Neuropsychopharmacology (2004) 29, 319–326
© 2004 Nature Publishing Group All rights reserved 0893-133X/04 $25.00
www.neuropsychopharmacology.org

In Vivo Evidence that 5-HT$_{2C}$ Receptor Antagonist but not Agonist Modulates Cocaine-Induced Dopamine Outflow in the Rat Nucleus Accumbens and Striatum

Sylvia Navailles[1], Philippe De Deurwaerdère[1], Grégory Porras[1] and Umberto Spampinato*[,1]

[1]*UMR CNRS, 5541-Université Victor Segalen Bordeaux 2, Bordeaux Cedex, France*

During recent years, much attention has been devoted at investigating the modulatory role of central 5-HT$_{2C}$ receptors on dopamine (DA) neuron activity, and it has been proposed that these receptors modulate selectively DA exocytosis associated with increased firing of DA neurons. In the present study, using *in vivo* microdialysis in the nucleus accumbens (NAc) and the striatum of halothane-anesthetized rats, we addressed this hypothesis by assessing the ability of 5-HT$_{2C}$ agents to modulate the increase in DA outflow induced by haloperidol and cocaine, of which the effects on DA outflow are associated or not with an increase in DA neuron firing, respectively. The intraperitoneal administration of cocaine (10–30 mg/kg) induced a dose-dependent increase in DA extracellular levels in the NAc and the striatum. The effect of 15 mg/kg cocaine was potentiated by the mixed 5-HT$_{2C/2B}$ antagonist SB 206553 (5 mg/kg i.p.) and the selective 5-HT$_{2C}$ antagonist SB 242084 (1 mg/kg i.p.) in both brain regions. The mixed 5-HT$_{2C/2B}$ agonist, Ro 60-0175 (1 mg/kg i.p.), failed to affect cocaine-induced DA outflow, but reduced significantly the increase in DA outflow induced by the subcutaneous administration of 0.1 mg/kg haloperidol. The obtained results provide evidence that 5-HT$_{2C}$ receptors exert similar effects in both the NAc and the striatum, and they modulate DA exocytosis also when its increase occurs independently from an increase in DA neuron impulse activity. Furthermore, they show that 5-HT$_{2C}$ agonists, at variance with 5-HT$_{2C}$ antagonists, exert a preferential control on the impulse-stimulated release of DA.

Neuropsychopharmacology (2004) **29,** 319–326, advance online publication, 15 October 2003; doi:10.1038/sj.npp.1300329

Keywords: *In vivo* dopamine release; cocaine; haloperidol; striatum; accumbens; 5-HT$_{2C}$ receptors

INTRODUCTION

During recent years, a growing body of evidence has highlighted the potential of central serotonin$_{2C}$ (5-HT$_{2C}$) receptors for improved treatment of neuropsychiatric disorders related to dopamine (DA) neuron dysfunctions, such as Parkinson's disease, schizophrenia, or drug addiction (Wood *et al*, 2001; Jones and Blackburn 2002). In this context, much attention has been devoted at investigating their modulatory role on DA neuron activity. However, even though compelling evidences indicate that 5-HT$_{2C}$ receptors do affect basal DA neuron activity, their influence on DA function in case of altered DA transmission remains unclear.

*Correspondence: Professor U Spampinato, UMR CNRS, 5541-Université Victor Segalen Bordeaux 2, 146 rue Léo Saignat, Boîte Postale 31, 33076 Bordeaux Cedex, France, Tel: +33 557 57 15 44, Fax: +33 556 90 02 78,
E-mail: Umberto.Spampinato@lnpb.u-bordeaux2.fr
Received 29 April 2003; revised 10 July 2003; accepted 10 September 2003
Online publication: 17 September 2003 at http://www.acnp.org/citations/Npp09170303183/default.pdf

5-HT$_{2C}$ receptors are expressed along the mesocortico-limbic and nigrostriatal DA pathways (Pazos *et al*, 1985; Eberle-Wang *et al*, 1997), and have been shown to exert phasic and tonic inhibitory controls on both basal DA neuronal firing and basal DA release in the nucleus accumbens (NAc), the striatum and the frontal cortex (Di Giovanni *et al*, 1999; Gobert *et al*, 2000). 5-HT$_{2C}$ receptors have been also shown to modulate stimulated DA release although in restricted conditions. Specifically, 5-HT$_{2C}$ antagonists potentiate the increase in DA release induced by drugs stimulating DA neuron firing rate, such as haloperidol, morphine, or phencyclidine (Hutson *et al*, 2000; Lucas *et al*, 2000; Porras *et al*, 2002b), but not that induced by drugs eliciting an impulse-independent outflow of DA such as amphetamine (Porras *et al*, 2002b). Furthermore, it has been shown that the nonselective 5-HT$_{2C}$ agonist DOI does not affect cocaine-induced increase in accumbal DA outflow (Willins and Meltzer, 1998). Considering that, at variance with morphine or haloperidol, DA exocytosis induced by cocaine is not triggered by an increase in DA neuron firing (Pitts and Marwah, 1988; White, 1990; Benwell *et al*, 1993), it has been proposed that 5-HT$_{2C}$ receptors selectively modulate the

impulse-dependent release of DA in the NAc and the striatum (Lucas *et al*, 2000; Porras *et al*, 2002b), but only when DA release is associated with increased firing of DA neurons (Willins and Meltzer, 1998).

This picture however becomes less clear when looking at recent data reporting the effect of 5-HT$_{2C}$ agents on behavioral and neurochemical DA responses elicited by cocaine. Indeed, it has been shown that locomotor stimulant and reinforcing properties of cocaine, two behavioral responses classically related to increased mesolimbic DA transmission, are sensitive to both 5-HT$_{2C}$ agonists and antagonists (McCreary and Cunningham, 1999; Grottick *et al*, 2000; Fletcher *et al*, 2002). In addition, it has been reported that 5-HT$_{2C}$ receptor knockout mice exhibit an enhanced locomotor response to cocaine, an effect associated with an increase in DA outflow in the NAc, but not in the striatum (Rocha *et al*, 2002). In the absence of neurochemical studies assessing the effect of selective 5-HT$_{2C}$ ligands on cocaine-induced DA outflow, the data reported above challenge the hypothesis that 5-HT$_{2C}$ receptors regulate selectively DA exocytosis associated with increased DA neuron firing. Furthermore, in contrast with previous data (De Deurwaerdère and Spampinato, 2001; Porras *et al*, 2002b), the study by Rocha *et al* (2002) raises the possibility that 5-HT$_{2C}$ receptors may exert a selective control of mesoaccumbens DA pathway activity. This point deserves attention in light of the therapeutic potential of 5-HT$_{2C}$ agents in drug addiction (Grottick *et al*, 2000). Indeed, an independent modulation of the mesoaccumbens DA pathway would avoid the emergence of extrapyramidal side effects related to altered nigrostriatal DA transmission (De Deurwaerdère *et al*, 1998; Wood *et al*, 2001).

Thus, the present study was performed to examine the ability of systemically injected selective 5-HT$_{2C}$ receptor agonist and antagonists to modulate the increase in DA extracellular levels induced by cocaine, and to determine the extent to which this control operates selectively in the NAc with respect to the striatum. To have a further insight into the role of DA neuron firing activity, the influence of 5-HT$_{2C}$ agonism was also studied on the impulse-stimulated release of DA induced by haloperidol. Experiments were performed using *in vivo* microdialysis in halothane-anesthetized rats, an experimental procedure permitting simultaneous monitoring of DA outflow in the ipsilateral NAc and striatum (De Deurwaerdère *et al*, 1998).

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (IFFA CREDO, Lyon, France) weighing 330–380 g were used. Animals were kept at a constant room temperature (21 $\pm$ 2°C) and relative humidity (60%) with a 12 light/dark cycle (dark from 8 p.m.) and had free access to water and food. All animals use procedures conformed to International European Ethical Standards (86/609-EEC) and the French National Committee (*décret* 87/848) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Drugs

The following compounds were used: Ro 60-0175.HCl (*S*-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine hydrochloride) kindly donated by Dr P Weber (F Hoffmann-La Roche, Basel, Switzerland); SB 206553 (5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f] indole), and SB 242084 (6-chloro-5-methyl-1-[6-(2-methylpiridin-3-yloxy)pyridin-3-yl carbamoyl] indoline) generously provided by Dr M Wood (Psychiatry CEDD, GlaxoSmithKline, Harlow, UK); cocaine hydrochloride (Calaire Chimie, Calais, France); haloperidol (4-[4-(*p*-chlorophenyl)-4-hydroxypiperidino]-4′-fluorobutyrophenone) as the commercially available solution (Haldol 5 mg/ml, Janssen Pharmaceutica, Beerse, Belgium). All others chemicals and reagents were the purest commercially available (VWR, Strasbourg, France; Sigma, Illkirch, France).

Microdialysis

Surgery and perfusion procedures were performed as previously described (Porras *et al*, 2002b), with minor modifications. Briefly, rats were anesthetized with a mixture of halothane and nitrous oxide-oxygen (2%; 2 : 1 v/v). After tracheotomy for artificial ventilation, the animals were placed in a stereotaxic frame, and their rectal temperature was monitored and maintained at 37.3°C $\pm$ 0.1 with a heating pad. Two microdialysis probes, 2 and 4 mm long, (CMA/11, 240 μm outer diameter, Cuprophan; Carnegie Medicin, Phymep, Paris, France) were implanted simultaneously using a dual probe holder (Carnegie Medicin, Phymep) in the right NAc and striatum (coordinates from interaural point: anteroposterior [AP] = 11, lateral [L] = 1.3, ventral [V] = 2 and AP = 9.8, L = 3.3, V = 2.7, respectively) according to the atlas of Paxinos and Watson (1986). Probes were perfused at a constant flow rate of 2 μl/min by means of a microperfusion pump (CMA 111, Carnegie Medicin, Phymep) with artificial cerebrospinal fluid (aCSF) containing (in mM): 154.1 Cl^-, 147 Na^+, 2.7 K^+, 1 Mg^{2+}, and 1.2 Ca^{2+}, adjusted to pH 7.4 with 2 mM sodium phosphate buffer. Dialysates (30 μl) were collected on ice every 15 min. The *in vitro* recovery of the probes was about 10% for DA. At the end of each experiment, the brain was removed and fixed in NaCl (0.9%)/paraformaldehyde solution (10%). The location of the probes was determined histologically on serial coronal sections (60 μm) stained with cresyl violet, and only data obtained from rats with correctly implanted probes were included in the results.

Chromatographic Analysis

Dialysate samples were immediately analyzed by reverse-phase high-performance liquid chromatography (HPLC) coupled with electrochemical detection, as previously described (Bonhomme *et al*, 1995). The mobile phase (containing (in mM) 70 NaH_2PO_4, 0.1 Na_2EDTA, 0.7 triethylamine, and 0.1 octylsulfonic acid plus 10% methanol, adjusted to pH 4.8 with orthophosphoric acid) was delivered at 1 ml/min flow rate (system LC-10AD-*VP*, Shimadzu, France) through a Hypersyl column (C18; 4.6 $\times$ 150 mm, particle size 5 μm; Touzard & Matignon, Paris, France). Detection of DA was carried out with a

coulometric detector (Coulochem II, ESA, Paris, France) coupled to a dual-electrode analytical cell (model 5014, ESA). The potential of the electrodes was set at −175 and +175 mV. Output signals were recorded on a computer (system class VP-4, Shimadzu, France). Under these conditions, the sensitivity for DA was 0.5 ρg/30 μl with a signal/noise ratio of 3 : 1.

Pharmacological Treatments

Pharmacological treatments were performed after the stabilization of DA levels in the perfusate. A stable baseline, defined as three consecutive samples in which DA contents varied by less than 10% in both structures, was generally obtained 135 min after the beginning of the perfusion (stabilization period).

Cocaine, diluted in NaCl 0.9%, was administered intraperitoneally at 10, 15, or 30 mg/kg in a volume of 1 ml/kg. Each 5-HT$_{2C}$ compound was injected intraperitoneally in a volume of 2 ml/kg, 15 min before cocaine. The 5-HT$_{2C/2B}$ receptor agonist Ro 60-0175 was dissolved in a physiological saline (NaCl 0.9%) and administered at 1 mg/kg. The selective 5-HT$_{2C}$ receptor antagonist SB 242084, dissolved in a mixture of physiological saline (NaCl 0.9%) containing hydroxypropyl-β-cyclodextrin (8% by weight) plus citric acid (25 mM), was administered at 1 mg/kg. The 5-HT$_{2C/2B}$ receptor antagonist SB 206553, diluted in a 99 : 1 v/v mixture of apyrogenic water and lactic acid, was administered at 5 mg/kg. In another set of experiments, Ro 60-0175 was injected 15 min before the DA receptor antagonist haloperidol, administered subcutaneously at 0.01 and 0.1 mg/kg in a volume of 1 ml/kg.

The doses of the different compounds used were chosen on the basis of previous studies to keep both selectivity and efficiency toward the targeted sites (Kennett *et al*, 1996, 1997; Gobert *et al*, 2000; Grottick *et al*, 2000; Lucas *et al*, 2000; Porras *et al*, 2002a, b). All drug doses were calculated as the free base. In each experimental group, animals received either drugs or their appropriate vehicle.

Statistical Analysis

DA contents in each sample were expressed as the percentage of the average baseline level calculated from the three fractions preceding any treatment. Data correspond to the mean ± SEM values of the percentage obtained in each experimental group. Drug overall effect was calculated as the average of DA content from dialysates collected after drug treatment administration (time 15–180 and 15–120 for cocaine and haloperidol experiments, respectively).

A one-way ANOVA (using group as the main factor) was used to determine for each experiment statistical differences between groups with time as repeated measures for the samples corresponding to the treatment effect. When significant ($p<0.05$), the ANOVA was followed by the Fisher's protected least-significance difference test (PLSD) to allow adequate multiple comparisons between groups. For each experiment, statistical differences in baseline DA levels among groups were assessed by a one-way ANOVA (using group as the main factor).

RESULTS

Basal Extracellular DA Concentrations in Dialysates from the Nac and the Striatum

All measurements were performed 150 min after the beginning of perfusion, by which time a steady state was achieved. Absolute basal levels of DA in dialysate simultaneously collected from the NAc and the striatum did not differ across the different experimental groups (see legend to figures for statistics) throughout the course of the study and were (mean ± SEM, without adjusting for probe recovery) 7.1 ± 1.1 pg/30 μl and 17.2 ± 1.6 pg/30 μl for the NAc and the striatum, respectively ($n=129$ animals).

Dose–Response Effect of Cocaine on DA Extracellular Levels

The effect of the intraperitoneal administration of 10, 15, and 30 mg/kg of cocaine on DA extracellular levels in the NAc and the striatum is shown in Figure 1. Cocaine induced a dose-dependent increase in DA outflow in both the NAc (one-way ANOVA $F_{3,22}=71$, $p<0.001$) and the striatum (one-way ANOVA $F_{3,22}=46$, $p<0.001$). The overall effect elicited by 10, 15, and 30 mg/kg of cocaine reached respectively, 189 ± 19%, 448 ± 46%, and 1290 ± 135% of baseline in the NAc and 160 ± 7%, 304 ± 36%, and 793 ± 93% of baseline in the striatum. In the context of our statistical analysis, only the effect elicited by the higher doses of cocaine reached statistical significance ($p<0.001$, Fisher's PLSD test for 15 and 30 mg/kg in both brain regions).

Effect of SB 206553 and SB 242084 on Cocaine-Induced Increase in DA Extracellular Levels

Figure 2 illustrates the effect of the 5-HT$_{2C/2B}$ antagonist SB 206553 (5 mg/kg i.p.; upper panels) and the selective 5-HT$_{2C}$ antagonist SB 242084 (1 mg/kg i.p.; lower panels) on the increase in DA extracellular levels induced by 15 mg/kg of cocaine in the NAc and the striatum. The increase in accumbal and striatal DA outflow induced by cocaine ($p<0.001$, Fisher's PLSD test after a one-way ANOVA;

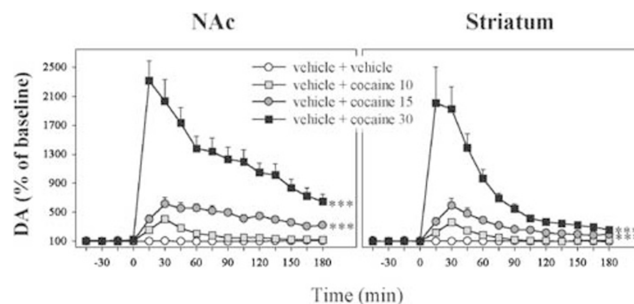

Figure 1 Time-course effect of cocaine (10, 15, and 30 mg/kg i.p.) on DA extracellular levels in the NAc and the striatum. Cocaine was injected at time zero. Data, obtained from four to eight animals per group, are presented as the mean ± SEM percentages of the baseline calculated from three samples preceding the injection of cocaine. Absolute basal levels of DA did not differ among groups in the NAc (one-way ANOVA $F_{3,22}=1.7$, $p>0.05$, NS) and the striatum (one-way ANOVA $F_{3,22}=1.2$, $p>0.05$, NS). ***$p<0.001$ *vs* the vehicle + vehicle group (Fisher's PLSD test).

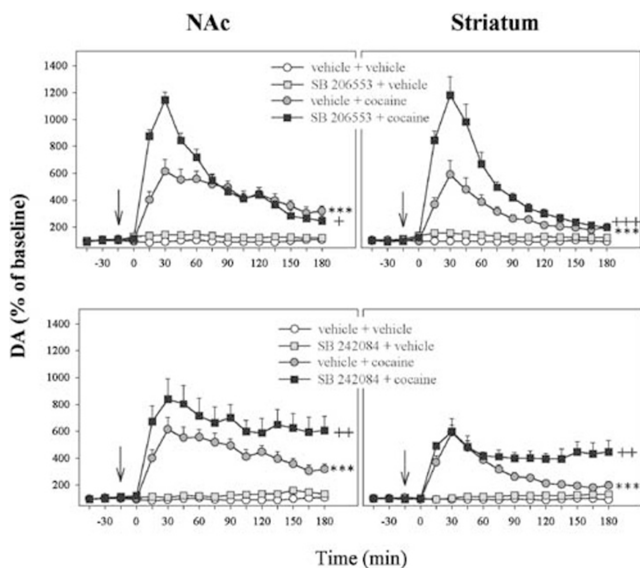

Figure 2 Time-course effect of the 5-HT$_{2C/2B}$ antagonist SB 206553 (5 mg/kg i.p.; upper panels) and the selective 5-HT$_{2C}$ antagonist SB 242084 (1 mg/kg i.p.; lower panels) on basal and cocaine-induced increase in DA extracellular levels in the NAc and the striatum. The 5-HT$_{2C}$ compounds were injected (vertical arrows) 15 min before the intraperitoneal administration of 15 mg/kg cocaine (time zero). Data, obtained from six to eight animals per group, are presented as the mean $\pm$ SEM percentages of the baseline calculated from the three samples preceding the first drug administration. Absolute basal levels of DA did not differ among groups in the NAc (one-way ANOVA $F_{3,23} = 1.2$, $p > 0.05$, NS; $F_{3,23} = 2.4$, $p > 0.05$, NS, for SB206553/cocaine and SB242084/cocaine experiments, respectively) and the striatum (one-way ANOVA $F_{3,23} = 0.5$, $p > 0.05$, NS; $F_{3,23} = 1.6$, $p > 0.05$, NS for SB206553/cocaine and SB242084/cocaine experiments, respectively). $^{***}p < 0.001$ *vs* the vehicle + vehicle group; $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ *vs* the vehicle + cocaine group (Fisher's PLSD test).

$F_{3,23} = 37.5$, $p < 0.001$ for the NAc; and $F_{3,23} = 30.9$, $p < 0.001$ for the striatum) was significantly potentiated by SB 206553 ($p < 0.05$ and < 0.001 *vs* cocaine-treated rats, Fisher's PLSD test, for the NAc and the striatum respectively), and reached its maximum 30 min after cocaine administration (Figure 2, upper panels).

Similarly, the increase in DA outflow induced by 10 mg/kg of cocaine ($p < 0.001$, Fisher's PLSD test after a one-way ANOVA; $F_{3,16} = 142$, $p < 0.001$ for the NAc; and $F_{3,16} = 197$, $p < 0.001$ for the striatum) was significantly potentiated by SB 206553 ($p < 0.001$ *vs* cocaine-treated rats, Fisher's PLSD test, for both the NAc and the striatum). Indeed, in the presence of SB 206553, DA extracellular levels stimulated by cocaine reached an overall increase of 364 $\pm$ 38% and of 276 $\pm$ 17% in the NAc and the striatum respectively. In both regions, the effect peaked 30 min after cocaine administration (937 $\pm$ 240 and 954 $\pm$ 174% of basal values for the NAc and the striatum respectively), and thereafter decreased progressively to about 286% (NAc) and 200% (striatum) of baseline at the end of the experiment (data not shown).

According to previous reports (Di Giovanni *et al*, 1999; Gobert *et al*, 2000), SB 206553 elicited a rapid increase in DA efflux *per se* in both brain regions, reaching approximately 45% above basal values 30–45 min after its injection. However, this effect, because of its small magnitude, did not reach statistical significance in the context of our statistical analysis (Fisher's PLSD test).

As shown in the lower panels of Figure 2, the enhancement of DA extracellular levels induced by 15 mg/kg of cocaine ($p < 0.001$, Fisher's PLSD test after one-way ANOVA; $F_{3,23} = 23.1$, $p < 0.001$ for the NAc; and $F_{3,23} = 22.9$, $p < 0.001$ for the striatum) was significantly increased by SB 242084 pretreatment ($p < 0.01$ *vs* cocaine-treated rats, Fisher's PLSD test, in both the NAc and the striatum). The time-course effect of this interaction was slightly different in both brain regions, the potentiation occurring later in the striatum.

Finally, in both the NAc and the striatum, SB 242084 elicited a progressive and long-lasting increase in DA efflux *per se* that reached almost 30% above control values at the end of the experimental period. However, as in the case of SB 206553, this effect did not reach statistical significance in the context of our statistical analysis (Fisher's PLSD test).

Effect of Ro 60-0175 on Cocaine-Induced Increase in DA Extracellular Levels

Figure 3 reports the effect of the 5-HT$_{2C/2B}$ agonist Ro 60-0175 (1 mg/kg i.p.) on the enhancement of DA extracellular levels induced by 15 mg/kg of cocaine in the NAc and the striatum. Cocaine-stimulated DA outflow ($p < 0.001$, Fisher's PLSD test after one-way ANOVA; $F_{3,25} = 17.2$, $p < 0.001$ for the NAc; and $F_{3,25} = 12.7$, $p < 0.001$ for the striatum) was not affected by Ro 60-0175 in both brain regions (NS, Fisher's PLSD test). Moreover, Ro 60-0175 by itself did not modify basal DA efflux in both the NAc and the striatum (NS, Fisher's PLSD test).

Effect of Ro 60-0175 on Haloperidol-Induced Increase in DA Extracellular Levels

Figure 4 reports the effect of the 5-HT$_{2C/2B}$ agonist Ro 60-0175 (1 mg/kg i.p.) on the increase in DA outflow elicited by the subcutaneous administration of 0.1 mg/kg haloperidol. In the NAc, DA extracellular levels enhanced by haloperidol reached a plateau (199 $\pm$ 14% of basal values) 75 min after its injection ($p < 0.001$, Fisher's PLSD test after a one-way

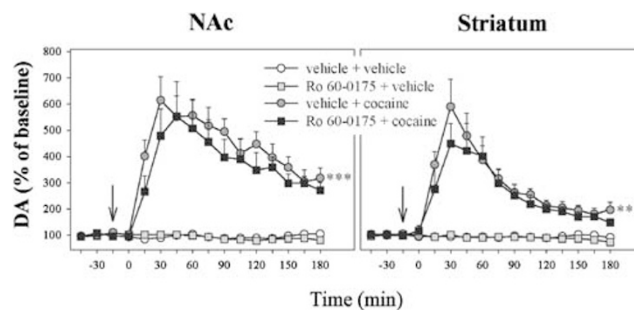

Figure 3 Time-course effect of the 5-HT$_{2C/2B}$ agonist Ro 60-0175 (1 mg/kg i.p.) on basal and cocaine-induced increase in DA extracellular levels in the NAc and the striatum. Ro 60-0175 was injected (vertical arrow) 15 min before the intraperitoneal administration of 15 mg/kg cocaine (time zero). Data, obtained from six to eight animals per group, are presented as the mean $\pm$ SEM percentages of the baseline calculated from the three samples preceding the first drug administration. Absolute basal levels of DA did not differ among groups in the NAc (one-way ANOVA $F_{3,25} = 2.8$, $p > 0.05$, NS) and the striatum (one-way ANOVA $F_{3,25} = 0.7$, $p > 0.05$, NS). $^{***}p < 0.001$ *vs* the vehicle + vehicle group (Fisher's PLSD test).

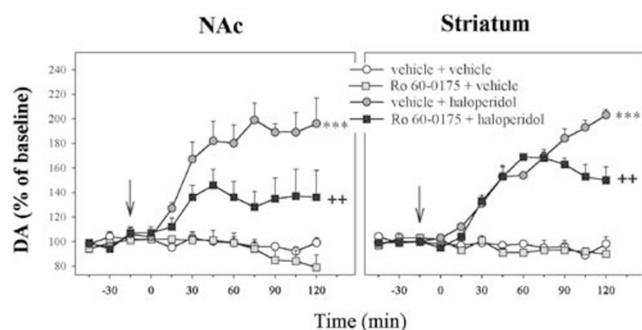

Figure 4 Time-course effect of the 5-HT$_{2C/2B}$ agonist Ro 60-0175 (1 mg/kg i.p.) on basal and haloperidol-induced increase in DA extracellular levels in the NAc and the striatum. Ro 60-0175 was injected (vertical arrow) 15 min before the intraperitoneal administration of 0.1 mg/kg haloperidol (time zero). Data, obtained from five to seven animals per group, are presented as the mean ± SEM percentages of the baseline calculated from the three samples preceding the first drug administration. Absolute basal levels of DA did not differ among groups in the NAc (one-way ANOVA $F_{3,16} = 2.3$, $p > 0.05$, NS) and the striatum (one-way ANOVA $F_{3,16} = 0.6$, $p > 0.05$, NS). ***$p < 0.001$ *vs* the vehicle + vehicle group; $^{++}p < 0.01$ *vs* the vehicle + haloperidol group (Fisher's PLSD test).

ANOVA, $F_{3,16} = 27.1$, $p < 0.001$) whereas, in the striatum, they increased progressively to reach 203 ± 5% of basal values at the end of the experimental period ($p < 0.001$, Fisher's PLSD test after a one-way ANOVA, $F_{3,16} = 227$, $p < 0.001$). The administration of Ro 60-0175 reduced the enhancement of DA outflow induced by haloperidol in the NAc and the striatum ($p < 0.01$ *vs* haloperidol-treated rats, Fisher's PLSD test). At variance with the NAc, the inhibitory effect of Ro 60-0175 in the striatum appeared during the last 45 min of the experimental period.

In another set of experiments, we have studied the effect of the same regimen of Ro 60-0175 on DA outflow stimulated by the subcutaneous administration of 0.01 mg/kg haloperidol. DA dialysate content reached its maximum 30 and 60 min after haloperidol injection in the NAc (135 ± 15%, $p < 0.001$, Fisher's PLSD test after a one-way ANOVA, $F_{3,19} = 11.6$, $p < 0.001$) and the striatum (160 ± 5%, $p < 0.001$, Fisher's PLSD test after a one-way ANOVA, $F_{3,19} = 16.4$, $p < 0.001$), respectively (data not shown). Ro 60-0175 did not affect haloperidol-stimulated DA outflow in both brain regions (NS, Fisher's PLSD). Finally, in each experiment, Ro 60-0175 failed to affect basal DA outflow *per se* in the NAc and the striatum (NS, Fisher's PLSD test).

DISCUSSION

In this study, we provide evidence that 5-HT$_{2C}$ antagonists potentiate the enhancement of DA outflow induced by cocaine in the rat NAc and striatum, and that 5-HT$_{2C}$ agonist, while ineffective on cocaine-induced DA outflow, reduces haloperidol-stimulated DA release in both brain regions. When considering the cellular mechanisms underlying the increase in DA outflow induced by cocaine and haloperidol, these results show that 5-HT$_{2C}$ receptors are able to modulate DA exocytosis also when it is not triggered by an increase in DA neuron impulse activity. Furthermore, they indicate that 5-HT$_{2C}$ agonists, at variance with 5-HT$_{2C}$ antagonists, exert a preferential inhibitory control on impulse-stimulated DA release.

As previously reported (Di Chiara and Imperato, 1988; Porras *et al*, 2002a), cocaine elicits a significant and dose-dependent increase in DA extracellular levels in both the NAc and the striatum. The systemic administration of the 5-HT$_{2B/2C}$ antagonist SB 206553 potentiates the increase in DA outflow induced by cocaine (10 and 15 mg/kg) in both brain regions. As discussed elsewhere (Di Giovanni *et al*, 1999; Porras *et al*, 2002b), it is unlikely that the 5-HT$_{2B}$-antagonist component of SB 206553 participates in the observed effects. Indeed, we have shown that the effect of cocaine on accumbal and striatal DA outflow is also potentiated by the selective 5-HT$_{2C}$ antagonist, SB 242084. However, the time course of the effects elicited by SB 206553 and SB 242084 on both basal and cocaine-stimulated DA outflow is different, the effect of SB 242084 being delayed. Distinct responses to these 5-HT$_{2C}$ antagonists have been already reported (De Deurwaerdère and Spampinato, 2001), and attributed to both pharmacokinetic and pharmacodynamic interactions (Gobert *et al*, 2000). Thus, our findings, in line with a recent study reporting a facilitation of cocaine-induced DA outflow in the NAc of mice lacking 5-HT$_{2C}$ receptors (Rocha *et al*, 2002), indicate that endogenous 5-HT inhibits cocaine-stimulated DA outflow via 5-HT$_{2C}$ receptor stimulation.

The finding that 5-HT$_{2C}$ antagonists modulate cocaine-induced increase in DA outflow dampens the proposal that 5-HT$_{2C}$ receptors affect selectively DA exocytosis originating from an increase in the impulse flow of DA neurons (Willins and Meltzer, 1998; Porras *et al*, 2002b). Indeed, the elevation of DA extracellular levels induced by cocaine is not triggered by an increase in DA neuron firing: it is consequent to DA transporter blockade, and is associated with a decrease in DA neuron impulse activity induced by the stimulatory action of DA itself on somatodendritic D_2 autoreceptors (Pitts and Marwah, 1988; White, 1990; Benwell *et al*, 1993). Considering that 5-HT$_{2C}$ receptor blockade is known to disinhibit DA neuron firing (Di Giovanni *et al*, 1999; Gobert *et al*, 2000), it is tempting to suggest that the enhancement of cocaine-stimulated DA outflow elicited by 5-HT$_{2C}$ antagonists could result from their opposite action on DA neuron firing (ie blockade of 5-HT$_{2C}$ receptors could reverse the inhibitory effect of cocaine on DA neuron firing). Favoring this hypothesis, previous studies have shown that the increase in DA outflow induced by DA reuptake blockers other than cocaine is sensitive to increased DA neuron impulse activity (Westerink *et al*, 1989). Also, blockade of 5-HT$_2$ receptors has been shown to counteract the ability of 5-HT to enhance the autoinhibitory control exerted by DA on D_2 receptors (Brodie and Bunney, 1996). Thus, our results, together with the above considerations, indicate that 5-HT$_{2C}$ receptor antagonists may modulate DA exocytosis also when it is not associated with an increase in DA neuron firing. In addition to its ability to block DA transporter, cocaine is also a potent inhibitor of the 5-HT transporter, thereby increasing 5-HT extracellular levels and reducing 5-HT neuron firing (Cunningham and Lakoski, 1990; Teneud *et al*, 1996). It is possible that the raise in 5-HT extracellular concentration strengthens the inhibitory tone of 5-HT$_{2C}$ receptors on DA neuron activity. Alternatively, blockade of the tonic inhibitory control of

5-HT$_{2C}$ receptors may unmask excitatory and phasic influences on DA outflow exerted by endogenous 5-HT via other 5-HT receptors, as already shown in case of concomitant increase in DA and 5-HT transmission (Kankaanpää *et al*, 2002; Bubar *et al*, 2003; Porras *et al*, 2003). Additional experiments are warranted to evaluate this possibility.

At variance with 5-HT$_{2C}$ antagonists, stimulation of 5-HT$_{2C}$ receptors by Ro 60-0175 affects neither basal nor cocaine-stimulated DA outflow, whatever the brain area considered. A similar result has been reported in a previous study showing that the nonselective 5-HT$_2$ agonist DOI fails to alter the effect of 10 mg/kg cocaine in the NAc of freely moving rats (Willins and Meltzer, 1998). It is unlikely that the lack of effectiveness of Ro 60-0175 is due to inappropriate dose and route administration, or to the 5-HT$_{2C}$ receptor occupancy by cocaine-induced endogenous 5-HT outflow. Indeed, behavioral data have shown that a similar regimen dramatically reduced, via a 5-HT$_{2C}$ receptor-dependent mechanism, locomotor hyperactivity and self-administration induced by nicotine, ethanol, and cocaine (Grottick *et al*, 2000, 2001; Tomkins *et al*, 2002). More likely, the failure of Ro 60-0175 to affect cocaine-induced DA outflow is related to the cellular mechanisms underlying their interaction on DA neurons. To further address this point, we have studied the effect of Ro 60-0175 on DA outflow induced by haloperidol, a drug that, at variance with cocaine, increases DA release as a consequence of increased DA neuron firing induced by DA-D$_2$ autoreceptor blockade (Mereu *et al*, 1984; Di Chiara and Imperato, 1988). Interestingly, we found that, in both the NAc and the striatum, 1 mg/kg Ro 60-0175 has no influence on basal and 0.01 mg/kg haloperidol-stimulated DA release, but inhibits the increase in DA release induced by 0.1 mg/kg haloperidol. These findings are in line with recent studies in the rat NAc and prefrontal cortex showing that 5-HT$_{2C}$ agonists exert a preferential effect on impulse-stimulated release of DA (Willins and Meltzer, 1998; Pozzi *et al*, 2002), probably consequent to their property to inhibit DA neuron firing (Gobert *et al*, 2000). In addition, the fact that the effect of 0.01 mg/kg haloperidol is insensitive to Ro 60-0175 suggests that, as already shown for 5-HT$_{2C}$ antagonism (Lucas *et al*, 2000), the degree of DA neuron activation is a critical factor for the expression of this interaction. Indeed, we have already shown that the facilitatory effect of SB 206553 on haloperidol-stimulated striatal DA release is no longer observed when haloperidol is administered at doses inducing a maximal increase in DA neuron firing rate (Lucas *et al*, 2000). By the above-discussed data, it is conceivable that the inhibitory effect of cocaine on DA neuron firing precludes the action of 5-HT$_{2C}$ agonists on cocaine-induced DA outflow. Altogether, these data suggest that DA neuron impulse activity is the main cellular mechanism targeted by 5-HT$_{2C}$ agents to control DA exocytosis (Willins and Meltzer, 1998; Porras *et al*, 2002b). Electrophysiological studies are warranted to confirm this hypothesis.

The simultaneous monitoring of accumbal and striatal DA outflow allow us to show that, as already reported for other drugs increasing DA release (Porras *et al*, 2002b), 5-HT$_{2C}$ agents exert similar influence on basal and cocaine-stimulated DA outflow (10 and 15 mg/kg) in both the NAc and the striatum. However, a recent study in 5-HT$_{2C}$ receptor knockout mice has reported that cocaine-stimulated DA outflow is selectively potentiated in the NAc with respect to the striatum (Rocha *et al*, 2002). It is possible that, given the different experimental context (5-HT$_{2C}$ receptor gene inactivation *vs* acute antagonist administration), adaptative responses subsequent to chronic deletion of 5-HT$_{2C}$ receptors in mice may have altered differentially the responsiveness of nigrostriatal and mesoaccumbal DA neurons to cocaine. For instance, previous studies in rats have shown that chronic, but not acute, pharmacological blockade of 5-HT$_{2C}$ receptors leads to a selective inhibition of the mesoaccumbal DA neuron firing with respect to the nigrostriatal one (Blackburn *et al*, 2002).

Finally, the obtained results raise the issue of the role of DA neurons in the effects of 5-HT$_{2C}$ ligands on DA-dependent behavioral responses induced by cocaine. Indeed, while 1 mg/kg Ro 60-0175 does not modulate cocaine-stimulated accumbal DA outflow in both anesthetized (this study) and freely moving rats (unpublished observation), it potently reduces the hyperlocomotive, discriminative stimulus, and reinforcing properties of cocaine (Grottick *et al*, 2000). Furthermore, although 5-HT$_{2C}$ antagonists increase both behavioral (McCreary and Cunningham, 1999; Fletcher *et al*, 2002) and neurochemical (present study) effects of cocaine, in some cases their potentiative effects do not follow the same time course. For instance, the facilitatory effect of SB 242084 on locomotor hyperactivity (Fletcher *et al*, 2002) occurs earlier than that observed on DA outflow (present study). Similarly, Hutson *et al* (2000) have reported different time-course effects of SB 242084 on phencyclidine-induced accumbal DA release and locomotor activity. Furthermore, it has been shown that SB 206553, without effect on amphetamine-stimulated DA outflow (Porras *et al*, 2002b), potentiates amphetamine-induced locomotor hyperactivity (Bankson and Cunningham, 2002). These data together point out that, as already shown for 5-HT$_{1A}$ receptors (Müller *et al*, 2002), 5-HT$_{2C}$ receptors may inhibit cocaine-induced DA-dependent behaviors independently from an action on DA outflow itself, thereby controlling DA transmission by acting downstream from DA neurons. This conclusion is supported by anatomical findings showing that 5-HT$_{2C}$ receptors are mainly expressed by non-DA neurons in various brain structures innervated by DA neurons (Eberle-Wang *et al*, 1997). Consistent with previous studies reporting that 5-HT$_{2C}$ receptors modulate cellular activity in various brain areas involved in the control of DA-dependent behaviors (Fox *et al*, 1998; De Deurwaerdère and Chesselet, 2000; Di Giovanni *et al*, 2001; Filip and Cunningham, 2002), it has been recently shown that stimulation of frontocortical 5-HT$_{2C}$ receptors reduces cocaine-induced locomotor hyperactivity (Filip and Cunningham, 2003). Moreover, from a molecular point of view, recent data have reported that 5-HT$_2$ receptors regulate the phosphorylation of the DA and cyclic 3′–5′ adenosine monophosphate-regulated phosphoprotein (DARPP-32), a protein located in dopaminoceptive neurons and involved in the mediation of the reinforcing effects of cocaine by processes acting independently from changes of DA outflow (Svenningsson *et al*, 2002; Zachariou *et al*, 2002).

In conclusion, the present study provides evidence that 5-HT$_{2C}$ receptors are able to modulate DA exocytosis in both

the NAc and the striatum, also when it occurs independently from an increase in DA neuron impulse activity. Furthermore, the obtained results, in agreement with previous findings (Pozzi *et al*, 2002), indicate that the phasic inhibitory control exerted by 5-HT_{2C} agonists occurs preferentially on impulse-stimulated release of DA. When considering the ability of 5-HT_{2C} agonists to inhibit DA-dependent behaviors induced by cocaine (Grottick *et al*, 2000), our findings also indicate that 5-HT_{2C} receptor stimulation can modulate altered DA transmission independently from an action on DA neuron activity itself. These findings add further insight into the mechanisms underlying the 5-HT_{2C} receptors/DA neuron interaction and its involvement in the DA effects of cocaine, thus providing a neurochemical basis for a better understanding of the therapeutic potential of 5-HT_{2C} agonists for treating cocaine abuse and dependence (Grottick *et al*, 2000; Rocha *et al*, 2002).

ACKNOWLEDGEMENTS

This work was supported by grants from Centre National de la Recherche Scientifique and Bordeaux 2 University. S Navailles was a fellowship recipient from the Ministère de la Recherche et de l'Enseignement Supérieur during the course of this study. We are grateful to Dr M Wood (Psychiatry CEDD, GlaxoSmithKline, Harlow, UK) for the generous gift of SB 206553 and SB 242084 and Dr P Weber (F Hoffmann-La Roche, Basel, Switzerland) for the gift of Ro 60-0175. We thank D Moison for the technical assistance.

REFERENCES

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.

Benwell ME, Balfour DJ, Lucchi HM (1993). Influence of tetrodotoxin and calcium on changes in extracellular dopamine levels evoked by systemic nicotine. *Psychopharmacology* **112**: 467–474.

Blackburn TP, Minabe Y, Middlemiss DN, Shirayama Y, Hashimoto K, Ashby Jr CR (2002). Effect of acute and chronic administration of the selective 5-HT2C receptor antagonist SB-243213 on midbrain dopamine neurons in the rat: an *in vivo* extracellular single cell study. *Synapse* **46**: 129–139.

Bonhomme N, De Deurwaerdère P, Le Moal M, Spampinato U (1995). Evidence for 5-HT4 receptor subtype involvement in the enhancement of striatal dopamine release induced by serotonin: a microdialysis study in the halothane-anesthetized rat. *Neuropharmacology* **34**: 269–279.

Brodie MS, Bunney EB (1996). Serotonin potentiates dopamine inhibition of ventral tegmental area neurons *in vitro*. *J Neurophysiol* **76**: 2077–2082.

Bubar MJ, McMahon LR, De Deurwaerdère P, Spampinato U, Cunningham KA (2003). Selective serotonin reuptake inhibitors enhance cocaine-induced locomotor activity and dopamine release in the nucleus accumbens. *Neuropharmacology* **44**: 342–353.

Cunningham KA, Lakoski JM (1990). The interaction of cocaine with serotonin dorsal raphe neurons. Single-unit extracellular recording studies. *Neuropsychopharmacology* **3**: 41–50.

De Deurwaerdère P, Chesselet MF (2000). Nigrostriatal lesions alter oral dyskinesia and c-*fos* expression induced by the serotonin agonist m-CPP in adult rats. *J Neurosci* **20**: 5170–5178.

De Deurwaerdère P, Spampinato U (2001). The nigrostriatal dopamine system: a neglected target for 5-HT2C receptors. *Trends Pharmacol Sci* **22**: 502–504.

De Deurwaerdère P, Stinus L, Spampinato U (1998). Opposite change of *in vivo* dopamine release in the rat nucleus accumbens and striatum that follows electrical stimulation of dorsal raphe nucleus: role of 5-HT3 receptors. *J Neurosci* **18**: 6528–6538.

Di Chiara G, Imperato A (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci* **85**: 5274–5278.

Di Giovanni G, De Deurwaerdère 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 Giovanni G, Di Matteo V, La Grutta V, Esposito E (2001). m-Chlorophenylpiperazine excites non-dopaminergic neurons in the rat substantia nigra and ventral tegmental area by activating serotonin-2C receptors. *Neuroscience* **103**: 111–116.

Eberle-Wang K, Mikeladze Z, Uryu K, Chesselet MF (1997). Pattern of expression of the serotonin2C receptor messenger RNA in the basal ganglia of adult rats. *J Comp Neurol* **384**: 233–247.

Filip MM, Cunningham KA (2002). Serotonin 5-HT(2C) receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. *Pharmacol Biochem Behav* **71**: 745–756.

Filip MM, Cunningham KA (2003). Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin 5-HT2C receptors in rat prefrontal cortex. *J Pharmacol Exp Ther* **306**: 734–743.

Fletcher PJ, Grottick AJ, Higgins GA (2002). 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.

Fox SH, Moser B, Brotchie JM (1998). Behavioral effects of 5-HT_{2C} receptor antagonism in the substantia nigra pars reticulata of the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Exp Neurol* **151**: 35–49.

Gobert A, Rivet JM, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas JP *et al* (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.

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

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.

Hutson PH, Barton CL, Jay M, Blurton P, Burkamp F, Clarkson R *et al* (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.

Jones BJ, Blackburn TP (2002). The medical benefit of 5-HT research. *Pharmacol Biochem Behav Apr* **71**: 555–568.

Kankaanpää A, Meririnne E, Seppala T (2002). 5-HT3 receptor antagonist MDL 72222 attenuates cocaine- and mazindol-, but not methylphenidate-induced neurochemical and behavioral effects in the rat. *Psychopharmacology* **159**: 341–350.

Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T *et al* (1996). *In vitro* and *in vivo* profile of SB 206553, a potent

5-HT2C/5-HT2B receptor antagonist with anxiolytic-like properties. *Br J Pharmacol* **117**: 427–434.

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

Lucas G, De Deurwaerdère P, Caccia S, Spampinato U (2000). The effect of serotonergic agents on haloperidol-induced striatal dopamine release *in vivo*: opposite role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes and significance of the haloperidol dose used. *Neuropharmacology* **39**: 1053–1063.

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

Mereu G, Fanni B, Gessa GL (1984). General anesthetics prevent dopaminergic neuron stimulation by neuroleptics. In: Usdin E, Carlsson A, Dahlstrom A, Engel J (eds). *Catecholamines: Neuropharmacology and Central Nervous System—Theoretical Aspects*. Alan R Liss Inc.: New York. pp. 353–358.

Müller CP, De Souza Silva MA, DePalma G, Tomaz C, Carey RJ, Huston JP (2002). The selective serotonin(1A)-receptor antagonist WAY 100635 blocks behavioral stimulating effects of cocaine but not ventral striatal dopamine increase. *Behav Brain Res* **134**: 337–346.

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

Pazos A, Cortés R, Palacios JM (1985). Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. *Brain Res* **346**: 231–249.

Pitts DK, Marwah J (1988). Cocaine and central monoaminergic neurotransmission: a review of electrophysiological studies and comparison to amphetamine and antidepressants. *Life Sci* **42**: 949–968.

Porras G, De Deurwaerdère P, Moison D, Spampinato U (2003). Conditional involvement of striatal serotonin3 receptors in the control of *in vivo* dopamine outflow in the rat striatum. *Eur J Neurosci* **17**: 771–781.

Porras G, Di Matteo V, De Deurwaerdère P, Esposito E, Spampinato U (2002a). Central serotonin4 receptors selectively regulate the impulse-dependent exocytosis of dopamine in the rat striatum: *in vivo* studies with morphine, amphetamine and cocaine. *Neuropharmacology* **43**: 1099–1109.

Porras G, Di Matteo V, Fracasso C, Lucas G, De Deurwaerdère P, Caccia S *et al* (2002b). 5-HT_{2A} and 5-$HT_{2C/2B}$ receptor subtypes modulate dopamine release induced *in vivo* by amphetamine and morphine in both the rat nucleus accumbens and striatum. *Neuropsychopharmacology* **26**: 311–324.

Pozzi L, Acconcia S, Ceglia I, Invernizzi RW, Samanin R (2002). Stimulation of 5-hydroxytryptamine (5-HT(2C)) receptors in the ventrotegmental area inhibits stress-induced but not basal dopamine release in the rat prefrontal cortex. *J Neurochem* **82**: 93–100.

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

Svenningsson P, Tzavara ET, Liu F, Fienberg AA, Nomikos GG, Greengard P (2002). DARPP-32 mediates serotonergic neurotransmission in the forebrain. *Proc Natl Acad Sci USA* **99**: 3188–3193.

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.

Tomkins DM, Joharchi N, Tampakeras M, Martin JR, Wichmann J, Higgins GA (2002). An investigation of the role of 5-HT(2C) receptors in modifying ethanol self-administration behaviour. *Pharmacol Biochem Behav* **71**: 735–744.

Westerink BHC, Hofsteede RM, Tuntler J, De Vries JB (1989). Use of calcium antagonism for the characterization of drug-evoked dopamine release from the brain of conscious rats determined by microdialysis. *J Neurochem* **52**: 722–729.

White FJ (1990). Electrophysiological basis of the reinforcing effects of cocaine. *Behav Pharmacol* **1**: 303–315.

Willins DL, Meltzer HY (1998). Serotonin 5-HT_{2C} agonists selectively inhibit morphine-induced dopamine efflux in the nucleus accumbens. *Brain Res* **781**: 291–299.

Wood MD, Heidbreder C, Reavill C, Ashby Jr CR, Middlemiss DN (2001). 5-HT2C receptor antagonists: potential in schizophrenia. *Drug Dev Res* **54**: 88–94.

Zachariou V, Benoit-Marand M, Allen PB, Ingrassia P, Fienberg AA, Gonon F *et al* (2002). Reduction of cocaine place preference in mice lacking the protein phosphatase 1 inhibitors DARPP 32 or Inhibitor 1. *Biol Psychiatry* **51**: 612–620.