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ORIGINAL INVESTIGATION

The effects of the 5-HT_{2C} receptor antagonist SB242084 on locomotor activity induced by selective, or mixed, indirect serotonergic and dopaminergic agonists

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Abstract
Rationale The 5-HT_{2C} receptor modulates mesolimbic dopamine (DA) function and the expression of DA-dependent behaviors, including stimulant-induced hyperactivity. The 5-HT_{2C} receptor may also be involved in drug-induced locomotion that is 5-HT-dependent.
Objectives This study investigated the effects of the 5-HT_{2C} receptor antagonist 6-chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB242084) on hyperlocomotion induced by psychomotor stimulants with selective, or mixed, actions on serotonergic and/or dopaminergic systems.
Materials and methods Male Sprague–Dawley rats were treated in the presence or absence of SB242084 with releasers/reuptake inhibitors of DA (amphetamine and methylphenidate), 5-HT (fenfluramine and citalopram), or both 5-HT and DA (MDMA and cocaine). In addition, the effects of SB242084 combined with nicotine, morphine, or the 5-$HT_{1A/1B}$ receptor agonist RU24969 were examined. Locomotor activity was recorded for 2 h.
Results SB242084 potentiated hyperactivity induced by MDMA (2.5–5 mg/kg), amphetamine (0.5 mg/kg), fenfluramine (5 mg/kg), cocaine (10 mg/kg), and methylphenidate (5 mg/kg). SB242084 modestly potentiated nicotine-induced (0.2–0.4 mg/kg) and morphine-induced (2.5 mg/kg) hyperactivity. SB242084 failed to influence hyperactivity induced by RU24969 (0.5–1 mg/kg) or citalopram (10–20mg/kg).
Conclusion SB242084 potentiated the locomotor stimulant effects of both indirect DA and 5-HT agonists. This potentiation may reflect two distinct mechanisms. The first involves direct enhancement of DA activity as shown by potentiation of the effects of amphetamine and methylphenidate. The second mechanism reflects an unmasking of stimulatory 5-HT receptors activated by 5-HT releasers (possibly 5-$HT_{1B/2A}$) through blockade of inhibitory 5-HT_{2C} receptors. The failure of SB242084 to potentiate the effect of citalopram might reflect differences between changes in synaptic levels of 5-HT produced by release compared to reuptake inhibition.

Keywords 5-HT_{2C} receptor · Amphetamine · Cocaine · Dopamine · Locomotor activity · Methylphenidate · MDMA · SB242084 · Serotonin

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Introduction

Over the last several years evidence has emerged indicating that the 5-HT_{2C} receptor subtype exerts a modulatory influence over the functioning of the mesocorticolimbic dopamine (DA) system originating in the ventral tegmental

area. The moderately selective 5-HT_{2C} receptor agonist Ro 60-0175 [(*S*)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine] (Martin et al. 1998) reduces the firing rate of mesolimbic DA neurons originating in the ventral tegmental area, leading to a reduction in DA release in terminal regions of the nucleus accumbens and frontal cortex (Di Matteo et al. 2000; Gobert et al. 2000). These effects are reversed by the selective 5-HT_{2C} receptor antagonist 6-chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB242084; Bromidge et al. 1997) (Di Matteo et al. 2000; Gobert et al. 2000). In addition, SB242084 alone increases the burst-firing of dopaminergic neurons in the ventral tegmental area leading to increased release of DA in the nucleus accumbens (Di Matteo et al. 1999). Thus, it appears that 5-HT_{2C} receptors may exert a tonic inhibitory influence over the activity of ascending DA neurons (Di Matteo et al. 2002).

The results of behavioral studies are consistent with this modulatory role of 5-HT_{2C} receptors on DA function. Much of this work has examined the expression of behaviors induced by cocaine, which are thought to be dopaminergically mediated, after treatment with 5-HT_{2C} receptor ligands. Recent studies have now shown that 5-HT_{2C} receptor activation reduces cocaine-stimulated locomotor activity, cocaine self-administration, the ability of a priming injection of cocaine to reinstate drug seeking behavior, and the discriminative stimulus properties of cocaine (Frankel and Cunningham 2004; Grottick et al. 2000). In contrast, blockade of 5-HT_{2C} receptors increases cocaine-induced locomotion, self-administration, and cocaine-primed reinstatement of responding (Filip et al. 2004; Fletcher et al. 2002a). Mice lacking the 5-HT_{2C} receptor also show increased responses to cocaine in tests of locomotion and self-administration (Rocha et al. 2002). The amphetamine derivative MDMA stimulates locomotor activity, and this effect is partially mediated by DA release (Gold et al. 1989; Kehne et al. 1996). In agreement with findings obtained with cocaine, 5-HT_{2C} receptor blockade also enhanced the locomotion induced by MDMA (Fletcher et al. 2002b; McCreary and Cunningham 1999).

The results of studies involving cocaine and MDMA are consistent with the notion that blocking 5-HT neurotransmission at the level of the 5-HT_{2C} receptor enhances DA-dependent function and behavior. However, both cocaine and MDMA also increase 5-HT neurotransmission by blocking the 5-HT transporter and by releasing 5-HT from neuronal terminals, respectively (Bradberry et al. 1993; Kankaanpaa et al. 1998; Ritz and Kuhar 1989; Teneud et al. 1996). At least in the case of MDMA, a direct role for increased 5-HT function in mediating locomotor stimulation induced by this drug has been shown (Bankson and Cunningham 2002; Callaway et al. 1990; Fletcher et al. 2002b; Kehne et al. 1996). This suggests that 5-HT_{2C} receptor blockade may influence MDMA- and/or cocaine-mediated behavioral effects directly via a serotonergic mechanism.

The main aim of the present study was to examine the effects of pharmacological blockade of 5-HT_{2C} receptors using SB242084 on the expression of locomotor activity elicited by a variety of manipulations that are known to have different effects on 5-HT and/or DA function. The first experiment compared the effects on locomotor activity of SB242084 given before treatment with a mixed DA and 5-HT releaser, MDMA with those resulting from treatment with the selective DA releaser amphetamine, or the selective 5-HT releaser fenfluramine. A parallel experiment examined the effects of SB242084 given in conjunction with a mixed 5-HT and DA reuptake blocker, cocaine, with those resulting from a combination of SB242084 with the selective DA reuptake blocker, methylphenidate, or the selective 5-HT reuptake blocker, citalopram. To further examine whether SB242084 modifies the expression of DA-dependent locomotor activation, we examined the interactions between SB242084 and morphine, or nicotine, both of which stimulate locomotion in part via activation of the mesolimbic DA system (Benwell and Balfour 1992; Joyce and Iversen 1979; Kelly and Iversen 1976; Reavill and Stolerman 1990). Finally, the effect of SB242084 against the mixed direct 5-$HT_{1A/1B}$ receptor agonist RU24969 (Barnes and Sharp 1999) was examined.

Materials and methods

Animals and housing

Adult male Sprague–Dawley rats weighing 225–250 g at the beginning of the experiments were used. The rats were housed in pairs in clear polycarbonate cages under a 12-h light/dark cycle (lights on at 8 A.M.). Food and water were freely available in the home cages.

Apparatus

All experiments were conducted in a custom-built locomotor activity monitor. This system consisted of 16 clear polycarbonate cages, measuring 25-cm-wide, 20-cm-high, and 45-cm-long. An array of six infrared photocells was attached outside the longer sides of the cages. The photocells were spaced 7.5 cm apart and 2 cm above the floor of the cage. The equipment was housed in a room different to the colony room.

Effects of SB242084 on drug-induced locomotor activity

Separate groups of rats were used to investigate the effects of pretreatment with 0.5 mg/kg of SB242084 or its vehicle

on changes in locomotor activity induced by amphetamine (0, 0.25, and 0.5 mg/kg; n=8; and 0, 0.5, and 1 mg/kg; n=8), methylenedioxymethamphetamine (0, 2.5, and 5 mg/kg; n=8), *d*-fenfluramine (0, 1, and 5 mg/kg; n=10), cocaine (0, 5, and 10 mg/kg; n=8), methylphenidate (0, 2.5, and 5 mg/kg; n=10), citalopram (0, 10, and 20 mg/kg; n=10), and RU24969 (0, 0.5 and 1 mg/kg; n=8). In all of these experiments, before any drug testing all rats were familiarized with the activity monitors by placing them in the cages for a 2-h period on each of three consecutive days. Two additional groups of rats were used to examine the interaction between SB242084 and nicotine (0, 0.2, and 0.4 mg/kg; n=12) and morphine (0, 2.5, and 5 mg/kg; n=12). Before testing began on these animals they were exposed to nicotine or morphine to induce tolerance to the depressant effects of these drugs. In the case of the nicotine group, rats received ten daily injections of 0.4 mg/kg of nicotine in the home cage; in the case of the morphine group they received four daily injections of 10 mg/kg of morphine in the home cage. Forty-eight hours after the last injection rats were familiarized with the activity monitors by placing them in the cages for a 2-h period on each of three consecutive days.

In all experiments on the test days rats were placed in the activity cages for 30 min and then injected with 0.5 mg/kg of SB242084 or its vehicle (see below). Rats were then returned to the activity cages for a further 30 min. At the end of this period rats were injected with the appropriate dose of the test drug and were replaced in the activity cages for 2 h. The number of photocell interruptions was recorded every 10 min. For all drugs, a repeated measures design was used whereby each rat was tested under all six possible combinations of SB242084 or its vehicle plus the two doses of the test drugs and the appropriate vehicle. Treatment combinations were administered in a semirandomized fashion with each combination given to at least one animal on each day. For each compound testing was usually conducted on Mondays and Thursdays or Tuesdays and Fridays.

The dose of SB242084 was chosen based on a consideration of the literature and on in-house data showing blockade of a number of 5-HT_{2C}-mediated responses by SB242084 (e.g., Fletcher et al. 2004; Martin et al. 2002).

Statistics

Data for all studies were analyzed using repeated measures three-way analysis of variance with SB242084 pretreatment, drug treatment, and time bin (10 min) as factors. Where appropriate, a significant three-way interaction was further analyzed by conducting separate two-way analyses of variance with SB242084 pretreatment and drug treatment as factors at each 10-min time point. Post hoc comparisons between means were conducted using the Fisher's least significant difference test.

Drugs

SB242084 was synthesized in the Department of Chemistry, Vernalis Research, Wokingham, UK, prepared in 0.9% saline solution containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid, and was injected by the IP route. Cocaine hydrochloride (BDH, Toronto, Ontario, Canada), *d*-amphetamine sulfate (Bureau of Drug Surveillance, Ottawa, Ontario, Canada), fenfluramine HCl (Servier), RU24969 [5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1H-indole; National Institute on Drug Abuse (NIDA) Drug Supply Program], and morphine sulfate (Bureau of Drug Surveillance, Ottawa, Ontario, Canada) were all dissolved in 0.9% saline and injected IP. MDMA [(±)-3,4-methylenedioxymethamphetamine HCl; NIDA Drug Supply Program] was dissolved in 0.9% saline and injected SC. Nicotine (Sigma-Aldrich, Oakville, Ontario, Canada) was dissolved in saline with the pH adjusted to 7.0 and was injected SC.

Results

Interactions between SB242084 and MDMA, amphetamine, or fenfluramine

Figure 1 shows the effects on locomotor activity of combining SB242084 with MDMA, amphetamine, or fenfluramine. MDMA significantly increased locomotor activity ($F_{2,14}$=96.5, p<0.001); and the main effect of SB242084 was also significant ($F_{1,7}$=27.38, p<0.001). The interaction between SB242084 and MDMA was significant ($F_{2,14}$=14.67, p<0001) and post hoc testing showed a significant enhancement of the effects of both doses of MDMA, measured over the 2-h test period by pretreatment with SB242084.

For the experiment involving amphetamine at doses 0.25 and 0.5 mg/kg, analysis of variance confirmed that amphetamine stimulated locomotion ($F_{2,14}$=28.36, p<0.001). The significant main effect of SB242084 ($F_{1,7}$=6.19, p<0.05) reflects the fact that overall activity levels were greater under SB242084 treatment. While both interaction terms involving SB242084 and amphetamine were not significant (p>0.08), post hoc tests indicated that the stimulant effect of 0.5 mg/kg of amphetamine measured over the 2-h testing period was enhanced under SB242084 treatment. Analysis of data for a second experiment involving amphetamine (0.5 and 1 mg/kg) showed that SB242084 potentiated the effect of 0.5 mg/kg but not 1 mg/kg (data not shown). In the experiment involving

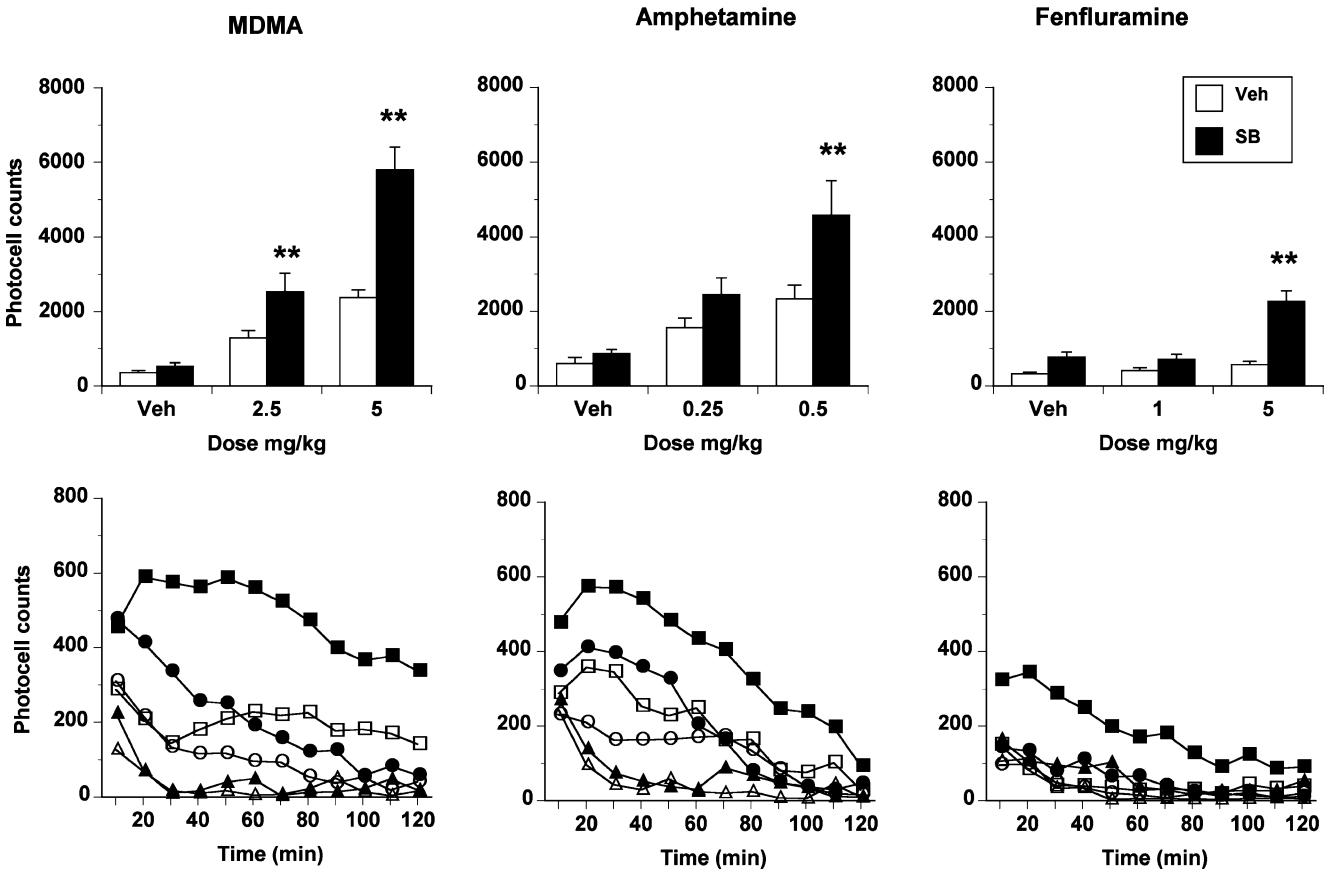

Fig. 1 The effects on locomotor activity of combining 0.5 mg/kg of SB242084 (*filled symbols* and *bars*) or its vehicle (*open symbols* and *bars*) with various doses of MDMA (n=8), amphetamine (n=8), or fenfluramine (n=10). The *upper panel* shows the mean±SEM photocell counts over a 2-h test period. The *lower panel* depicts the mean number of photocell counts in each 10-min period of the 2-h test; SEMs are omitted for clarity. For each test drug, the vehicle condition is represented by *triangles*, the low dose of the drug by *circles*, and the high dose by *squares*. **p<0.01 compared to the *Veh*—same dose condition

fenfluramine the main effects of fenfluramine ($F_{2,18}$=23.25, p<0.001) and SB242084 ($F_{1,9}$=60.60, p<0.001) were both significant as was the interaction between these two factors ($F_{2,18}$=11.54, p<0.001). By itself, fenfluramine did not alter locomotor activity, but the combination of SB242084 and 5 mg/kg of fenfluramine markedly increased photocell counts compared to fenfluramine or vehicle conditions alone.

Interactions between SB242084 and cocaine, methylphenidate, or citalopram

Figure 2 shows the effects on locomotor activity of combining SB242084 with cocaine, methylphenidate, or citalopram. Cocaine significantly increased locomotor activity ($F_{2,14}$=16.94, p<0.001); and the main effect of SB242084 was also significant ($F_{1,7}$=6.24, p<0.05). The two-way interaction between these factors was not significant ($F_{2,14}$=3.00, p<0.08), although the overall three-way interaction was significant ($F_{22,154}$=3.59, p<0001). Further analysis showed that the interaction between SB242084 and cocaine was significant at the 10-, 20-, and 30-min time points (p<0.05) but not at other times. SB242084 enhanced the effects of only the 10-mg/kg dose of cocaine.

For the experiment involving methylphenidate, analysis of variance revealed significant main effects of methylphenidate ($F_{2,18}$=30.08, p<0.001) and SB242084 ($F_{1,9}$=10.29, p<0.01). The interaction between these two factors was also significant ($F_{2,18}$=5.11, p<0.02), although this varied as a function of time ($F_{22,198}$=3.43, p<0.001). Further analysis of this effect showed that the interactions between methylphenidate and SB242084 were significant at the 20-, 30-, 40-, and 50-min time points (smallest $F_{2,18}$=3.67, p<0.05). Post hoc tests conducted on the data for the total activity scores showed that SB242084 enhanced the locomotor activity induced by 5 mg/kg of methylphenidate; the effect for 2.5 mg/kg was not significant (p=0.06).

In the study involving citalopram, neither the main effect of SB242084 nor any of the interaction terms involving SB242084 were significant (all p>0.3). Thus, SB242084 treatment had no effect in this experiment. The main effect of citalopram was significant ($F_{2,16}$=9.26, p<0.01). Further

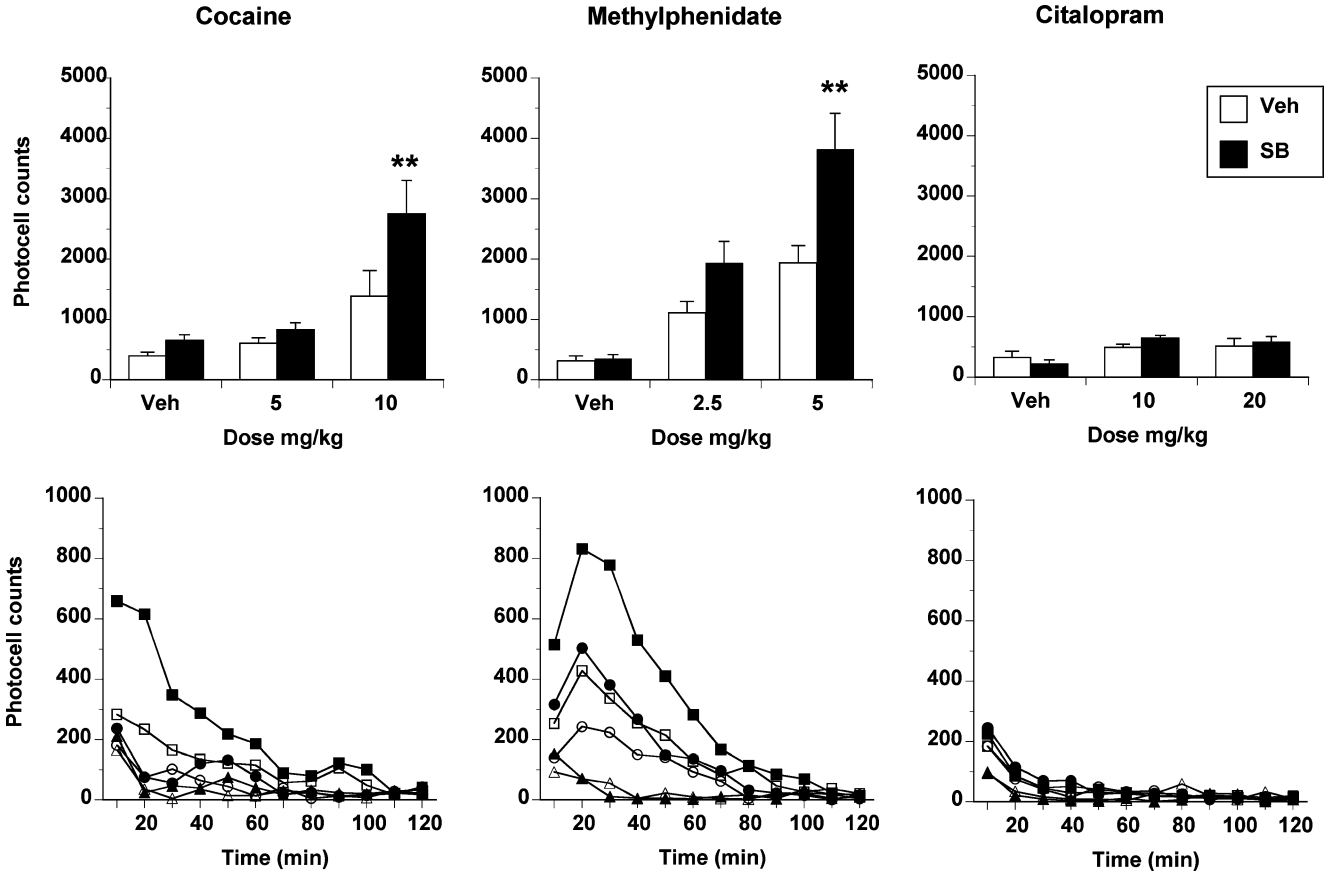

Fig. 2 The effects on locomotor activity of combining 0.5 mg/kg of SB242084 (*filled symbols* and *bars*) or its vehicle (*open symbols* and *bars*) with various doses of cocaine (n=8), methylphenidate (n=10), or citalopram (n=10). The *upper panel* shows the mean±SEM photocell counts over a 2-h test period. The *lower panel* depicts the mean number of photocell counts in each 10-min period of the 2−h test; SEMs are omitted for clarity. For each test drug the vehicle condition is represented by *triangles*, the low dose of the drug by *circles*, and the high dose by *squares*. **p<0.01 compared to the *Veh*—same dose condition

analysis of this effect revealed that when collapsed across both the time and SB242084 factors, activity scores for both 10 and 20 mg/kg of citalopram treatment were significantly higher than for saline treatment; however, it is clear that any stimulant effect of citalopram was very minor in comparison to the other compounds.

Interactions between SB242084 and nicotine or morphine

Figure 3 shows the effects of SB242084 on nicotine-stimulated activity. Analysis of variance indicated significant main effects of nicotine ($F_{2,22}$=33.30, p<0.001) and SB242084 ($F_{1,11}$=45.13, p<0.001). In these rats, SB242084 enhanced the stimulant effects of nicotine as reflected in the significant interaction between SB242084 and nicotine ($F_{2,22}$=6.25, p<0.001) and the overall three-way interaction ($F_{22,242}$=2.32, p<0.001). Post hoc testing revealed that SB242084 potentiated the effects of both doses of nicotine.

Figure 3 also shows the effects of SB242084 on morphine-stimulated activity. Both the main effect of morphine ($F_{2,22}$=88.3, p<0.001) and the main effect of SB242084 were significant ($F_{1,11}$=18.32, p<0.01). The SB242084 × morphine interaction was significant ($F_{2,22}$=5.52, p<0.05), indicating that SB242084 potentiated the effect of morphine. Post hoc testing on the data for the full session revealed that this effect was seen only at the 2.5-mg/kg dose of morphine.

Interactions between SB242084 and RU24969

Figure 4 shows the effects on locomotor activity of combining SB242084 with the 5-HT_{1A}/5-HT_{1B} receptor agonist RU24969. Analysis of variance showed a significant main effect of RU24969 only ($F_{1,7}$=0.03, p<0.001). Treatment with SB242084 did not alter the response to RU24969.

Discussion

The selective 5-HT_{2C} receptor antagonist SB242084 potentiated the locomotor activation induced by several drugs

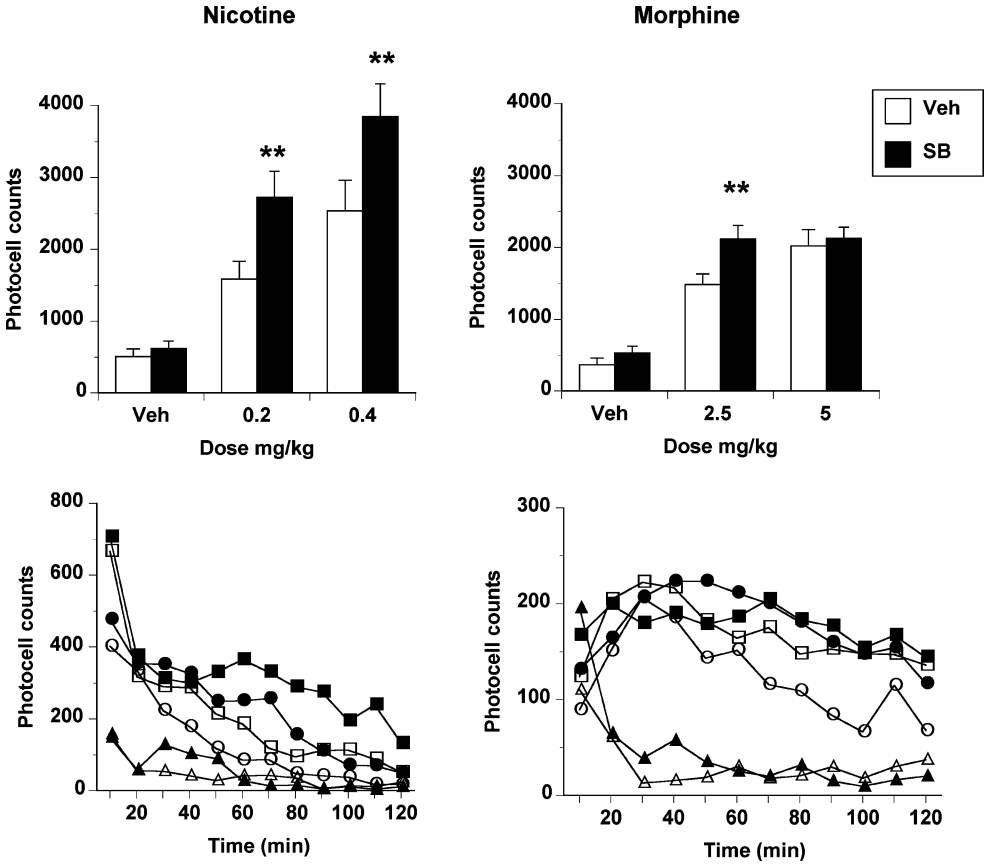

Fig. 3 The effects on locomotor activity of combining 0.5 mg/kg of SB242084 (*filled symbols* and *bars*) or its vehicle (*open symbols* and *bars*) with various doses of nicotine (n=12) or morphine (n=12). The *upper panel* shows the mean±SEM photocell counts over a 2-h test period. The *lower panel* depicts the mean number of photocell counts in each 10-min period of the 2-h test; SEMs are omitted for clarity. For each test drug the vehicle condition is represented by *triangles*, the low dose of the drug by *circles*, and the high dose by *squares*. (Note the scale difference on the *Y*-axis for the time course graphs). **p<0.01 compared to the *Veh*—same dose condition

from distinct pharmacological classes and with diverse neuropharmacological actions, including cocaine, MDMA, amphetamine, methylphenidate, nicotine, and morphine. This list can also be expanded to include phencyclidine (Hutson et al. 2000). One possible explanation for the ability of SB242084 to potentiate the effects of these drugs is that this effect results from pharmacokinetic interactions. Cytochrome P450 enzymes are responsible for metabolism of many drugs, including those tested in the present experiments, and so interference with the activity of these enzymes could alter the behavioral response to these drugs simply through changes in plasma exposure level. Several lines of evidence suggest that pharmacokinetic interactions are unlikely to account for the effects of SB242084. Firstly, in vitro SB242084 has negligible affinity for several major cytochrome P450 enzymes responsible for metabolism of cocaine, amphetamine, nicotine, morphine, and fenfluramine (Bromidge et al. 1997). Secondly, SB242084 does not alter brain levels of MDMA (Fletcher et al. 2002b), amphetamine, or cocaine (unpublished observations) at the doses used in the present studies. Thirdly, mice lacking the 5-HT_{2C} receptor gene also show enhanced stimulant and reinforcing effects of cocaine (Rocha et al. 2002). These converging findings make it likely that the effect of SB242084 to increase the stimulant effects of a variety of drugs reflects its neuropharmacological action to block 5-HT_{2C} receptors.

Consistent with earlier reports, 5-HT_{2C} receptor blockade enhanced the locomotor stimulatory effects of cocaine (Fletcher et al. 2002a; McCreary and Cunningham 1999) and MDMA (Bankson and Cunningham 2002; Fletcher et al. 2002b). Locomotor activity stimulated by both drugs is blocked or attenuated by destruction of DA terminals in the nucleus accumbens (Kelly and Iversen 1976; Gold et al. 1989), pointing to a role for elevated DA function in mediating the motor stimulant effects of cocaine and MDMA. In the case of cocaine this results from blockade of the DA transporter (Ritz and Kuhar 1989), and in the case of MDMA from increased release of DA (Gold et al. 1989; Kankaanpaa et al. 1998; Nash and Nichols 1991). Given that 5-HT_{2C} receptor blockade increases the firing rate of DA neurons in the ventral tegmental area (VTA) (Di Giovanni et al. 1999; Di Matteo et al. 1999), it is possible that SB242084 potentiates the effects of cocaine and MDMA by enhancing the ability of these drugs to elevate extracellular levels of DA. In keeping with this explanation, SB242084 increased the motor stimulant effects of the DA reuptake inhibitor methylphenidate and the DA releaser amphetamine. At the doses used, methylphenidate and amphetamine increase extracellular DA levels with no effect on 5-HT (Kuczenski and Segal 1989, 1997, 2001). Thus, 5-HT_{2C} receptor blockade enhances the motor stimulant effects of drugs that selectively increase extracellular levels of DA.

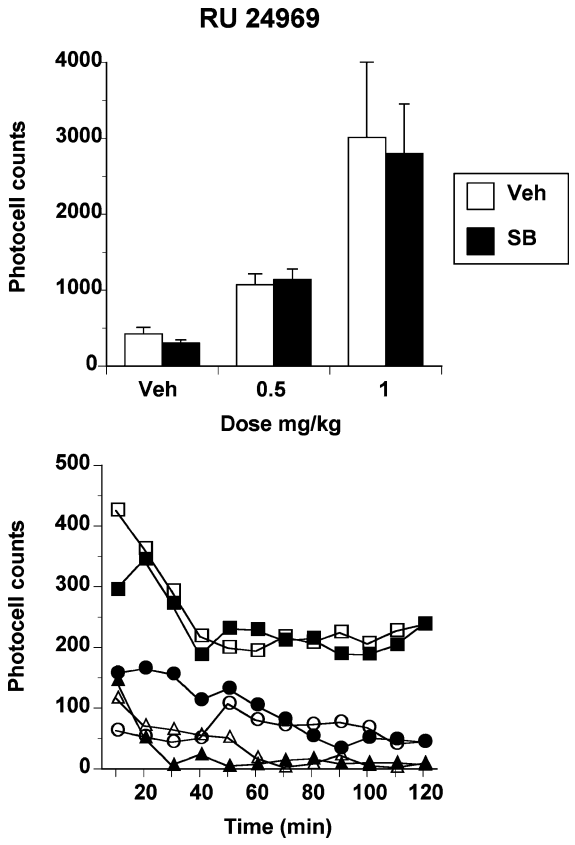

Fig. 4 The effects on locomotor activity of combining 0.5 mg/kg of SB242084 (*filled symbols* and *bars*) or its vehicle (*open symbols* and *bars*) with various doses of RU24969 (n=8) The *upper panel* shows the mean±SEM photocell counts over a 2-h test period. The *lower panel* depicts the mean number of photocell counts in each 10-min period of the 2-h test; SEMs are omitted for clarity. For each test drug the vehicle condition is represented by *triangles*, the low dose of the drug by *circles*, and the high dose by *squares*

Recently, SB242084 was shown to potentiate the increase in extracellular levels of DA in the nucleus accumbens and the striatum elicited by 15 mg/kg of cocaine (Navailles et al. 2004); this provides direct evidence for the postulated dopaminergic mechanism by which 5-HT_{2C} receptor blockade increases the effect of cocaine. In contrast, the mixed 5-$HT_{2B/2C}$ antagonist SB206553, which enhances the motor stimulant effect of 0.5 mg/kg of amphetamine (Bankson and Cunningham 2002), did not alter the DA-elevating effects of 2 mg/kg of amphetamine (Porras et al. 2002). The studies reported here show that the ability of SB242084 to enhance the stimulant effect of amphetamine depends on the dose of amphetamine; the response to 0.5 mg/kg was enhanced in two separate experiments, but the response to 1 mg/kg of amphetamine was unaffected. These data suggest that any modulatory effect of 5-HT_{2C} receptor blockade on the behavioral effects of amphetamine may be overwhelmed in the face of a high degree of DA release.

Nicotine and morphine increase locomotion by indirectly activating mesolimbic DA neurons originating in the VTA (Benwell and Balfour 1992; Joyce and Iversen 1979; Reavill and Stolerman 1990). SB242084 increased the stimulant effects of both drugs, although the interaction with nicotine seemed more pronounced than that with morphine. These findings are in broad keeping with demonstrations that the motor stimulant effect of nicotine and the ability of morphine and nicotine to elevate DA levels in the nucleus accumbens are reduced by 5-HT_{2C} receptor agonists (Di Matteo et al. 2004; Grottick et al. 2001; Willins and Meltzer 1998). Again, given that SB242084 modulates the activity of DA neurons in the VTA, a plausible explanation for the increased response to nicotine and morphine under 5-HT_{2C} receptor blockade is that this involves an increased capacity of morphine and nicotine to elevate DA levels in the nucleus accumbens. This possibility is supported, at least in the case of morphine, by the finding that SB206553 enhanced the ability of morphine to increase DA release (Porras et al. 2002).

In addition to releasing DA, MDMA also releases 5-HT (e.g., Kankaanpaa et al. 1998). The more selective 5-HT releaser fenfluramine, when given alone, had no effect on locomotion, but pretreatment with SB242084 led to strong locomotor activation. These results confirm an earlier report using *d*-fenfluramine (Higgins et al. 2001). At the doses used, fenfluramine has little impact on DA activity (Balcioglu and Wurtman 1998; Shoaib et al. 1997), suggesting that released 5-HT, acting presumably via non-5-HT_{2C} receptors, elicits motoric activation. One candidate receptor is the 5-HT_{2A} receptor, as implied from findings that the selective 5-HT_{2A} receptor antagonist M100907 blunts the locomotor response to MDMA (Fletcher et al. 2002b; Herin et al. 2005; Kehne et al. 1996) and a *d*-fenfluramine/SB242084 combination (Higgins et al. 2001). A second candidate 5-HT receptor subtype for mediating the enhanced stimulant effects of MDMA/SB242084 combination and fenfluramine/SB242084 combination is the 5-HT_{1B} receptor. This is supported by the observation that mixed 5-$HT_{1A/1B}$ receptor agonists such as RU24969 and CGS12066B increase locomotor activity in rodents (Cheetham and Heal 1993; Green et al. 1984), and by the fact that the 5-$HT_{1B/1D}$ receptor antagonist GR127935 attenuates the stimulant effect of MDMA (Bankson and Cunningham 2002; Fletcher et al. 2002b; McCreary et al. 1999).

Cocaine elevates 5-HT function by inhibiting 5-HT reuptake. Like MDMA, the stimulant effects of cocaine are attenuated by 5-HT_{1B} and 5-HT_{2A} receptor antagonists (Castanon et al. 2000; Filip et al. 2004; Fletcher et al. 2002a; McMahon and Cunningham 2001), suggesting that activation of these receptors by endogenous 5-HT, subsequent to reuptake inhibition, is part of the mechanism by which cocaine increases locomotion. In the present experiments a selective 5-HT reuptake inhibitor citalopram

(SSRI) did not alter locomotion even in the presence of 5-HT_{2C} receptor blockade. Both fenfluramine and citalopram selectively increase synaptic 5-HT levels (Boothman et al. 2006; Bymaster et al. 2002; Millan et al. 2000), as opposed to the more generalized monoamine changes induced by cocaine and MDMA. Given the significant fenfluramine/SB242084 interaction (see also Higgins et al. 2001), the lack of an equivalent interaction between citalopram and SB242084 may relate to qualitative or quantitative differences in synaptic 5-HT changes produced by selective 5-HT reuptake inhibition, compared to the 5-HT-releasing property of fenfluramine (Fuller et al. 1988). Thus, despite its capacity to elevate extracellular 5-HT (Boothman et al. 2006; Millan et al. 2000) the magnitude of this effect induced by citalopram may have been below a threshold for induction of locomotor activation via 5-HT_{2A} and/or 5-HT_{1B} receptor activation, even in the presence of concomitant 5-HT_{2C} receptor block. It is interesting to note that in the mouse citalopram elicits a mild hyperactivity that is potentiated by SB242084 (Millan et al. 2003). Combined with reports of a synergistic effect between citalopram and SB242084 on extracellular 5-HT levels in the hippocampus and immobility time in the mouse tail suspension test (Boothman et al. 2006; Cremers et al. 2004), it is clear that 5-HT_{2C} receptor blockade can potentiate certain SSRI-mediated effects.

In addition to their effects on DA and 5-HT systems, amphetamine, methylphenidate, cocaine, and MDMA increase noradrenaline (NA) neurotransmission (Florin et al. 1994; Kuczenski and Segal 1997; Rothman et al. 2001). SB242084 elevates extracellular levels of NA (Millan et al. 1998) and so it is possible that some of the effects of SB242084 involve interactions between 5-HT and NA. Selective NA reuptake inhibitors do not stimulate activity (Brocco et al. 2002; Davids et al. 2002), although the motor-stimulant effect of amphetamine is blocked by prazosin and clonidine, and is enhanced by propranolol (Vanderschuren et al. 2003). In contrast, none of these NA receptor antagonists alter cocaine-stimulated activity (Vanderschuren et al. 2003). Thus, the role of NA in mediating the effects of psychomotor stimulants is not precisely known, although it is likely to be complex. Therefore, the most parsimonious explanation for the effect of SB242084 on drug-induced locomotor activation is that it involves dopaminergic and/or serotonergic mechanisms.

As a final part to these studies, we investigated the interaction between SB242084 and the direct 5-$HT_{1A/1B}$ receptor agonist RU24969. The failure of SB242084 to potentiate the RU24969-induced hyperactivity suggests a certain pharmacological specificity to the effect of SB242084 on the other drugs tested in this report. This lack of effect of SB242084 on the stimulant effect of RU24969 fails to support an interesting interaction reported by Dalton et al. (2004). These authors found that in mice the hyperlocomotion elicited by joint activation of 5-HT_{1A} and 5-HT_{1B} receptors through 8-OH-DPAT and CP-94,253 cotreatment is dependent on intact 5-HT_{2C} receptor function. The different results between that study and the present one may reflect either species differences or differences among the pharmacological approaches used to influence these 5-HT receptor subtypes.

Overall, these experiments suggest that 5-HT_{2C} receptor blockade alters drug-induced locomotion by two distinct mechanisms. The first is a modulation of DA neural activity. Evidence for this mechanism is shown by the ability of SB242084 to potentiate the effects of amphetamine and methylphenidate, two drugs that selectively and directly increase DA neurotransmission, as well as the effects of nicotine and morphine, two drugs that indirectly activate mesolimbic DA neurons. The second may involve an unmasking of a serotonergic mechanism, as evidenced by the potentiation of the selective 5-HT releaser fenfluramine. The failure to see a similar interaction with citalopram may suggest that the degree of increased synaptic 5-HT tone is a critical factor for hyperlocomotion. The interaction between MDMA and SB242084 could reflect the recruitment of both mechanisms, which may explain why the greatest magnitude of response noted across all experiments was apparent after the MDMA/SB242084 combination. On the basis of the present data, showing a lack of interaction between SB242084 and the SSRI citalopram, it seems that the ability of SB242084 to enhance the stimulant effect of cocaine, at least in the rat, is likely due to the dopaminergic mechanism, rather than unmasking of a serotonergic mechanism.

The fact that blockade of 5-HT_{2C} receptors enhances the stimulant effects of a number of drugs of abuse indicates a possible endogenous modulation by 5-HT_{2C} receptors of the effects of these drugs. The 5-HT_{2C} receptor is the only known G protein-coupled receptor that undergoes RNA editing (Burns et al. 1997). This posttranscriptional modification gives rise to functionally distinct receptor isoforms (Burns et al. 1997). For some of these isoforms basal receptor activity is reduced resulting from less efficient G protein coupling (Fitzgerald et al. 1999; Herrick-Davis et al. 1999; Niswender et al. 1998, 1999). Distinct patterns of 5-HT_{2C} RNA editing have been linked to depression, suicide (Gurevich et al. 2002; Niswender et al. 2001), and schizophrenia (Sodhi et al. 2001). Perhaps distinct patterns of 5-HT_{2C} receptor isoforms resulting from altered RNA editing may contribute to individual differences in responsivity to drugs of abuse and vulnerability to addiction.

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References

Balcioglu A, Wurtman RJ (1998) Effects of fenfluramine and phentermine (fen-phen) on dopamine and serotonin release in rat striatum: in vivo microdialysis study in conscious animals. Brain Res 813:67–72

Bankson MG, Cunningham KA (2002) Pharmacological studies of the acute effects of (+)-3,4-methylenedioxymethamphetamine on locomotor activity: role of 5-HT(1B/1D) and 5-HT(2) receptors. Neuropsychopharmacology 26:40–52

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

Benwell ME, Balfour DJ (1992) The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br J Pharmacol 105:849–856

Boothman LJ, Mitchell SN, Sharp T (2006) Investigation of the SSRI augmentation properties of 5-HT(2) receptor antagonists using in vivo microdialysis. Neuropharmacology 50(6):726–732

Bradberry CW, Nobiletti JB, Elsworth JD, Murphy B, Jatlow P, Roth RH (1993) Cocaine and cocaethylene: microdialysis comparison of brain drug levels and effects on dopamine and serotonin. J Neurochem 60:1429–1435

Brocco M, Dekeyne A, Veiga S, Girardon S, Millan MJ (2002) Induction of hyperlocomotion in mice exposed to a novel environment by inhibition of serotonin reuptake. A pharmacological characterization of diverse classes of antidepressant agents. Pharmacol Biochem Behav 71:667–680

Bromidge SM, Duckworth M, Forbes IT, Ham P, King FD, Thewlis KM, Blaney FE, Naylor CB, Blackburn TP, Kennett GA, Wood MD, Clarke SE (1997) 6-Chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB-242084): the first selective and brain penetrant 5-HT2C receptor antagonist. J Med Chem 40:3494–3496

Burns CM, Chu H, Rueter SM, Hutchinson LK, Canton H, Sanders-Bush E, Emeson RB (1997) Regulation of serotonin-2C receptor G-protein coupling by RNA editing. Nature 387: 303–308

Bymaster FP, Zhang W, Carter PA, Shaw J, Chernet E, Phebus L, Wong DT, Perry KW (2002) Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. Psychopharmacology (Berl) 160:353–361

Callaway CW, Wing LL, Geyer MA (1990) Serotonin release contributes to the locomotor stimulant effects of 3,4-methylenedioxymethamphetamine in rats. J Pharmacol Exp Ther 254:456–464

Castanon N, Scearce-Levie K, Lucas JJ, Rocha B, Hen R (2000) Modulation of the effects of cocaine by 5-HT1B receptors: a comparison of knockouts and antagonists. Pharmacol Biochem Behav 67:559–566

Cheetham SC, Heal DJ (1993) Evidence that RU 24969-induced locomotor activity in C57/B1/6 mice is specifically mediated by the 5-HT1B receptor. Br J Pharmacol 110:1621–1629

Cremers TI, Giorgetti M, Bosker FJ, Hogg S, Arnt J, Mork A, Honig G, Bogeso KP, Westerink BH, den Boer H, Wikstrom HV, Tecott LH (2004) Inactivation of 5-HT(2C) receptors potentiates consequences of serotonin reuptake blockade. Neuropsychopharmacology 29:1782–1789

Dalton GL, Lee MD, Kennett GA, Dourish CT, Clifton PG (2004) mCPP-induced hyperactivity in 5-HT2C receptor mutant mice is mediated by activation of multiple 5-HT receptor subtypes. Neuropharmacology 46:663–671

Davids E, Zhang K, Kula NS, Tarazi FI, Baldessarini RJ (2002) Effects of norepinephrine and serotonin transporter inhibitors on hyperactivity induced by neonatal 6-hydroxydopamine lesioning in rats. J Pharmacol Exp Ther 301:1097–1102

Di Giovanni G, De Deurwaerdere P, Di Mascio M, Di Matteo V, Esposito E, Spampinato U (1999) Selective blockade of serotonin-2C/2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. Neuroscience 91:587–597

Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E (1999) SB 242084, a selective serotonin2C receptor antagonist, increases dopaminergic transmission in the mesolimbic system. Neuropharmacology 38:1195–1205

Di Matteo V, Di Giovanni G, Di Mascio M, Esposito E (2000) Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin (2C) receptors. Brain Res 865:85–90

Di Matteo V, Cacchio M, Di Giulio C, Esposito E (2002) Role of serotonin(2C) receptors in the control of brain dopaminergic function. Pharmacol Biochem Behav 71:727–734

Di Matteo V, Pierucci M, Esposito E (2004) Selective stimulation of serotonin2c receptors blocks the enhancement of striatal and accumbal dopamine release induced by nicotine administration. J Neurochem 89:418–429

Filip M, Bubar MJ, Cunningham KA (2004) Contribution of serotonin (5-hydroxytryptamine; 5-HT) 5-HT2 receptor subtypes to the hyperlocomotor effects of cocaine: acute and chronic pharmacological analyses. J Pharmacol Exp Ther 310:1246–1254

Fitzgerald LW, Iyer G, Conklin DS, Krause CM, Marshall A, Patterson JP, Tran DP, Jonak GJ, Hartig PR (1999) Messenger RNA editing of the human serotonin 5-HT2C receptor. Neuropsychopharmacology 21:82S–90S

Fletcher PJ, Grottick AJ, Higgins GA (2002a) Differential effects of the 5-HT(2A) receptor antagonist M100907 and the 5-HT (2C) receptor antagonist SB242084 on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. Neuropsychopharmacology 27:576–586

Fletcher PJ, Korth KM, Robinson SR, Baker GB (2002b) Multiple 5-HT receptors are involved in the effects of acute MDMA treatment: studies on locomotor activity and responding for conditioned reinforcement. Psychopharmacology (Berl) 162:282–291

Fletcher PJ, Chintoh AF, Sinyard J, Higgins GA (2004) Injection of the 5-HT2C receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. Neuropsychopharmacology 29: 308–318

Florin SM, Kuczenski R, Segal DS (1994) Regional extracellular norepinephrine responses to amphetamine and cocaine and effects of clonidine pretreatment. Brain Res 654:53–62

Frankel PS, Cunningham KA (2004) m-Chlorophenylpiperazine (mCPP) modulates the discriminative stimulus effects of cocaine through actions at the 5-HT2C receptor. Behav Neurosci 118:157–162

Fuller RW, Snoddy HD, Robertson DW (1988) Mechanisms of effects of d-fenfluramine on brain serotonin metabolism in rats: uptake inhibition versus release. Pharmacol Biochem Behav 30:715–721

Gobert A, Rivet JM, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas JP, Cistarelli L, Melon C, Millan MJ (2000) Serotonin(2C) receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. Synapse 36:205–221

Gold LH, Hubner CB, Koob GF (1989) A role for the mesolimbic dopamine system in the psychostimulant actions of MDMA. Psychopharmacology (Berl) 99:40–47

Green AR, Guy AP, Gardner CR (1984) The behavioural effects of RU 24969, a suggested 5-HT1 receptor agonist in rodents and the effect on the behaviour of treatment with antidepressants. Neuropharmacology 23:655–661

Grottick AJ, Fletcher PJ, Higgins GA (2000) Studies to investigate the role of 5-HT(2C) receptors on cocaine- and food-maintained behavior. J Pharmacol Exp Ther 295:1183–1191

Grottick AJ, Corrigall WA, Higgins GA (2001) Activation of 5-HT (2C) receptors reduces the locomotor and rewarding effects of nicotine. Psychopharmacology (Berl) 157:292–298

Gurevich I, Tamir H, Arango V, Dwork AJ, Mann JJ, Schmauss C (2002) Altered editing of serotonin 2C receptor pre-mRNA in the prefrontal cortex of depressed suicide victims. Neuron 34:349–56

Herin DV, Liu S, Ullrich T, Rice KC, Cunningham KA (2005) Role of the serotonin 5-HT2A receptor in the hyperlocomotive and hyperthermic effects of (+)-3,4-methylenedioxymethamphetamine. Psychopharmacology (Berl) 178:505–513

Herrick-Davis K, Grinde E, Niswender CM (1999) Serotonin 5-HT2C receptor RNA editing alters receptor basal activity: implications for serotonergic signal transduction. J Neurochem 73:1711–1717

Higgins GA, Ouagazzal AM, Grottick AJ (2001) Influence of the 5-HT(2C) receptor antagonist SB242,084 on behaviour produced by the 5-HT(2) agonist Ro60-0175 and the indirect 5-HT agonist dexfenfluramine. Br J Pharmacol 133:459–466

Hutson PH, Barton CL, Jay M, Blurton P, Burkamp F, Clarkson R, Bristow LJ (2000) Activation of mesolimbic dopamine function by phencyclidine is enhanced by 5-HT(2C/2B) receptor antagonists: neurochemical and behavioural studies. Neuropharmacology 39:2318–2328

Joyce EM, Iversen SD (1979) The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neurosci Lett 14:207–212

Kankaanpaa A, Meririnne E, Lillsunde P, Seppala T (1998) The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens. Pharmacol Biochem Behav 59:1003–1009

Kehne JH, Ketteler HJ, McCloskey TC, Sullivan CK, Dudley MW, Schmidt CJ (1996) Effects of the selective 5-HT2A receptor antagonist MDL 100,907 on MDMA-induced locomotor stimulation in rats. Neuropsychopharmacology 15:116–124

Kelly PH, Iversen SD (1976) Selective 6OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. Eur J Pharmacol 40:45–56

Kuczenski R, Segal D (1989) Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis. J Neurosci 9:2051–2065

Kuczenski R, Segal DS (1997) Effects of methylphenidate on extracellular dopamine, serotonin, and norepinephrine: comparison with amphetamine. J Neurochem 68:2032–2037

Kuczenski R, Segal DS (2001) Locomotor effects of acute and repeated threshold doses of amphetamine and methylphenidate: relative roles of dopamine and norepinephrine. J Pharmacol Exp Ther 296:876–883

Martin JR, Bos M, Jenck F, Moreau J, Mutel V, Sleight AJ, Wichmann J, Andrews JS, Berendsen HH, Broekkamp CL, Ruigt GS, Kohler C, Delft AM (1998) 5-HT2C receptor agonists: pharmacological characteristics and therapeutic potential. J Pharmacol Exp Ther 286:294–913

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

McCreary AC, Cunningham KA (1999) Effects of the 5-HT2C/2B antagonist SB 206553 on hyperactivity induced by cocaine. Neuropsychopharmacology 20:556–564

McCreary AC, Bankson MG, Cunningham KA (1999) Pharmacological studies of the acute and chronic effects of (+)-3, 4-methylenedioxymethamphetamine on locomotor activity: role of 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B/1D) receptors. J Pharmacol Exp Ther 290:965–973

McMahon LR, Cunningham KA (2001) Antagonism of 5-hydroxytryptamine(2a) receptors attenuates the behavioral effects of cocaine in rats. J Pharmacol Exp Ther 297:357–363

Millan MJ, Dekeyne A, Gobert A (1998) Serotonin (5-HT)2C receptors tonically inhibit dopamine (DA) and noradrenaline (NA), but not 5-HT, release in the frontal cortex in vivo. Neuropharmacology 37:953–955

Millan MJ, Gobert A, Rivet JM, Adhumeau-Auclair A, Cussac D, Newman-Tancredi A, Dekeyne A, Nicolas JP, Lejeune F (2000) Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic, but not serotonergic, transmission by blockade of alpha2-adrenergic and serotonin2C receptors: a comparison with citalopram. Eur J Neurosci 12:1079–1095

Millan MJ, Veiga S, Girardon S, Brocco M (2003) Blockade of serotonin 5-HT1B and 5-HT2A receptors suppresses the induction of locomotor activity by 5-HT reuptake inhibitors, citalopram and fluvoxamine, in NMRI mice exposed to a novel environment: a comparison to other 5-HT receptor subtypes. Psychopharmacology (Berl) 168:397–409

Nash JF, Nichols DE (1991) Microdialysis studies on 3,4-methylenedioxyamphetamine and structurally related analogues. Eur J Pharmacol 200:53–58

Navailles S, De Deurwaerdere P, Porras G, Spampinato U (2004) In vivo evidence that 5-HT2C receptor antagonist but not agonist modulates cocaine-induced dopamine outflow in the rat nucleus accumbens and striatum. Neuropsychopharmacology 29:319–326

Niswender CM, Sanders-Bush E, Emeson RB (1998) Identification and characterization of RNA editing events within the 5-HT2C receptor. Ann N Y Acad Sci 861:38–48

Niswender CM, Copeland SC, Herrick-Davis K, Emeson RB, Sanders-Bush E (1999) RNA editing of the human serotonin 5-hydroxytryptamine 2C receptor silences constitutive activity. J Biol Chem 274:9472–9478

Niswender CM, Herrick-Davis K, Dilley GE, Meltzer HY, Overholser JC, Stockmeier CA, Emeson RB, Sanders-Bush E (2001) RNA editing of the human serotonin 5-HT2C receptor. alterations in suicide and implications for serotonergic pharmacotherapy. Neuropsychopharmacology 24:478–491

Porras G, Di Matteo V, Fracasso C, Lucas G, De Deurwaerdere P, Caccia S, Esposito E, Spampinato U (2002) 5-HT2A and 5-HT2C/2B receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. Neuropsychopharmacology 26:311–24

Reavill C, Stolerman IP (1990) Locomotor activity in rats after administration of nicotinic agonists intracerebrally. Br J Pharmacol 99:273–278

Ritz MC, Kuhar MJ (1989) Relationship between self-administration of amphetamine and monoamine receptors in brain: comparison with cocaine. J Pharmacol Exp Ther 248:1010–1017

Rocha BA, Goulding EH, O'Dell LE, Mead AN, Coufal NG, Parsons LH, Tecott LH (2002) Enhanced locomotor, reinforcing, and neurochemical effects of cocaine in serotonin 5-hydroxytryptamine 2C receptor mutant mice. J Neurosci 22:10039–10045

Rothman RB, Baumann MH, Dersch CM, Romero DV, Rice KC, Carroll FI, Partilla JS (2001) Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. Synapse 39:32–41

Shoaib M, Baumann MH, Rothman RB, Goldberg SR, Schindler CW (1997) Behavioural and neurochemical characteristics of phentermine and fenfluramine administered separately and as a mixture in rats. Psychopharmacology (Berl) 131:296–306

Sodhi MS, Burnet PW, Makoff AJ, Kerwin RW, Harrison PJ (2001) RNA editing of the 5-HT(2C) receptor is reduced in schizophrenia. Mol Psychiatry 6:373–379

Teneud LM, Baptista T, Murzi E, Hoebel BG, Hernandez L (1996) Systemic and local cocaine increase extracellular serotonin in the nucleus accumbens. Pharmacol Biochem Behav 53:747–752

Vanderschuren LJ, Beemster P, Schoffelmeer AN (2003) On the role of noradrenaline in psychostimulant-induced psychomotor activity and sensitization. Psychopharmacology (Berl) 169:176–185

Willins DL, Meltzer HY (1998) Serotonin 5-HT2C agonists selectively inhibit morphine-induced dopamine efflux in the nucleus accumbens. Brain Res 781:291–299