5-Hydroxytryptamine2C Receptor Contribution to \( m \)-Chlorophenylpiperazine and \( N \)-Methyl-\( \beta \)-carboline-3-carboxamide-Induced Anxiety-Like Behavior and Limbic Brain Activation


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ABSTRACT

Activation of 5-hydroxytryptamine2C (5-HT\(_{2C}\)) receptors by the 5-HT\(_{2}\) receptor agonist \( m \)-chlorophenylpiperazine (\( m \)-CPP) elicits anxiety in humans and anxiety-like behavior in animals. We compared the effects of \( m \)-CPP with the anxiogenic GABA\(_{A}\) receptor inverse agonist \( N \)-methyl-\( \beta \)-carboline-3-carboxamide (FG-7142) on both anxiety-like behavior and regional brain activation using functional magnetic resonance imaging (fMRI) in the rat. We also determined whether the selective 5-HT\(_{2C}\) receptor antagonist SB 242084 [6-chloro-2,3-dihydro-5-methyl-N-[6-[[2-methyl-3-pyridinyl]oxy]-3-pyridinyl]-1H-indole-1-carboxamide dihydrochloride] would blunt \( m \)-CPP or FG-7142-induced neuronal activation. Both \( m \)-CPP (3 mg/kg i.p.) and FG-7142 (10 mg/kg i.p.) elicited anxiety-like behavior when measured in the social interaction test, and pretreatment with SB 242084 (1 mg/kg i.p.) completely blocked the behavioral effects of both anxiogenic drugs. Regional brain activation in vivo in response to anxiogenic drug challenge was determined by blood oxygen level-dependent (BOLD) fMRI using a powerful 9.4T magnet. Region of interest analyses revealed that \( m \)-CPP and FG-7142 significantly increased BOLD signals in brain regions that have been linked to anxiety, including the amygdala, dorsal hippocampus, and medial hypothalamus. These BOLD signal increases were blocked by pretreatment with SB 242084. In contrast, injection of \( m \)-CPP and FG-7142 resulted in BOLD signal decreases in the medial prefrontal cortex that were not blocked by SB 242084. In conclusion, the brain activation signals produced by anxiogenic doses of both \( m \)-CPP and FG-7142 are mediated at least partially by the 5-HT\(_{2C}\) receptor, indicating that this receptor is a key component in anxiogenic neural circuitry.

The 5-hydroxytryptamine 2C (5-HT\(_{2C}\)) receptor has been implicated in mood and anxiety disorders and is a target for development of novel anxiolytic drugs (Wood, 2003). Selective and nonselective 5-HT\(_{2C}\) receptor antagonists reduce anxiety-like behavior in several animal models of anxiety (Stutzmann et al., 1991; Kennett et al., 1995; Griebel et al., 1997). For example, SB 242084 [6-chloro-2,3-dihydro-5-methyl-N-[6-[[2-methyl-3-pyridinyl]oxy]-3-pyridinyl]-1H-indole-1-carboxamide dihydrochloride], a potent and selective 5-HT\(_{2C}\) receptor antagonist, is anxiolytic in the social interaction test and the Geller-Seifter conflict test of anxiety (Kennett et al., 1997). Although 5-HT\(_{2C}\) receptor antagonism is anxiety-like, agonist-induced activation of 5-HT\(_{2C}\) receptors is anxiogenic. Activation of 5-HT\(_{2C}\) receptors by 5-HT\(_{2}\) receptor agonists, such as \( m \)-chlorophenylpiperazine (\( m \)-CPP) and 6-chloro-2-[1-piperazinyl] pyrazine (MK-212), elicits anxiety in humans and anxiety-like behavior in animals (Charney et al., 1987; Lowy and Meltzer, 1988; Kahn and Wetzler, 1991; Gatch, 2003; de Mello Cruz et al., 2005). For example, \( m \)-CPP administration to mice resulted in less time spent on the open arms of an elevated plus maze (Benjamin et al., 1990), and in rats, anxiety-like behavior has been reported in the...
light-dark box, the open-field test, and the social interaction test (Bilkei-Gorzo et al., 1998; Bagdy et al., 2001; Martin et al., 2002; Campbell and Merchant, 2003). The 5-HT_{2C} receptor antagonist SB 242084 blocks the anxiogenic effects of mCPP in rats (Bagdy et al., 2001; Campbell and Merchant, 2003). In addition, indirect activation of 5-HT_{2C} receptors contributes to the anxiogenic effects following acute treatment with selective serotonin reuptake inhibitors (Bagdy et al., 2001). Taken together, these studies suggest that 5-HT_{2C} receptor activation is an important component of anxiogenesis.

Recent advances in functional magnetic resonance imaging (fMRI) technology allow the study of pharmacologically-induced regional activation in the living rat brain (Chen et al., 2005; Ireland et al., 2005; Steward et al., 2005). Blood oxygen level-dependent (BOLD) fMRI is a method that has distinct advantages over other in vivo imaging techniques. A prominent advantage of fMRI is the ability to collect temporal data of a drug’s effect in different brain regions in a single animal. BOLD-induced brain activation has been used to examine the effects of pharmacological agents, such as amphetamine (Dixon et al., 2005), cocaine (Febo et al., 2005), and citalopram (Steward et al., 2005).

mCPP has been advanced as a useful pharmacological probe for fMRI studies of regional activation induced by 5-HT_{2C} receptor activation (Houston et al., 2001; Anderson et al., 2002). However, mCPP has significant affinities for several serotonin receptors in addition to the 5-HT_{2C} receptor, clouding the interpretation of in vivo studies (Hamik and Peroutka, 1989; Campbell and Merchant, 2003). In the current report, we used SB 242084, a selective 5-HT_{2C} receptor antagonist (Kennett et al., 1997), to evaluate the contribution of the 5-HT_{2C} receptor to mCPP-induced anxiety-like behavior and regional BOLD signal changes within the amygdala, hippocampus, hypothalamus, and the medial prefrontal cortex. To determine whether a 5-HT_{2C} receptor-dependent process contributes to drug-induced anxiety-like behavior, in general, animals were injected with the GABA_A receptor inverse agonist N-methyl-β-carboline-3-carboxamide (FG-7142), a potent anxiogenic agent (Dorow et al., 1983).

The pattern of regional activation produced by FG-7142 was compared with that observed in animals that were pretreated with the 5-HT_{2C} receptor antagonist SB 242084.

Materials and Methods

Drugs. mCPP (Tocris Bioscience, Ellisville, MO) was administered at a dose of 3 mg/kg i.p. FG-7142 complexed with hydroxypropyl-β-cyclodextrin (Sigma-Aldrich, St Louis, MO) was injected at a dose of 10 mg/kg i.p. SB 242084, a generous gift from SmithKline Beecham (Harlow, UK), was administered at a dose of 1 mg/kg i.p. mCPP and FG-7142 were dissolved in saline (0.9%). SB 242084 was sonicated to a suspension using 10% Tween 80 in saline.

Social Interaction Paradigm. Anxiety-like behavior of rats was measured using a modified social interaction paradigm (Varlinskaya and Spear, 2002). The social interaction paradigm is a test that measures the total social interactions of a drug-treated rat paired with a control rat that is unfamiliar to the drug-treated rat. Six groups, each consisting of six adult male Sprague-Dawley rats (250–300 g), were used: vehicle, mCPP, FG-7142, SB 242084, SB 242084/mCPP (pretreatment 10 min before mCPP administration), or SB 242084/FG-7142 (pretreatment 10 min before FG-7142 administration). The day before testing, each of the manipulated (drug-treated) rats was familiarized to the social interaction chamber for approximately 30 min under low-light conditions. On the day of testing, rats were injected with drug or vehicle and, 30 min later, placed into a 45 x 30 x 20-cm clear acrylic chamber bisected by a clear central partition containing a 9 x 7-cm opening. Each drug-treated familiarized rat was paired with a nonfamiliarized control rat. Behavioral testing was conducted during the dark cycle between 7:00 PM and 11:00 PM under low-light conditions. Behavior was recorded by video/camcorder for future scoring by observers blind to the treatment conditions. The social interaction paradigm was performed with the FG-7142 test (Bilkei-Gorzo et al., 1998; Bagdy et al., 2001; Martin et al., 2002). The 5-HT_{2C} receptor antagonist (Kennett et al., 1997), to evaluate the contribution of the 5-HT_{2C} receptor to mCPP-induced anxiety-like behavior and regional BOLD signal changes within the amygdala, hippocampus, hypothalamus, and the medial prefrontal cortex. To determine whether a 5-HT_{2C} receptor-dependent process contributes to drug-induced anxiety-like behavior, in general, animals were injected with the GABA_A receptor inverse agonist N-methyl-β-carboline-3-carboxamide (FG-7142), a potent anxiogenic agent (Dorow et al., 1983).

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The pattern of regional activation produced by FG-7142 was compared with that observed in animals that were pretreated with the 5-HT_{2C} receptor antagonist SB 242084.
ments, SB 242084 was injected after the baseline period, and m-CPP or FG-7142 was injected 10 min later. Functional images were acquired using a fast gradient-echo sequence [TR (repetition time) = 200 ms, TE (echo time) = 12 ms, NEX (number of excitations) = 2, 64 x 64 x 8 matrix, 30 x 30 x 2 mm FOV (field of view)]. Functional images following drug administration were superimposed on anatomical images taken before drug injection to identify regions of interest (ROI), i.e., areas that show significant changes in the BOLD signal.

Data Analysis. All fMRI data analyses were performed using MATLAB software (version 7.0.4; MathWorks, Inc., Natick, MA). Activation maps were generated for each data set using a sliding t test, comparing the time-averaged data over a period of 300 s (5 min) from the postinjection period to a comparable preinjection period. For each animal, the effects of baseline drift and high-frequency noise in the fMRI signal were removed by polynomial curve fitting and low-pass data filtering, respectively. To assess changes in specific brain regions, ROI analyses were performed using a rat brain atlas as a guide for structural identification (Paxinos and Watson, 1982). ROIs were defined to include the dorsal hippocampus, the amygdaloid complex, the hypothalamus (at the level of mediodorsal and paraventricular nuclei), and the medial prefrontal cortex (mPFC). For group analyses, ROI BOLD signal intensities ($\Delta S/S_0$) from individual animals were averaged across animals. Area under the curve (AUC) was calculated for the postinjection period of each animal in each drug treatment group. The values from both the left and right side of the brain were averaged, and data were analyzed using one-way ANOVA with Tukey’s multiple comparison post hoc test.

Results

m-CPP and FG-7142-Induced Behavioral Changes Are Blocked by SB 242084. Acute administration of both m-CPP and FG-7142 significantly reduced the total amount of time spent in social interaction compared with vehicle-injected animals (Fig. 1A; ANOVA: $F_{(2,15)} = 130.9, p < 0.0001$). The doses used were chosen based upon previous experiments performed using m-CPP and FG-7142 in the social interaction test as a behavioral measure for anxiety-like behavior in rats (Short and Maier, 1993; Rex et al., 2004). Pretreatment with SB 242084 significantly blocked the anxiogenic effects of m-CPP in the social interaction test (SB 242084/m-CPP; Fig. 1B; ANOVA: $F_{(3,20)} = 27.23, p < 0.0001$). The dose of SB 242084 was chosen based upon previous rodent behavioral experiments (Martin et al., 2002; Campbell and Merchant, 2003; Knapp et al., 2004). Post hoc analysis revealed that the m-CPP treatment group was significantly different from vehicle, SB 242084/m-CPP, and SB 242084 alone. In addition, pretreatment with SB 242084 10 min before FG-7142 injection completely blocked the anxiogenic effects of FG-7142 as indicated in Fig. 1C (SB 242084/FG-7142; ANOVA: $F_{(3,20)} = 19.75, p < 0.0001$). Post hoc analysis revealed that the FG-7142 treatment group was significantly different from vehicle, SB 242084/FG-7142, and SB 242084 alone.

m-CPP-Induced BOLD Signal Changes in Limbic Brain Regions. m-CPP administration increased BOLD signals in limbic brain regions associated with anxiety: the amygdala, hippocampus, and hypothalamus (Table 1). Temporal changes in the BOLD signal following m-CPP injection are illustrated in Fig. 2A, which shows activation maps in a series of 5-min increments for the entire 40-min postinjection period. The maximal intensity of the BOLD signal increases was observed approximately 15 min postinjection.

Injection of m-CPP resulted in activation of the amygdala, with increases in the BOLD signal within 1 min after m-CPP challenge that continued to rise over the next 15 min until a plateau was reached (Fig. 3A). The ability of m-CPP to activate the amygdala was completely blocked by pretreatment with SB 242084 (Fig. 3B; ANOVA: $F_{(3,20)} = 15.40, p < 0.0001$). BOLD signal increases induced by m-CPP are significantly different from those observed from vehicle, SB 242084/m-CPP, and SB 242084 alone.

m-CPP also elicited BOLD signal increases in the hippocampus and hypothalamus that were blocked by pretreatment with SB 242084. BOLD signal increases within the hippocampus (Fig. 4A) and hypothalamus (Fig. 5A) were observed 1 min after m-CPP injection and plateaued within 15 min. Statistical analyses demonstrated that m-CPP-induced BOLD signal increases in dorsal hippocampus were blocked by pretreatment with SB 242084 (Fig. 4B; ANOVA: $F_{(3,20)} = 7.680, p = 0.0013$), as were BOLD signal increases in the medial nuclei of the hypothalamus (Fig. 5B; ANOVA: $F_{(3,20)} = 17.22, p < 0.0001$) and the paraventricular nuclei of
Signal changes in the ROIs measured.

For both p/H11021 changes were significantly different from vehicle (indicated by AUC analysis of ROI data from part A.

Fig. 3.

m-CPP-induced BOLD signal increases in the amygdala are blocked by SB 242084. A, averaged BOLD signal changes (ΔS/S₀, n = 6 per treatment group) for vehicle (●), m-CPP (3 mg/kg, ■), SB 242084 (1 mg/kg) pretreatment before m-CPP (3 mg/kg, SB/m, ◆), and SB 242084 alone (1 mg/kg, SB, ▲). The time of drug injection is 0 on the x-axis. B, AUC analysis of ROI data from part A. m-CPP-induced BOLD signal changes were significantly different from vehicle (indicated by *, p < 0.001), SB/m (indicated by †, p < 0.001), and SB alone (indicated by ‡, p < 0.001).

In contrast to the other anxiety-related sites, m-CPP caused BOLD signal decreases in the mPFC, which were not blocked by pretreatment with SB 242084 (data not shown; ANOVA: F(3,20) = 5.923, p = 0.0046). Although vehicle, m-CPP, and SB 242084 alone were significantly different from each other, there was no significant difference between the m-CPP and SB 242084/m-CPP treatment conditions.

FG-7142-Induced BOLD Signal Changes in Limbic Brain Regions. FG-7142 administration resulted in BOLD signal increases in limbic brain regions associated with anxiety: the amygdala, hippocampus, and hypothalamus (Table 1). Temporal analyses of BOLD signal changes following FG-7142 injection are illustrated as activation maps in a series of 5-min increments for the 40-min postinjection period (Fig. 6). The intensity of the BOLD signal increases remained near maximum throughout the 40-min scan, indicating a long duration of action (Fig. 6). Like m-CPP, injection of FG-7142 generated BOLD signal increases in the amygdala, hippocampus, and medial hypothalamus. The BOLD signal increases observed 15 min after FG-7142 injection appeared less intense than those for the same time point following m-CPP injection, with the exception of the hippocampal area, where FG-7142-induced BOLD signal increases were more robust.

![Image](https://via.placeholder.com/150)

Fig. 3. m-CPP-induced BOLD signal increases in the amygdala are blocked by SB 242084. A, averaged BOLD signal changes (ΔS/S₀, n = 6 per treatment group) for vehicle (●), m-CPP (3 mg/kg, ■), SB 242084 (1 mg/kg) pretreatment before m-CPP (3 mg/kg, SB/m, ◆), and SB 242084 alone (1 mg/kg, SB, ▲). The time of drug injection is 0 on the x-axis. B, AUC analysis of ROI data from part A. m-CPP-induced BOLD signal changes were significantly different from vehicle (indicated by *, p < 0.001), SB/m (indicated by †, p < 0.001), and SB alone (indicated by ‡, p < 0.001).
ROI analyses of amygdala following injection of vehicle, FG-7142, SB 242084/FG-7142, or SB 242084 alone are shown in Fig. 7. BOLD signal increases were observed within 1 min following drug injection (Fig. 7A). Unlike m-CPP, BOLD signal increases following FG-7142 injection did not plateau during the 40-min postinjection period, and this was observed in all of the ROIs examined. Longer scans were performed with FG-7142 in an additional three rats, and the BOLD signal increases did not plateau within a 60-min postinjection period (data not shown), indicating different pharmacokinetic profiles for m-CPP and FG-7142. Statistical analysis of amygdala data demonstrates that the FG-7142 BOLD signal increases were blocked by pretreatment with SB 242084 (Fig. 7B; ANOVA: F(3,20) = 19.63, p < 0.0001). FG-7142 is significantly different from vehicle, SB 242084/FG-7142, and SB 242084 alone. SB 242084/FG-7142 appeared to decrease the BOLD signal; however, this was not significantly different from vehicle or SB 242084 alone. FG-7142 generated strong BOLD signal increases within the hippocampus (Fig. 8A) and medial hypothalamus (Fig. 9A) that were evident within 1 min. Statistical analysis demonstrated that FG-7142-induced BOLD signal increases in hippocampus were blocked by pretreatment with SB 242084 (Fig. 8B; ANOVA: F(3,20) = 9.678, p = 0.0004), as were BOLD signal increases in the medial nuclei of the hypothalamus (Fig. 9B; ANOVA: F(3,20) = 11.87, p = 0.0001), but not in the paraventricular nuclei of hypothalamus (data not shown). FG-7142-induced BOLD signal increases in both hippocampus and hypothalamus differed from vehicle, SB 242084/FG-7142, and SB 242084 alone. Although SB 242084/FG-7142 and SB 242084 appeared to decrease the BOLD signal in hypothalamus, these were not significantly different from vehicle or each other.

As with m-CPP, BOLD signal decreases were observed in the mPFC following injection of FG-7142 (data not shown; ANOVA: F(3,20) = 4.563, p = 0.0011). Although vehicle, m-CPP, and SB 242084 alone were significantly different from

Fig. 5. m-CPP-induced BOLD signal increases in the hypothalamus are blocked by SB 242084. A, averaged BOLD signal changes (ΔS/S₀, n = 6 per treatment group) for vehicle (●), m-CPP (3 mg/kg, ▼), SB 242084 (1 mg/kg) pretreatment before m-CPP (3 mg/kg, SB/m, □), and SB 242084 alone (1 mg/kg, SB, △) within the hypothalamus. The time of drug injection is 0 on the x-axis. B, AUC analysis of ROI data from part A. m-CPP-induced BOLD signal changes were significantly different from vehicle (indicated by * p < 0.001), SB/m (indicated by † p < 0.001), and SB alone (indicated by ‡, p < 0.001).

Fig. 6. Temporal activation maps following FG-7142 injection. Representative activation maps showing BOLD signal increases from 5 to 40 min following FG-7142 injection. A p-value gradient on the right illustrates color-coordinated levels of BOLD signal significance.

Fig. 7. FG-7142-induced BOLD signal increases in the amygdala are blocked by SB 242084. A, averaged BOLD signal changes (ΔS/S₀, n = 6 per treatment group) for vehicle (●), FG-7142 (10 mg/kg, FG, ▼), SB 242084 (1 mg/kg) pretreatment before FG-7142 (10 mg/kg, SB/FG, □), and SB 242084 alone (1 mg/kg, SB, △). The time of drug injection is 0 on the x-axis. B, AUC analysis of ROI data from part A. FG-7142-induced BOLD signal changes were significantly different from vehicle (indicated by * p < 0.001), SB/FG (indicated by †, p < 0.001), and SB alone (indicated by ‡, p < 0.001).

Fig. 8. FG-7142-induced BOLD signal increases in the hippocampus are blocked by SB 242084. A, averaged percent BOLD signal changes (ΔS/S₀, n = 6 per treatment group) for vehicle (●), FG-7142 (10 mg/kg, FG, ▼), SB 242084 (1 mg/kg) pretreatment before FG-7142 (10 mg/kg, SB/FG, □), and SB 242084 alone (1 mg/kg, SB, △). The time of drug injection is 0 on the x-axis. B, AUC analysis of ROI data from part A. FG-7142-induced BOLD signal changes were significantly different from vehicle (indicated by * p < 0.001), SB/FG (indicated by †, p < 0.001), and SB alone (indicated by ‡, p < 0.001).
and SB alone (indicated by ‡, from vehicle (indicated by •), FG-7142 (10 mg/kg, FG, □), SB 242084 (1 mg/kg) pretreatment before FG-7142 (10 mg/kg, SB/FG, ○), and SB 242084 alone (1 mg/kg, SB, △) within the hypothalamus. The time of drug injection is 0 on the x-axis. A, AUC analysis of ROI data from part A. FG-7142-induced BOLD signal changes were significantly different from vehicle (indicated by ‡, p < 0.01), SB/FG (indicated by †, p < 0.001), and SB alone (indicated by •, p < 0.001). There was no significant difference between the FG-7142 and SB 242084/FG-7142 treatment conditions in the mPFC.

Discussion

The present results point to the 5-HT<sub>2C</sub> receptor as integral to the anxiety-like behavior and limbic brain activation produced by two anxiogenic drugs with different mechanisms of action: m-CPP and FG-7142. In fMRI studies, we focused on brain regions that are key elements in a distributed brain network subserving anxiety: amygdala, hippocampus, medial hypothalamus, paraventricular nucleus, and the medial prefrontal cortex. This is the first study to characterize the 5-HT<sub>2C</sub> receptor contribution to m-CPP-induced BOLD signal changes. Because the 5-HT<sub>2C</sub> receptor has been suggested to modulate GABA neurotransmission (Huidobro-Toro et al., 1996; Feng et al., 2001; Serrats et al., 2005), we also determined whether pharmacological blockade of the 5-HT<sub>2C</sub> receptor would modify BOLD signal changes induced by another anxiogenic drug, FG-7142, which targets the GABA<sub>A</sub> receptor.

In agreement with previous studies (Short and Maier, 1993; Rex et al., 2004), we found that m-CPP and FG-7142 were both anxiogenic in the social interaction test. In addition, we found that pretreatment with a selective 5-HT<sub>2C</sub> receptor antagonist blocked the behavioral effects of m-CPP and FG-7142. Although previous studies have reported that SB 242084 blocks m-CPP-induced behaviors (Kennett et al., 1997; Bagdy et al., 2001; Campbell and Merchant, 2003), our observation that SB 242084 blocks FG-7142-elicted anxiety-like behavior is novel and underscores the possible central role for the 5-HT<sub>2C</sub> receptor in anxiety. Although our behavioral study did not reveal an anxiolytic effect of SB 242084, the experimental conditions were optimized for demonstrating a reduction in social interaction (anxiogenic-like behavior), rather than an increase (anxiolytic-like behavior).

Both m-CPP and FG-7142 activated the amygdala, hippocampus, and the medial and paraventricular nuclei of the hypothalamus, as measured by fMRI. The m-CPP-induced BOLD signal increases are consistent with recent report by Stark et al. (2006). m-CPP-induced BOLD signal increases in each area plateaued ~15 min after injection. An anxiolytic dose of SB 242084 blocked BOLD signal increases generated by m-CPP in each of the ROIs. Taken together, these data suggest that m-CPP-induced activation of the amygdala, the hippocampus, and the hypothalamus is mediated by the 5-HT<sub>2C</sub> receptor. FG-7142 produced a BOLD signal pattern that was similar, but not identical, to that seen after m-CPP challenge, with increases observed in the amygdala, hippocampus, and hypothalamus. However, unlike m-CPP, the BOLD signal produced by FG-7142 did not plateau within the 40-min period following drug administration, probably reflecting a difference in pharmacokinetics between the two anxiogenic agents. This is the first fMRI study to characterize FG-7142-induced BOLD signal changes, and in general, the pattern of FG-7142-induced BOLD signal increases is consistent with a previous c-Fos study (Singewald et al., 2003). A key, novel finding is that SB 242084 pretreatment blocked FG-7142-induced BOLD signal increases in all but two of ROIs, suggesting that the 5-HT<sub>2C</sub> receptor is integral to FG-7142-elicted activation of limbic brain regions that subserve anxiety. Both m-CPP and FG-7142 elicited BOLD signal decreases in the mPFC, suggesting that the BOLD signal activation is region-specific and not an overall nonspecific effect. Although blockade of the BOLD activation with a 5-HT<sub>2C</sub> receptor-selective antagonist suggests that the 5-HT<sub>2C</sub> receptor is a key component of m-CPP and FG-7142-induced brain activation, it would be important to use multiple 5-HT receptor antagonists to investigate the possible role of additional 5-HT receptors.

Our studies were performed in rats lightly anesthetized with isoflurane. Because movement-associated artifacts would preclude any analysis of BOLD signal data (Hajnal et al., 1994), it was not possible to perform these experiments in the unanesthetized rat. It is likely that isoflurane dampened brain metabolic activity. Both inhalation and injectable anesthetics have been shown to alter cerebral glucose metabolism (Dudley et al., 1982; Ori et al., 1986). Use of anesthesia may also alter synaptic transmission, resulting in an overall increase in inhibitory transmission throughout the brain (Richards, 1995). Despite the potential confounds associated with isoflurane, the increases that we observed in BOLD signals are consistent with previous c-Fos studies reporting anxiety-induced activation of the amygdala and other regions (Singewald et al., 2003). Because heart rate and blood gases remained stable throughout the fMRI-scanning period, regardless of the experimental treatment, it is unlikely that changes in these parameters resulted in any BOLD signal artifacts.

In summary, the findings of this study indicate that the brain activation that accompanies a behavioral state of anxiety critically involves the 5-HT<sub>2C</sub> receptor. Consistent with this conclusion, SB 243213, an inverse agonist at the 5-HT<sub>2C</sub> receptor, is anxiolytic in rodent behavioral tests and blocks anxiety-like behavior in ethanol-withdrawn rats (Knapp et al., 2004; Overstreet et al., 2003). Interestingly, SB 243213 shows no evidence of tolerance or dependence (Kennett et al., 1997; Wood et al., 2001). Because tolerance and withdrawal symptoms are inherent problems with long-term benzodiaz-
epine use ( Bateson, 2002 ), 5-HT 2C receptor antagonists may prove to be superior anxiolytic agents. The current evidence that a 5-HT 2C receptor antagonist blocks the anxiety-like behavior elicited by anxiogenic agents with different mechanisms of action provides additional evidence for 5-HT 2C receptor antagonists as potential targets for treatment of anxiety disorders.

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References


