateral nucleus of the amygdala produced ultrasonic vocalization and increased the latency to investigate new objects in rats exposed to an open-field, an anxiogenic-like effect prevented by intraperitoneal (IP) pretreatment with the selective 5-HT_{2C} antagonist SB-24084 (Campbell & Merchant, 2003). In the same line of evidence, our recent plus-maze results (Cruz et al., 2005) with basolateral amygdala infusion of ritanserin, a mixed 5-HT2 blocker that exhibits higher affinity at 5-HT2C than 5-HT2A receptors (Leysen et al., 1986; Leysen, 2004), show that this compound was able to prevent decreased open arm exploration induced by IP injection of MK-212. Interestingly, in this same study, ritanserin microinfusion into the basolateral amygdala was ineffective to change basal anxiety-like levels in saline-pretreated animals. It seems, therefore, that 5-HT_{2C} receptors within both the VH and basolateral nucleus of the amygdala play a similar role in mediating fear or anxiety-related behaviors. This view is supported by the existence of a bilateral neural projection between the VH and several nuclei of the amygdaloid complex (for a review, see Pitkanen, Pikkarainen, Nurminen, & Ylinen, 2000).

Deakin and Graeff (1991) have proposed a dual role of 5-HT action on anxiety mediation. According to this hypothesis, ascending fibers from the dorsal raphe nucleus might facilitate anxiety through actions on the amygdala, while inhibiting inborn fight/flight reactions in the periaqueductal gray. Although this model recognizes the participation of an anatomical projection from the MRN to the dorsal hippocampus in the resistance to chronic and inescapable aversive stimuli, no mention is made regarding the 5-HT projections from the DRN to the VH. Our results suggest that 5-HT_{2C} receptors located within the VH might modulate anxiety behavior in a similar way to that attributed to the amygdala by Deakin and Graeff in their model.

It is important to acknowledge that direct comparisons between the effects of selective lesions of either the VH or the amygdala have suggested these structures to be functionally distinct in the control of defensive behaviors. For example, VH but not amygdala lesions produced anxiolytic-like effects in widely used animal models of anxiety such as the EPM (Sommer et al., 2001; Kjelstrup et al., 2002; McHugh et al., 2004), the successive alleys test (McHugh et al., 2004) and the social interaction in rats (Decker, Curzon, & Brioni, 1995; McHugh et al., 2004). It is still unclear whether these differences are task-dependent or related to specific nuclei of the amygdala.

Results from Experiment 1 also showed the doses of 3.0 and 10.0 µg of RO-60-0175 to markedly reduce closed arm entries, a behavioral profile indicative of decreased locomotor activity in the EPM. This is in agreement with the well-documented locomotor-suppressant effects of systemically administered 5-HT2C agonists, including RO-60-0175 (Martin et al., 1998, Kennett et al., 2000; Martin et al., 2002).

As confirmed by the ANCOVA, the RO-60-0175-induced locomotor interference was observed exclusively at the two highest doses. This decrease in general activity practically abolished the occurrence of risk assessment-related behaviors. However, this effect cannot be interpreted as an anxiolytic-like action. As we previously reported (Cruz et al., 1994), a lack of risk assessment upon high anxiety levels that are accompanied by a significant decrease of closed arm entries most probably reflects decreased general exploration or even complete immobility inside the closed arms. Therefore, the present results also suggest that the VH 5-H_{T2C} receptors might play a role in mediating locomotor activity.

As far as we know, this is the first report in the literature that tested the behavioral effects of RO-60-0175 microinjected into the VH. The locomotor activity effect observed in the present study was also reported by Fletcher and colleagues (2004), who found that microinjections of the same dose range of RO-60-0175 into the ventral tegmental area also impaired locomotor activity. Therefore, it appears that 5-H_{T2C} in different brain areas might be involved in the mediation of locomotor activity. At least in part, this view is corroborated by our results, which found the group microinjected with RO-60-0175 outside the VH to show a trend toward reducing closed arm entries, although this effect was not statistically significant.

In Experiment 2, VH infusion of the selective 5-H_{T2C}agonist RS 102221 did not affect conventional parameters of EPM exploration. At the two highest doses, however, RS 102221 significantly reduced risk assessment. Considering that a reduction in risk assessment in the absence of locomotor effects is consistent with a selective anxiolytic-like action in the EPM (Cruz et al., 1994; Griebel et al., 1997a, 1997b), our results suggest that the VH 5-H_{T2C} receptor blockade might be associated with a reduction of anxiety-like states.

Frequency and/or duration of risk assessment-related behaviors from the closed arms have been widely used in the EPM scoring as a reliable and sensitive measure to detect anxiolytic-like effects of 5-HT compounds (Griebel et al., 1997a, 1997b; Setem et al., 1999; Griebel, Rodgers, Perrault, & Sanger, 2000), which does not necessarily change the conventional parameter of anxiety in this test. Factor analyses of spatiotemporal and ethologically derived measures of rats in the EPM indicated that although risk assessment and conventional anxiety measures loaded in the same factor, the former but not the latter also loaded in another factor seemingly related to decision-making processes or more cognitively oriented aspects of anxiety (Cruz et al., 1994). Therefore, it can be concluded from the present results that RS 102221 microinfusion into the VH induced an anxiolytic-like action as measured by the risk assessment in the EPM.

The possibility that the conventional anxiety measures might be less sensitive in detecting anxiolytic-like action of 5-HT2 antagonists upon low basal levels of anxiety in the EPM cannot be discounted (Rodgers & Dalvi, 1997). In fact, substantial experimental evidence indicates that, whereas 5-H_{T2C} agonists decrease open arm exploration,

an anxiogenic-like action that is prevented by selective and nonselective 5-H$_{T2C}$ antagonists, the blockade of 5-H$_{T2C}$receptors by itself had little or no effect on conventional anxiety parameters in the EPM (Griebel, 1996; Griebel et al., 1997a, 1997b; Setem et al., 1999, Jones et al., 2002; Martin et al., 2002). In line with this view, we reported that the nonselective blockade of 5-H$_{T2C}$ receptors in the basolateral nucleus of the amygdala prevented MK-212-induced decrease in open arm exploration, whereas the blockade of this receptor by itself was ineffective in saline pretreated animals (Cruz et al., 2005).

Finally, the use of inadequate doses cannot be totally excluded from the lack of clear effects of RS 102221 on open arm exploration. Although the effects of similar RS 102221 dose ranges have been investigated in different brain areas (e.g. McMahon et al., 2001; Filip & Cunningham, 2002), this is first study in which the effects of intra-VH RS 102221 infusion was investigated in anxiety-like behaviors of rats exposed to the EPM. Therefore, further experiments comparing the effects of other dose ranges and other selective 5-H$_{T2C}$ agonists and antagonists in both conventional and ethologically derived measures in the EPM could improve our knowledge about the involvement of 5-H$_{T2C}$ receptors on anxiety mediation.

Acknowledgment

Research supported by grants from the Brazilian National Research Council (CNPq, Brazil) to A.P.M. Cruz (350214/1998-0) and J. Landeira-Fernandez (522720/95-1).

References

Alves, S.H., Pinheiro, G., Motta, V., Landeira-Fernandez, J., & Cruz, A.P.M. (2004). Anxiogenic effects in the rat elevated plus-maze of 5-HT2C agonists into ventral but not dorsal hippocampus. *Behavioural Pharmacology, 15*, 37-43.

Andres, J.I., Alonso, J.M., Fernandez, J., Iturrino, L., Martinez, P., Meert, T.F., et al. (2002). 2-(Dimethylaminomethyl)-tetrahydrois oxazolopyridobenzapine derivates. Synthesis of a new 5-HT(2C) antagonist with potential anxiolytic properties. *Bioorganic and Medicinal Chemistry Letters, 12*, 3573-3577.

Azmitia, E.C., & Segal, M. (1978). An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. *Journal of Comparative Neurology, 179*, 641-667.

Backstrom, J.R., Westphal, R.S., Canton, H., & Sanders-Bush, E. (1995). Identification of 5-HT2C receptors as glycoproteins containing N-linked oligosaccharides. *Molecular Brain Research*, *33*, 311-318.

Bagdy, G., Graf, M., Anheuer, Z.E., Modos, E.A., & 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. *International Journal of Neuropsychopharmacology, 4*, 399-408.

Bannerman, D.M., Grubb, M., Deacon, R.M., Yee, B.K., Feldon J., & Rawlins J.N. (2003). Ventral hippocampal lesions affect anxiety but not spatial learning. *Behavioral Brain Research. 139*, 197-213.

Bannerman, D.M., Rawlins, J.N., McHugh, S.B., Deacon, R.M., Yee, B.K., Bast, T., et al. (2004). Regional dissociation within the hippocampus-memory and anxiety. *Neuroscience and Biobehavioral Reviews. 28*, 273-283.

Benjamin, D., Lal, H., & Meyerson, L.R. (1990). The effects of 5-HT1B characterizing in the mouse elevated plus-maze. *Life Sciences ,47*, 195-203.

Blackburn, T.P., Kemp, J.D., Martin, D.A., & Cox, B. (1984) Evidence that 5-HT agonist-induced rotational behavior in the rat is mediated via 5-HT$_1$ receptors. *Psychopharmacolog,y 83*, 163-165

Body, S., Asgari, K., Cheung, T.H., Bezzina, G., Fone, K.F., Glennon, J.C., et al. (2006). Evidence that the effect of 5-HT2 receptor stimulation on temporal differentiation is not mediated by receptors in the dorsal striatum. *Behavioral Processes, 71*, 258-267.

Boes, M., Jenck, F., Martin, J.R., Moreau, J-L., Sleight, A.J., Wichmann, J., et al. (1997). Novel agonists of 5-HT$_{2C}$ receptors. Synthesis and biological evaluation of substituted 2-(Indol-1-yl)-1-methylethylamines and 2-(Indeno[1,2-b]pyrrol-1-yl)-1-methylamines. Improved therapeutics for obsessive compulsive disorder. *Journal of Medicinal Chemistry, 40*, 2762-2769.

Bonhaus, D.W., Weinhardt, K.K., Taylor, M., DeSouza, A., McNeeley, P.M., Szczepanski, K., et al. (1997). RS-102221: a novel high-affinity and selective 5-HT$_{2C}$ receptor antagonist. *Neuropharmacology, 36*, 621-629.

Bull, E.J., Huston, P.H., & Fone, K.C. (2003). Reduced social interaction following 3,4-methylenedioxymethamphetamine is not associated with enhanced 5-HT2C receptor responsivity. *Neuropharmacology, 44*, 439-448.

Campbell, B.M., & Merchant, K.M. (2003). Serotonin 2C receptors within the basolateral amygdala induce acute fear-like responses in an open-field environment. *Brain Research, 993*, 1-9.

Clemett, D.A., Punhani, T., Duxon, M.S., Blackburn, T.P., & Fone, K.C. (2000) Immunohistochemical localization of the 5-HT$_{2C}$ receptor protein in the rat CNS. *Neuropharmacology, 39*, 123-132.

Clineschmidt, B.V. (1979). MK-212: a serotonin-like agonist in the CNS. *General Pharmacology, 10*, 287-290.

Cruz, A.P.M., Frei, F., & Graeff, F.G. (1994). Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacology Biochemistry and Behavior*, 171-176.

Cruz, A.P.M., Pinheiro, G., Alves, S.H., Ferreira, G., Mendes, M., Faria, L., et al. (2005). Behavioral effects of systemically administered MK-212 are prevented by ritanserin microinfusion into the basolateral amygdala of rats exposed to the elevated plus-maze. *Psychopharmacology, 182*, 345-354.

Cunningham, K.A., Callahan, P.M., & Appel J.B. (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 in Neurosciences, 17*, 17-24.

Davis, M., & Shi, C. (1999). The extended amygdala: are the central nucleus of the amygdala and the bed nucleus of the stria terminalis differentially involved in fear versus anxiety? *Annals of New York Academy of Sciences,* 877, 281-291.

Deacon, R.M.J., Croucher, A., & Rawlins, J.N.P. (2002). Hippocampal cytotoxic lesion on species-typical behaviours in mice. *Behavioral Brain Research, 132*, 203-213.

Deakin, J.F.W., & Graeff, F.G. (1991). 5-HT and mechanisms of defense. *Journal of Psychopharmacology, 5*, 305-315.

Decker, M.W., Curzon, P., & Brioni, J.D. (1995). Influence of separate and combined septal and amygdala lesions on memory, acoustic startle, anxiety and locomotor activity in rats. *Neurobiology of Learning and Memory, 64*, 156-168.

Degroot, A., & Treit, D. (2004). Anxiety is functionally segregated within the septo-hipppocampal system. *Brain Research, 1001*, 60-71.

Dekeyne, A., Girardon, S., & Milan, M.J. (1999). Discriminative stimulus properties of the novel (5-HT)2C receptor agonist, RO 60-0175, a pharmacological analysis. *Neuropharmacology, 38*, 415-423.

Durand, M., Mormèd, P., & Chaouloff, F. (2003). Wistar-Kyoto rats are sensitive to the hypomotor and anxiogenic effects of mCPP. *Behavioral Pharmacology, 14*, 173-177.

File, S.E. (1992). Behavioural detection of anxiolytic actions. In: *Experimental approaches to anxiety and depression*. Elliot JM, Heal DJ, Marsden CA (editors). London: John Wiley, pp 25-44.

Filip, M., & Cunningham, K.A. (2002). Serotonin 5-HT(2C) receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. *Pharmacology Biochemistry and Behavior, 71*, 745-756.

Filip, M., & Cunningham, K.A. (2003). Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin(2C) (5-HT(2C) receptors in rat prefrontal cortex. *Journal of Pharmacology and Experimental Therapeutics, 306*, 734-743.

Fletcher, P.J., Chintoh, A.F., Sinyard, J., & Higgins, G. (2004). Injection of the 5-HT_{2C} receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. *Neuropsychopharmacology, 29*, 308-318.

Fone, K.C., Shalders, K., Fox, Z.D., Arthur, R., & Marsden, C.A. (1996). Increased 5-HT2C receptor responsiveness occurs on rearing rats in social isolation. *Psychopharmacology, 123*, 46-352.

Garcia-Alcover, G., Segura, L.C., Garcia Pena, M., Martinez-Torres, A., & Miledi, R. (2006). Ontogenetic distribution of 5-HT2C, 5-HT2A, and 5-HT7 receptors in the rat hippocampus. *Gene Expression, 13*, 53-57.

Gibson, E.L., Barnfield, A.M., & 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, 457-65.

Gordon, J.A., & Hen, R. (2004). The serotonin system and anxiety. *Neuromolecular Medicine* 5, 27-40.

Graeff, F.G. (2002). On serotonin and experimental anxiety. *Psychopharmacology* 163, 467-476.

Graeff, F.G., Guimaraes, F.S., De Andraede, T.G., & Deakin, J.F. (1996). Role of 5-HT in stress, anxiety and depression. *Pharmacology Biochemistry and Behavior, 54*, 129-141.

Gray, J.A., & McNaughton, N., editors (2000). *The neuropsychology of anxiety, 2nd ed*. Oxford: Oxford University press.

Griebel, G., Rodgers, R.J., Perrault, G., & Sanger, D.J. (2000). The effects of compounds varying in selectivity as 5-HT(1A) receptor antagonists in three rat models of anxiety. *Neuropharmacology, 39*, 1848-1857.

Griebel, G., Perrault, G., & Sanger, D.J. (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, R.J., Ghislaine, P., & Sanger, D.J. (1997b). Risk assessment behaviour: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. *Pharmacology Biochemistry and Behavior, 57*, 817-827.

Griebel, G. (1996). Variability in the effects of 5-HT-related compounds in experimental models of anxiety: evidence for multiple mechanisms of 5-HT in anxiety or never ending story*? Polish Journal of Pharmacology, 48*, 129-136.

Griebel, G., Moreau, J.L., Jenck, F., Mutel, V., Martin, J.R., & 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. *Behavioural Pharmacology, 5*, 642-645.

Hajos-Korcsok, E., Robinson, D.D., Yu, J.C., Fitch, C.S., Walker, E., & Merchant, K.M. (2003). Rapid habituation of hippocampal serotonin and norepinephrine release and axiety-related behaviors, but not plasma corticosterone levels, to repeat footshock stress in rats. *Pharmacology Biochemistry and Behavior, 74*, 609-616.

Jacob, C.A., Cabral, A.H., Almeida, L.P., Magierek, V., Ramos, P.L., Zanoveli, J.M., et al. (2002). Chronic imipramine enhances 5-HT(1A) and 5-HT(2) receptors-mediated inhibition of panic-like behavior in the rat dorsal periaqueductal gray. *Pharmacology Biochemistry and Behavior, 72*, 761-766.

Jenck, F., Bos, M., Wichmann, J., Stadler, H., Martin, J.R., & Moreau, J.L. (1998). The role 5-HT2C in affective disorders. *Expert Opinion Investigational Drugs, 7*, 1587-1599.

Jones, B.J., & Blackburn, T.P. (2002). The medical benefit of 5-HT research. *Pharmacology Biochemistry and Behavior* 71, 55-568.

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

Kennett, G., Lightowler, S., Trail, B., Bright, F., & Bromidge, S. (2000). Effects of RO 600175, a novel 5-HT_{2C} receptor agonist, in three animal models of anxiety. *European Journal of Pharmacology, 387*, 197-204.

Kennett, G.A., Wood, M.D., Bright, F., Trail, B., Riley, G., Holland, et al. (1997). SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. *Neuropharmacology, 36*, 609-620.

Kennett, G.A., Whitton, P., Shah, K., & Curzon, G. (1989). Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT1C receptor antagonists. *European Journal of Pharmacology, 164*, 445-454.

Kjelstrup, K.G., Tuvnes, F.A., Steffenach, H.A., Murison, R., Moser, E.I., & Moser, M.B. (2002). Reduced fear expression after lesions of the ventral hippocampus. *Proceedings of the National Academy of Sciences USA, 99*, 10825-10830.

Knight, A.R., Misra, A., Quirk, K., Benwell, K., Revell, D., Kennett, G., et al. (2004). Pharmacological characterisation of the agonist radioligant binding site of 5-HT(2A), 5-HT2(B) and 5-HT(2C) receptors. *Naunyn Schmiedebergs Archives of Pharmacology, 370*, 114-123.

Kshama, D., Hrishikeshavan, H.J., Shanbhogue, R., & Munonyedi, U.S. (1990). Modulation of baseline behavior in rats by putative serotonergic agents in three ethoexperimental paradigms. *Behavioral and Neural Biology, 54*, 234-253.

LeDoux, J.E., Iwata, J., Cichetti, P., & Reis, D.J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *Journal of Neurosciences, 8*, 2517-2519.

Leysen, J.E. (2004). 5-HT_2 receptors. *Current Drug Targets - CNS Neurological Disorders l , 3*, 11-26.

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

Mammounas, L.A., Mullen, C.A., O'Hearn, E., & Molivier, M.E. (1991). Dual serotonergic projections to forebrain in the rat: morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivates. *Journal of Comparative Neurology, 314*, 558-586.

Martin, J.R., Ballard, T.M., & Higgins, G.A. (2002). Influence of the 5-HT2C receptor antagonist, SB-242084, in tests of anxiety. *Pharmacology Biochemistry and Behavior, 71*, 615-625.

Martin, J.R., Bos, M., Jenck, F., Moreau, J., Mutel, V., Sleight, A.J., et al. (1998). 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. *Journal of Pharmacology and Experimental Therapeutics, 286*, 913-924.

McHugh, S.B., Deacon, R.M., Rawlins, J.N., & Bannermen, D.M. (2004). Amygdala and ventral hippocampus contribute differentially to mechanisms of fear and anxiety. *Behavioral Neurosciences, 118*, 63-78.

McMahon, L.R., Filip, M., & Cunningham, K.A. (2001). Differential regulation of the mesoaccumbens circuit by serotonin 5-hydroxytryptamine (5-HT)2A and 5-HT2C receptors. *The Journal of J Neuroscience, 21*, 7781-7787.

McQuade, R., & Sharp, T. (1997). Functional mapping of dorsal and median raphe 5-hydroxytryptamine pathways in forebrain of the rat using microdialysis. *Journal of Neurochemistry, 62*, 791-796.

Millan, M.J. (2005). Serotonin 5-HT2C as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. *Therapie, 6*, 441-460.

Millan, M.J., Brocco, M., Gobert, A., & Dekeyne, A. (2005). Anxiolytic-like properties of agomelatine, an antidepressant with melatoninergic and serotonergic properties: role of 5-HT2C receptor blockade. *Psychopharmacology, 177*, 448-458.

Nic Dhonnchadha, B.A., Bourin, M., & Hascoet, M. (2003). Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. *Behavioral Brain Research, 140*, 203-214.

Paxinos, G., & Watson, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press.

Pitkanen, A., Pikkarainen, M., Nurminen, N., & Ylinen, A. (2000). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Annals of the New York Academy of Sciences, 911*, 369-391.

Pompeiano, M., Palácios, J.M., & Mengod, G. (1994). Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT$_{2C}$ receptors. *Molecular Brain Research, 23*, 163-178.

Porter, R.H.P., Benwell, K.R., Lamb, H., Malcolm, C.S., Allen, N.H., Revell, D.F., et al. (1999). Functional characterization of agonists at recombinant human 5-HT2A, 5-HT2B and 5-HT2C receptors in CHO-K1 cells. *British Journal of Pharmacology, 128*, 13-20.

Rainnie, D.G. (1999). Serotonergic modulation in the rat basolateral amygdala. *Journal of Neurophysiology, 82*, 69-85.

Rex, A., Voigt, J.P., & Fink, H. (2005). Anxiety but not arousal increases 5-Hydroxytryptamine release in the rat ventral hippocampus in vivo. *European Journal of Neuroscience, 22*, 1185-1189.

Ripol, N., Hascoet, M., & Bourin, M. (2006). Implication of 5-HT2A subtype in DOI activity in the four-plates test-retest paradigm in mice. *Behavioral Brain Research,166*, 131-139.

Rodgers, R.J., Cole, J.C., Cobain, M.R., Daly, P., Doran, P.J., Eells, J.R., et al. (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. *Behavioural Pharmacology, 3*, 621-634.

Setem, J., Pinheiro, A.P., Motta, V.A., Morato, S., & Cruz, A.P.M. (1999). Ethopharmacological analysis of 5-HT ligands on the rat elevated plus-maze. *Pharmacology Biochemistry and Behavior, 62*, 515-521.

Sommer, W., Moller, C., Wiklund, L., Thorsell, A., Rimondini, R., Nissbrandt, H., et al. (2001). Local 5,7-dihydroxytryptamine lesions of rat amygdala: Release of punished drinking, unaffected plus-maze behavior and ethanol consumption. *Neuropsychopharmacology, 24*, 430-440.

Vertes, R.P. (1991). A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. *The Journal of Comparative Neurology, 313*, 643-668.

Vickers, S.P., Easton, N., Malcolm, C.S., Allen, N.H., Porter, R.H., Bickerdike, M.J., et al. (2001). *Pharmacology Biochemistry and Behavior, 69*, 643-652.

Voigt, J.P., Rex, A., Sohr, R., & Fink, H. (1999). Hippocampal 5-HT and NE release in the transgenic rat TGR(mREN2)27 related to behavior on the elevated plus maze. *European Neuropsychopharmacology, 9*, 279-285.

Wallis, C.J., & Lal, H. (1998). A discriminative stimulus produced by 1-(3-chlorophenyl)-piperazine (mCPP) as a putative animal model of anxiety. *Progress in Neuropsychopharmacology and Biological Psychiatry, 22*, 547-565.

Wood, M.D. (2003). Therapeutic potential of 5-HT2C receptor antagonists in the treatment of anxiety disorders. *Current Drug Targets CNS and Neurology Disorders, 2*, 383-387.

Wright, I.K., Upton, N., & Marsden, C.A. (1992). Effect of established and putative anxiolytics on extracellular 5-HT and 5-HIAA in ventral hippocampus of rats during behaviour on the elevated X-maze. *Psychopharmacology, 109*, 338-346.

Zanoveli, J.M., Nogueira, R.L., & Zangrossi, H. Jr (2003). Serotonin in the dorsal periaqueductal gray modulates inhibitory avoidance and one-way escape behaviors in the elevated T-maze. *European Journal of Pharmacology, 473*, 153-161.