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

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Abstract

This study investigated the effects of two selective serotonin$_{2C}$ (5-hydroxytryptamine, 5-HT$_{2C}$) receptor-acting compounds into ventral hippocampus (VH) of rats exposed to the elevated plus-maze (EPM). In the first experiment, rats were exposed to the EPM 10 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 additio conventional parameters of open arm exploration (i.e. percentages of open arm entries and of time spent in these arms), risk assessm related behaviors were recorded as anxiety-like measures in EPM scoring. RO-60-0175 selectively decreased open arm explorati the dose of 1.0 µg, while inducing locomotor-suppressant effects at the two highest doses. In the second experiment, VH infusion 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 assessm in the closed ones. This behavioral profile of risk assessment is suggestive of an anxiolytic-like action. These results further corrobo our previous findings showing that VH 5-HT$_{2C}$ receptor activation elicits anxiogenic-like and locomotor-suppressant effects, and sugg that the selective blockade of this receptor is accompanied by an anxiolytic-like action as detected by ethologically derived measure the EPM. **Keywords:** anxiety, 5-HT$_{2C}$ receptors, RO-60-0175, RS 102221, ventral hippocampus, elevated plus-maze, risk assessm

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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; B Huston, & Fone , 2003; Durand, Mormèd, & Chaoul 2003). In fact, newly selective 5-HT$_{2C}$ agonists (e.g. R 60-0175) have been found to increase anxiety-rela behaviors (Griebel et al., 1997; Kennett, et al., 19 Kennett, Lightowler, S., Trail, Bright, & Bromidge, 20 Martin, Ballard, & Higgins, 2002; Millan, Brocco, Gob & Dekeyne , 2005), although null and even anxiolytic-l effects have also been reported (Nic Dhonnchadha, Bou & Hascoet, 2003; Rippol, Hascoet, & Bourin, 2006).

Despite growing insights into the neural mechanis through which 5-HT systems might influence defens behavior, the circuits responsible for the above findings well as the exact role of the 5-HT$_{2C}$ receptor in specific ty of anxiety remain unclear. For example, 5-HT$_{2C}$ agon increase anxiety-related behaviors in the basolateral nucl of the amygdala (Campbell & Merchant, 2003) but decre panic-related behaviors in the dorsal periaqueductal g (Jenck, Bos, Wichmann, Stadler, Martin, & Moreau, 19 Graeff, 2002; Jacob et al., 2002; Zanoveli, Nogueira, Zangrossi, 2003). Therefore, different brain structures t receive direct 5-HT projections from the dorsal raphe nucl might have a distinct contribution to anxiety mediation.

Besides the amygdala and the periaqueductal gr

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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

the 5-HT_{2C}-receptor (nM affinity for 5-HT_{2C} and > 2
100-fold lower for other receptors; Boes et al., 1997; Po
et al., 1999). Although RO-60-0175 presents considera
selectivity for 5-HT_{2A} and 5-HT_{2B} receptors (Vickers et
2001; Knight et al., 2004), this compound seems to prod
predominantly 5-HT_{2C}-receptor mediated behavior (Ma
et al., 1998). This conclusion is supported by results
which highly selective 5-HT_{2C} antagonists, such as S
242084 (Martin, Ballard, & Higgins, 2002), but not 5-H
or 5-HT_{2B} antagonists (Dekeyne, Girardon, & Milan, 19
prevent changes in behavior induced by R0-60-01
These patterns of results support the use of RO-60-0175
several laboratories as a reliable pharmacological tool
activating 5-HT_{2C} receptors.

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

Methods

Subjects

Experimentally naive male Wistar rats weighing 1
250 g were employed as subjects. The animals were b
and raised in the vivarium at the University of Brasi
One week before the study they were brought to
holding room of the laboratory facilities and housed
groups of two in polycarbonate cages measuring 30 × 3
50 cm. All the rats had free access to food and water. Ro
temperature was controlled (25 + 1°C) and light-dark cy
was maintained on a 12-h on-off cycle (07:00-19:00h lig
on). The experimental sessions were carried out during
light phase of the cycle. The experimental protocols w
conducted in conformity with the recommendations of
Brazilian Society of Neuroscience and Behavior (SBNe
which are based on the US National Institutes of Hea
Guide for the Care and Use of Laboratory Animals (revi
in 1996).

Surgery

Animals were anaesthetized with sodium thiopental
mg/kg IP) and placed in a stereotaxic frame with the h
level between bregma and lambda. A subcutaneous inject
of 2% lidocaine with vasoconstrictor was administered
the surgical area until a small bubble was formed. Each
was bilaterally implanted with a stainless steel guide cann
(o.d. 0.7 mm) aimed at 0.5 mm above the target area. Tak

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

described elsewhere (Cruz, Frei, & Graeff, 1994). T absolute number of closed arm entries was interpreted a reliable index of locomotor activity (File, 1992; Cruz al., 1994).

Experiment 2. In this experiment, four groups of r were exposed to the same EPM procedure described Experiment 1, 10 min after VH infusion either with vehi or the selective 5-HT$_{2C}$-receptor antagonist RS 10222 the doses of 0.75, 1.25 or 2.5 µg. These doses were cho on the basis of previous studies in which RS 102 was administered intracerebrally (McMahon, Filip, Cunningham, 2001; Filip & Cunningham, 2002, 20 Body et al., 2006).

Histology

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

Statistical analysis

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

Results

Histology

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

Behavioral testing

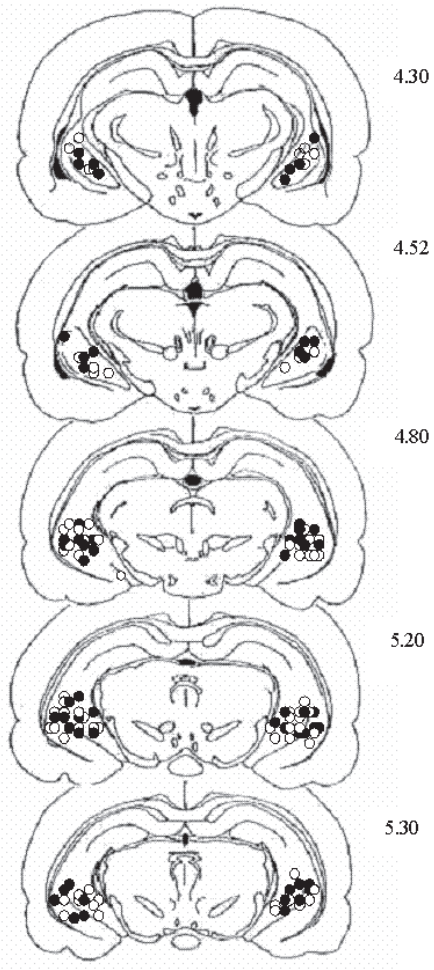

Figure 1. Composite of infusion sites aimed at the VH from Experiment 1 (white circles) and Experiment 2 (black circles). With the reference to the Paxinos and Watson (1986) atlas, the numbers on the right side of each plate indicate the distance in mm from bregma.

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

in these arms. In these two cases, the ANCOVA revealed significant effects of treatment when the closed arm entr were statistically controlled for ($p > .05$). Therefore, decrease in open arm exploration observed at the do of 3.0 and 10.0 µg was probably due to a locomo impairment.

Figure 2 (lower panel) illustrates the effects RO-60-0175 microinjections on risk assessme The ANOVA indicated a main effect of treatme

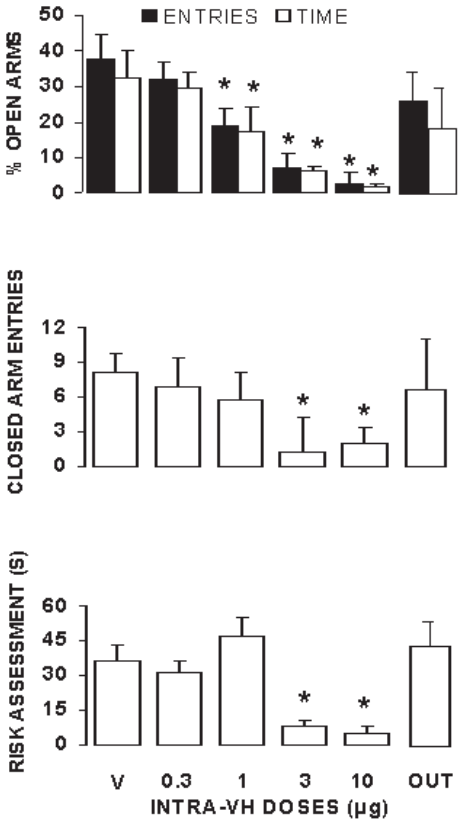

Figure 2. Mean (+SEM) percentage of open arm entries time (upper panel), closed arm entries (middle panel) and assessment (lower panel) among groups microinjected either v vehicle (n = 7) or RO-60-0175 at the doses of 0.3 µg (n = 8), 1 (n = 7), 3 µg (n = 9) and 10 µg (n = 7) into the VH. OUT (n = illustrates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

$F(5, 43) = 7.23$, $p < .05$, and post hoc comparis revealed a significant reduction of risk assessment at doses of 3.0 and 10.0 µg ($p < .05$ and .01, respectively). other significant differences were found, despite a trend increased risk-assessment at the dose of 1.0 µg. Again, ANCOVA failed to detect significant differences on r assessment parameters when the closed arm entries w used as covariate factor ($p > .05$).

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 ($p < .05$).

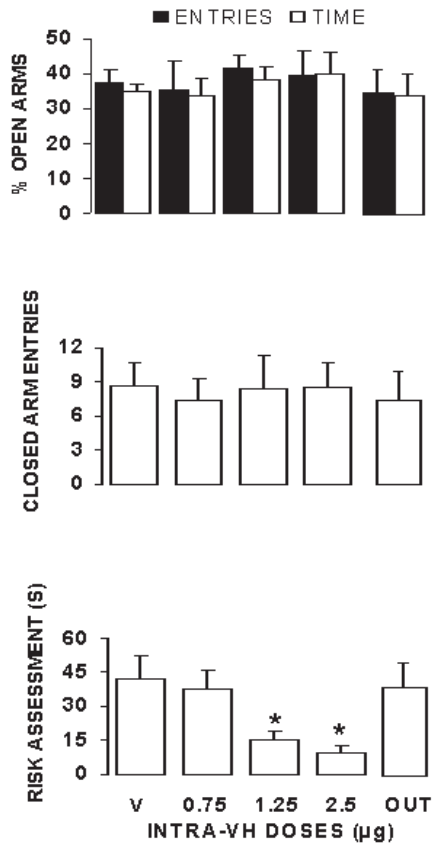

Figure 3. Mean (+SEM) percentage of open arm entries and time (upper panel), closed arm entries (middle panel) and risk assessment (lower panel) among groups microinjected either with vehicle (n = 7) or RS-102221 at the doses of 0.75 (n = 8), 1.25 (n = 7) and 2.5 µg (n = 10) into the VH. OUT (n = 7) 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

Pinheiro, Motta, Morato, & Cruz, 1999; Durand, Morm & Chaouloff, 2003), whereas its blockage by select 5-HT$_{2C}$ antagonists has been appraised as a potential tar for anxiolytic compounds (Kennett et al., 1997; Bag Graf, Anheuer, Modos, & Kantor, 2001; Andres et al., 20 Jones, Duxon, & King, 2002; Martin, Ballard, & Higgi 2002; Jones & Blackburn, 2002; Wood, 2003; Gord 2004; Millan, 2005). However, intriguing results rang from anxiolytic-like effects of 5-HT$_{2C}$ receptor agon (Nic Dhonnchadha et al., 2003) to little or null effects 5-HT$_{2C}$ receptor antagonists (Griebel, Perrault, & Sang 1997, Griebel, Rodgers, Ghislaine, & Sanger, 1997; Dhonnchadha et al., 2003) have also been reported in so animal models of anxiety. Moreover, many of these effe vary considerably in different postsynaptic 5-HT sites in brain (Jenck, Bos, Wichmann, Stadler, Martin, & More 1998; Graeff, Guimaraes, De Andraede, & Deakin, 19 Graeff, 2002; Zanoveli et al., 2003).

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

Because the VH is a postsynaptic 5-HT site nota implicated in anxiety (Gray & McNaughton, 20 Degroot & Treit, 2004; Rex et al., 2005), it is possi that the present RO-60-0175 effects in the VH mi involve 5-HT projections from the dorsal raphe nucl (Azmitia & Segal, 1978; Vertes, 1991). In this respect has been found that potentially dangerous situations su as a context previously associated to an aversive stimu (Hajos-Korcsok, 2003) or acute EPM exposure (Wrigh al., 1992; Voigt et al., 1999; Rex et al., 2005) marke enhance postsynaptic 5-HT levels in the VH. Converse selective VH lesions attenuate anxiety-related behavi in these animal models of anxiety (Kjelstrup et al., 20 Bannerman et al., 2003). Taking into account the prese of the 5-HT$_{2C}$ receptor subtype at a very high density in VH (Pompeiano et al., 1994; Backstrom et al., 1995; Fe et al., 1996, Clemett et al., 2000; Garcia-Alcover et 2006), it seems reasonable to assume that 5-HT-indu anxiety in the VH might be at least in part mediated 5-HT$_{2C}$-receptor activation.

(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

induced locomotor interference was observed exclusiv
at the two highest doses. This decrease in general activ
practically abolished the occurrence of risk assessme
related behaviors. However, this effect cannot
interpreted as an anxiolytic-like action. As we previou
reported (Cruz et al., 1994), a lack of risk assessment up
high anxiety levels that are accompanied by a signific
decrease of closed arm entries most probably refle
decreased general exploration or even complete immobi
inside the closed arms. Therefore, the present results a
suggest that the VH 5-H_{T2C} receptors might play a role
mediating locomotor activity.

As far as we know, this is the first report in
literature that tested the behavioral effects of RO-60-01
microinjected into the VH. The locomotor activity eff
observed in the present study was also reported by Fletc
and colleagues (2004), who found that microinjections of
same dose range of RO-60-0175 into the ventral tegmen
area also impaired locomotor activity. Therefore, it appe
that 5-H_{T2C} in different brain areas might be involved
the mediation of locomotor activity. At least in part, t
view is corroborated by our results, which found the gro
microinjected with RO-60-0175 outside the VH to sh
a trend toward reducing closed arm entries, although t
effect was not statistically significant.

In Experiment 2, VH infusion of the select
5-H_{T2C}agonist RS 102221 did not affect conventio
parameters of EPM exploration. At the two highest dos
however, RS 102221 significantly reduced risk assessme
Considering that a reduction in risk assessment in
absence of locomotor effects is consistent with a select
anxiolytic-like action in the EPM (Cruz et al., 19
Griebel et al., 1997a, 1997b), our results suggest that
VH 5-H_{T2C} receptor blockade might be associated wit
reduction of anxiety-like states.

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

The possibility that the conventional anxiety measu
might be less sensitive in detecting anxiolytic-like act
of 5-HT2 antagonists upon low basal levels of anxiety
the EPM cannot be discounted (Rodgers & Dalvi, 199

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.

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