

Contribution of serotonin (5-HT) 5-HT₂ receptor subtypes to the hyperlocomotor effects of cocaine: Acute and chronic pharmacological analyses

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Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; 5-HT₂R, serotonin₂ receptor; DA, dopamine; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; GABA, γ -aminobutyric acid; (+)-MDMA, (+)-3,4-methylenedioxymethamphetamine; NAc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area

ABSTRACT

The role of serotonin (5-hydroxytryptamine; 5-HT) 5-HT₂ receptor subtypes (5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R) in acute cocaine-evoked hyperactivity was compared to their contribution to the development and expression of locomotor sensitization upon repeated, intermittent treatment with cocaine (10 mg/kg/day for 5 days) in male Wistar rats. Cocaine-evoked hyperactivity was significantly enhanced by pretreatment with the preferential 5-HT_{2A}R agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and the 5-HT_{2C}R antagonist SDZ SER-082. The 5-HT_{2A}R antagonist SR 46349B and the preferential 5-HT_{2C}R agonist MK 212 (2 mg/kg) significantly attenuated acute cocaine-evoked hyperactivity; however, a lower dose of MK 212 (0.3 mg/kg) enhanced cocaine-evoked hyperactivity. The 5-HT_{2B}R agonist BW 723C86 and the 5-HT_{2B}R antagonist SB 204741 had no effect on cocaine-evoked hyperactivity. Repeated treatment with cocaine alone resulted in a two-fold increase in hyperactivity upon challenge with cocaine 5 days after termination of the cocaine regimen (sensitization). The 5-HT_{2A}R antagonist SR 46349B also blocked cocaine-evoked hyperactivity following repeated cocaine treatment, while the other 5-HT₂R ligands were ineffective. When any of the 5-HT₂R ligands was co-administered with cocaine during the treatment regimen (10 mg/kg/day for 5 days), the development of sensitization was unchanged as measured by the level of cocaine-evoked hyperactivity upon challenge 5 days after termination of the treatment. The present study implies that 5-HT_{2A}R and 5-HT_{2C}R exert oppositional influence upon hyperactivity evoked by acute administration of cocaine; this balance is altered following repeated cocaine administration.

Cocaine enhances dopamine (DA), serotonin (5-HT), and norepinephrine neurotransmission through inhibition of their respective reuptake inhibitors (Koe, 1976). Enhancement of DA, particularly within the DA mesoaccumbens (“reward”) pathway, is important in the locomotor stimulant, reinforcing, and discriminative stimulus effects of cocaine (Callahan et al., 1997; Delfs et al., 1990; Pettit et al., 1984). However, the 5-HT system has also been shown to play a vital role in the modulation of DA mesoaccumbens pathways (De Deurwaerdere and Spampinato, 1999; Di Matteo et al., 1999; Gobert et al., 2000; Schmidt et al., 1992) and has been implicated in the mediation of cocaine-evoked behaviors, including cocaine-induced hyperactivity (Bubar et al., 2003; Filip and Cunningham, 2002, 2003; Fletcher et al., 2002, 2004; McCreary and Cunningham, 1999; McMahan and Cunningham, 2001; McMahan et al., 2001).

The 5-HT_{2A} receptor (5-HT_{2A}R) and the 5-HT_{2C}R appear to have opposing influences on DA neurotransmission and psychostimulant-evoked behaviors. Microdialysis assays suggest that the 5-HT_{2A}R can enhance DA neurotransmission under “stimulated” conditions such as after amphetamine administration (Schmidt et al., 1992) or dorsal raphe nucleus stimulation (De Deurwaerdere and Spampinato, 1999), while the 5-HT_{2C}R appears to exert inhibitory control over brain DA pathways (De Deurwaerdere and Spampinato, 1999; Di Matteo et al., 1999; Gobert et al., 2000). In keeping with a potentiative role for the 5-HT_{2A}R over DA mesoaccumbens circuits, 5-HT_{2A}R antagonists have been shown to block the hyperlocomotor (Filip et al., 2001; McMahan and Cunningham, 2001) and discriminative stimulus effects (McMahan and Cunningham, 2001; but see Callahan and Cunningham, 1995; Meert and Janssen, 1992) as well as relapse to self-administration evoked by cocaine (Fletcher et al., 2002). Conversely,

systemic administration of brain-penetrant 5-HT_{2C}R antagonists has been shown to potentiate these same behavioral effects of cocaine (Fletcher et al., 2002; McCreary and Cunningham, 1999). These data suggest that the 5-HT₂R family may be functional and oppositional regulators of the neural substrates that control responsiveness to cocaine.

Modifications in serotonin function may be involved in the processes that underlie “behavioral sensitization” (Cunningham et al., 1992; Filip et al., 2001; Przegalinski et al., 2001). Behavioral sensitization is the enhancement of locomotor hyperactivity and stereotypies demonstrated upon challenge with cocaine during withdrawal from repeated, intermittent cocaine administration (for review, see Vanderschuren and Kalivas, 2000). This behavioral model has been employed extensively to analyze the neural modifications associated with chronic cocaine exposure and withdrawal (White and Kalivas, 1998).

The present series of experiments were conducted to compare the ability of selective agonists and antagonists for specific 5-HT₂R subtypes to modulate locomotor activity evoked by acute cocaine administration vs. their ability to modulate the acquisition or expression of locomotor sensitization to cocaine. A repeated cocaine treatment regimen of 10 mg/kg/day for 5 days has previously been shown to induce locomotor sensitization when expression is measured 5 days following the last treatment injection (Filip et al., 2001; Przegalinski et al., 2001). To examine the ability of the 5-HT₂R ligands to alter *acquisition* of cocaine sensitization, the ligands were administered prior to each daily injection of cocaine during the repeated treatment regimen, while the ability of the 5-HT₂R ligands to alter *expression* of sensitization was determined via administration of the ligands prior to challenge with cocaine 5 days after the termination

of repeated cocaine treatment. We hypothesized that the selective 5-HT_{2A}R antagonist SR46349B and the preferential 5-HT_{2C}R agonist MK 212 would limit acute cocaine (10 mg/kg)-evoked hyperactivity and the development and/or expression of sensitization to cocaine while the preferential 5-HT_{2A}R agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and the selective 5-HT_{2C}R antagonist SDZ SER-082 were expected to enhance these cocaine-evoked behaviors. Although DOI has moderate affinity for all three 5-HT₂R subtypes (see Table 1), the effects of DOI on acute cocaine-evoked hyperactivity are thought to be primarily mediated by the 5-HT_{2A}R, since the behavioral effects of DOI (e.g., wet dog shakes) are preferentially blocked by 5-HT_{2A}R, but not 5-HT_{2B/2C}R, antagonists (Kennett, 1993; Schreiber et al., 1995). Since 5-HT_{2B}R expression in the brain is low (Duxon et al., 1997), the 5-HT_{2B}R agonist BW 723C86 and the 5-HT_{2B}R antagonist SB 204741 were predicted to have little or no effect on acute cocaine-evoked hyperactivity or cocaine sensitization.

MATERIALS AND METHODS

ANIMALS

Male Wistar rats (N = 832; Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland) weighing 250-270 g at the beginning of the experiment were used. The rats were housed 8 per cage in standard plastic rodent cages (57cm x 35 cm x 20 cm) in a colony room maintained at $21 \pm 2^{\circ}\text{C}$ and at 40-50% humidity under a 12 hr light-dark cycle (lights on at 0700 hrs) and had continuous access to tap water and rodent chow except during experimental sessions. All experiments were conducted during the light phase of the light-dark cycle (between 0900-1500 hrs) and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approval from the Bioethics Commission as compliant with the Polish Law (21 August 1997).

DRUGS

The following drugs, their full chemical names (when relevant), the supplier, and the route of injections, respectively, were as follows: BW 723C86 [1-[5-(2-thienylmethoxy)-1*H*-3-indolyl]propan-2-amine HCl; Tocris Cookson, Bristol, UK; i.p.], cocaine HCl (Merck, Damstadt, Germany; i.p.), DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl; Sigma, St. Louis, MO, USA; i.p.], MK 212 [6-chloro-2-(1-piperazinyl)pyrazine HCl; Tocris Cookson, Bristol, UK; i.p.], SB 204741 [*N*-(1-methyl-5-indolyl)-*N'*-(3-methyl-5-isothiazolyl) urea; Tocris Cookson, Bristol, UK; i.p.], SDZ SER-082 [(+)-*cis*-4,5,7a,8,9,10,11,11a-octahydro-7*H*-10-methylindolo(1,7-BC)(2,6)naphthyridine fumarate; Tocris Cookson, Bristol, UK; i.p.] and SR 46349B [1(Z)-[2-

(dimethylamino)ethoxyimino]-1-(2-fluorophenyl)-3-(4-hydroxyphenyl)-2(E)-propene; Sanofi-Syhelabo, France; s.c.]. To achieve dissolution, DOI, MK 212 and SDZ SER-082 were dissolved in saline (0.9% NaCl), BW 723C86 and SB 204741 were suspended in aqueous 1% Tween solution, and SR 46349B was dissolved in 2-3 drops of ethanol and diluted as required in distilled water. All drugs were injected in a volume of 1 ml/kg. The doses of drugs were chosen based upon their functional selectivity at a particular 5-HT₂R (Cunningham et al., 1986; Gobert et al., 2000; Kennett et al., 1997; Rinaldi-Carmona et al., 1992; Schreiber et al., 1995); the affinity profiles for each of the 5-HT₂R ligands are presented in Table 1.

APPARATUS

Locomotor activity was monitored and quantified in clear plexiglass chambers (43 cm x 43 cm x 25 cm) housed inside Opto-varimex® activity monitors surrounded with a 15 x 15 array of photocell beams located 3 cm from the floor surface (Columbus Instruments, Columbus, OH, USA). Interruptions of these photobeams resulted in horizontal activity defined as distance traveled (expressed in cm). Records of horizontal activity were made by the control software (Columbus Instruments) for subsequent statistical evaluation.

PROCEDURES

Effects of 5-HT₂R ligands on acute cocaine-evoked locomotor activity. Rats were habituated to the test environment for 2 hrs/day on each of the 2 days before the start of the experiment, and on each test day for 1 hr before the start of the test session. Animals were tested only one time, and separate groups of animals (n=8/group) were pretreated

with either the 5-HT_{2A}R agonist DOI (0.1-1 mg/kg), 5-HT_{2A}R antagonist SR 46349B (0.25-1 mg/kg), 5-HT_{2B}R agonist BW 723C86 (3-10 mg/kg), 5-HT_{2B}R antagonist SB 204741 (1-3 mg/kg), 5-HT_{2C}R agonist MK 212 (0.1-2 mg/kg), 5-HT_{2C}R antagonist SDZ SER-082 (0.25-1 mg/kg), or the appropriate vehicle 30 min (DOI, BW 723C86, MK 212, SDZ SER-082) or 40 min (SR 46349B, SB 204741) prior to an i.p. treatment injection of either saline (1 ml/kg) or cocaine (10 mg/kg). Measurements of locomotor activity began immediately after the second (saline or cocaine) injection and lasted 60 min.

Effects of 5-HT₂R ligands on the acquisition of behavioral sensitization to cocaine.

On each day for 5 consecutive days, rats (n=8/group) were removed from their home cage, weighed and injected with either DOI (0.1-1 mg/kg), SR 46349B (0.25-1 mg/kg), BW 723C86 (3-10 mg/kg), SB 204741 (1-3 mg/kg), (0.1-2 mg/kg) or SDZ SER-082 (0.25-1 mg/kg) and returned to their home cage; 30-40 min later rats received an injection of cocaine (10 mg/kg) and were immediately returned to their home cage. Control rats (n=8/group) were injected with the appropriate vehicle (1 ml/kg; see Drugs above) prior to an injection of saline (1 ml/kg) or cocaine (10 mg/kg) each day for 5 consecutive days in a similar manner. All injections occurred between 1030 and 1330 hrs. On days 3 and 4 following the last repeated injection, rats were habituated to the test environment for 2 hrs/day. On the 5th day after the last repeated injection, rats were habituated to the test environment for 40 min. The rats were then removed from the test environment to receive a challenge of cocaine (10 mg/kg), immediately returned to the test environment, and locomotor activity was recorded for 60 min. Each rat underwent only one test session.

Effects of 5-HT₂R ligands on the expression of behavioral sensitization to cocaine.

Rats (n=8/group) were removed from their home cage, weighed, and injected with vehicle (1 ml/kg) or cocaine (10 mg/kg) and immediately returned to their home cage each day for 5 days. All injections occurred between 1030 and 1330 hrs. On days 3 and 4 following the last repeated injection, rats were habituated to the test environment for 2 hrs/day. On the 5th day after the last repeated injection, rats were habituated to the test environment and briefly removed from the test environment after 1 hr habituation to receive a pretreatment injection of an appropriate vehicle (1 ml/kg), DOI (0.1-1 mg/kg), SR 46349B (0.25-1 mg/kg), BW 723C86 (3-10 mg/kg), SB 204741 (1-3 mg/kg), MK 212 (0.1-2 mg/kg) or SDZ SER-082 (0.25-1 mg/kg) and returned to the test environment. Thirty min (DOI, BW 723C86, MK 212, SDZ SER-082) or 40 min later (SR 46349B, SB 204741), the rats received an i.p. treatment injection of either saline (1 ml/kg) or cocaine (10 mg/kg), were returned to the test environment, and their locomotor was activity recorded for 60 min. Each rat underwent only one test session.

DATA ANALYSIS

For analyses of acute administration of 5-HT₂R ligands on cocaine-induced hyperactivity, data are presented as mean horizontal distance traveled in centimeters (\pm S.E.M.) for the 60 min observation period. The data were analyzed using a two-way analysis of variance (ANOVA) for the factors of pretreatment [0 mg/kg (i.e., vehicle) and different doses of the 5-HT₂R ligand], treatment (0 or 10 mg/kg cocaine), and the pretreatment x treatment interaction. The Student-Newman-Keuls procedure was used to

analyze preplanned, pairwise comparisons; all comparisons were conducted with an experimentwise error rate of $\alpha=0.05$.

For the sensitization experiments, the dependent measure was horizontal activity (mean total distance traveled in cm \pm S.E.M.) observed during the 60-min challenge test 5 days after the last repeated treatment. Because group comparisons on challenge days were specifically defined before the start of the experiment, these planned comparisons were conducted in lieu of an overall F test in a multifactorial ANOVA; this analysis has been supported in a number of statistical texts (e.g., Keppel, 1973). Each experiment was subjected to a one-way ANOVA with levels of the treatment factor corresponding to the dose of 5-HT₂R ligand + cocaine administered either during the repeated treatment regimen (acquisition of sensitization) or upon challenge 5 days following repeated cocaine treatment (expression of sensitization). Subsequent *a priori* comparisons between means representing changes from baseline activity for horizontal activity were made using a Student's *t* test (SAS for Windows, Version 8.1), which were conducted with an experimentwise error rate of $\alpha=0.05$.

Results

Hyperactivity induced by acute cocaine administration

Effects of the 5-HT_{2A}R agonist DOI on cocaine-induced hyperactivity. A main effect of pretreatment ($F_{3,56} = 4.74$, $p < 0.01$), treatment ($F_{1,7} = 42.12$, $p < 0.001$), and a pretreatment x treatment interaction ($F_{3,56} = 2.90$, $p < 0.05$) were observed for total horizontal activity summed across the 1-hr session. DOI (0.1-1 mg/kg) administered prior to a systemic saline injection did not alter basal locomotor activity ($p > 0.05$). Pretreatment with DOI dose-dependently increased the horizontal activity induced by cocaine (10 mg/kg), reaching significance at the highest dose (1 mg/kg) of DOI tested ($p < 0.05$; Fig. 1A).

Effects of the 5-HT_{2A}R antagonist SR 46349B on cocaine-induced hyperactivity. A main effect of pretreatment ($F_{3,56} = 6.99$, $p < 0.001$), treatment ($F_{1,7} = 29.49$, $p < 0.001$), and a pretreatment x treatment interaction ($F_{3,56} = 2.95$, $p < 0.05$) were observed for total horizontal activity summed across the 1-hr session. Pretreatment with SR 46349B (0.25-1 mg/kg) dose-dependently attenuated cocaine-induced horizontal activity ($p < 0.05$); activity levels observed following pretreatment with 1 mg/kg of SR 46349B were significantly decreased ($p < 0.001$; Fig. 1B) to levels that were not significantly different from vehicle+saline controls ($p > 0.05$). SR 46349B (0.25-1 mg/kg) tested alone did not significantly alter basal locomotor activity ($p > 0.05$).

Effects of the 5-HT_{2B}R agonist BW 723C86 on cocaine-induced hyperactivity. A main effect of treatment ($F_{1,5} = 40.31$, $p < 0.001$), but not pretreatment ($F_{2,42} = 1.67$, $p > 0.05$) or a pretreatment x treatment interaction ($F_{2,42} = 1.58$, $p > 0.05$), was observed for total horizontal activity summed across the 1-hr session. Neither of the doses of BW 723C86

(3 and 10 mg/kg) significantly altered either basal or cocaine-induced horizontal activity ($p > 0.05$; Fig. 2A).

Effects of the 5-HT_{2B}R antagonist SB 204741 on cocaine-induced hyperactivity. A main effect of treatment ($F_{1,5} = 42.00, p < 0.001$), but not pretreatment ($F_{2,42} = 0.04, p > 0.05$) or a pretreatment x treatment interaction ($F_{2,42} = 0.33, p > 0.05$), was observed for total horizontal activity summed across the 1-hr session. Neither of the doses of SB 204741 (1 and 3 mg/kg) significantly altered basal or cocaine-induced horizontal activity ($p > 0.05$; Fig. 2B).

Effects of 5-HT_{2C}R agonist MK 212 on cocaine-induced hyperactivity. A main effect of pretreatment ($F_{4,70} = 5.08, p < 0.01$), treatment ($F_{1,9} = 44.39, p < 0.001$), and a pretreatment x treatment interaction ($F_{4,70} = 4.28, p < 0.01$) were observed for total horizontal activity summed across the 1-hr session. Pretreatment with 0.3 mg/kg of MK 212 enhanced cocaine-induced horizontal activity ($p < 0.05$), while 2 mg/kg of MK 212 significantly reduced cocaine-induced increases in locomotor activity ($p < 0.05$) to levels that were not significantly different from vehicle+saline controls ($p > 0.05$; Fig. 3A). However, 2 mg/kg of MK 212 significantly reduced basal locomotor activity ($p < 0.05$; Fig. 3A).

Effects of the 5-HT_{2C}R antagonist SDZ SER-082 on cocaine-induced hyperactivity. A main effect of pretreatment ($F_{3,56} = 15.97, p < 0.01$), treatment ($F_{1,7} = 64.25, p < 0.001$), and a pretreatment x treatment interaction ($F_{3,56} = 17.32, p < 0.001$) were observed for total horizontal activity summed across the 1-hr session. Pretreatment with SDZ SER-082 increased the horizontal activity induced by cocaine (10 mg/kg) in a dose-dependent manner; a significant enhancement was observed after 1 mg/kg of SDZ SER-082 ($p <$

0.001; Fig. 3B). SDZ SER-082 (0.25-1 mg/kg) did not alter basal locomotor activity ($p > 0.05$).

Cocaine Sensitization

Rats repeatedly treated with cocaine (5 days), displayed a ~two-fold increase in locomotor activity when challenged with cocaine (10 mg/kg) 5 days after the last treatment injection compared to the effect of the acute injection of cocaine (10 mg/kg) in vehicle-treated (5 days) rats (Figs. 4-6), indicating that behavioral sensitization was detected at 5 days after termination of the repeated cocaine treatment utilized in these experiments.

Effects of 5-HT₂R ligands on the development of cocaine sensitization. Rats received 5 daily pretreatments with vehicle or a 5-HT₂R ligand followed by an injection of saline or cocaine (10 mg/kg). Five days after termination of the repeated regimen, the animals were challenged with cocaine (10 mg/kg) and locomotor activity was measured. A main effect of treatment was observed for horizontal activity summed across the 60 min session in the experimental groups pretreated with DOI ($F_{4,34} = 10.767, p < 0.001$), SR 46349B ($F_{4,35} = 3.90, p < 0.05$), BW 723C86 ($F_{3,28} = 7.93, p < 0.001$), SB 204741 ($F_{3,28} = 3.68, p < 0.05$), MK 212 ($F_{5,42} = 6.03, p < 0.001$), SDZ SER-082 ($F_{4,34} = 3.48, p < 0.05$). Rats repeatedly dosed with vehicle+cocaine or any 5-HT₂R ligand+cocaine combination exhibited significantly higher levels of activity upon challenge with cocaine compared to animals repeatedly injected with vehicle+saline (sensitization; Table 2). In all cases, the degree of hyperactivity seen upon challenge with cocaine on the test day was similar regardless of the pharmacological regimen imposed during the repeated treatment ($p > 0.05$; Table 2).

Effects of 5-HT₂R ligands on expression of cocaine sensitization. Rats were treated daily with vehicle or cocaine (10 mg/kg/day for 5 days). Five days after the last injection, the animals were challenged with vehicle or a 5-HT₂R ligand followed by cocaine (10 mg/kg) and locomotor activity was measured.

Effects of the 5-HT_{2A}R agonist DOI on expression of cocaine sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60 min session ($F_{4,34} = 2.97, p < 0.05$). However, pretreatment with DOI (0.1–1 mg/kg) did not significantly alter hyperactivity expressed upon challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen ($p > 0.05$; Fig. 4A).

Effects of the 5-HT_{2A}R antagonist SR 46349B on expression of cocaine sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60 min session ($F_{4,35} = 17.21, p < 0.001$). Pretreatment with SR 46349B (0.25-1 mg/kg) dose-dependently reduced the hyperactivity induced upon challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen; pretreatment with 0.5 and 1.0 mg/kg of SR 46349B significantly suppressed cocaine-evoked hyperactivity ($p < 0.05$; Fig. 4B).

Effects of the 5-HT_{2B}R agonist BW 723C86 on expression of cocaine sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60 min session ($F_{3,27} = 12.44, p < 0.001$). BW 723C86 had no effect on the expression of sensitization since pretreatment with BW 723C86 (3 or 10 mg/kg) did not significantly alter hyperactivity induced by challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen ($p > 0.05$; Fig. 5A).

Effects of the 5-HT_{2B}R antagonist SB 204741 on expression of cocaine sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60 min session ($F_{3,28} = 4.87$, $p < 0.01$). None of the doses of SB 204741 significantly altered hyperactivity expressed upon challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen ($p > 0.05$; Fig. 5B).

Effects of the 5-HT_{2C}R agonist MK 212 on expression of cocaine sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60 min session ($F_{5,40} = 12.64$, $p < 0.001$). Pretreatment with MK 212 (0.1–1 mg/kg) did not significantly alter hyperactivity expressed upon challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen. However, the highest dose (2 mg/kg) of MK 212 significantly reduced hyperactivity induced by challenge with cocaine ($p < 0.01$; Fig. 6A); this dose of MK 212 also suppressed basal locomotor activation (see Fig. 3A).

Effects of the 5-HT_{2C}R antagonist SDZ SER-082 on expression of cocaine sensitization. A main effect of treatment was observed for total horizontal activity summed across the 60 min session ($F_{4,34} = 7.36$, $p < 0.001$). Pretreatment with SDZ SER-082 (0.25–1 mg/kg) did not significantly alter hyperactivity expressed upon challenge with cocaine (10 mg/kg) 5 days after termination of the repeated cocaine regimen ($p > 0.05$; Fig. 6B).

DISCUSSION

The present studies were conducted to compare the ability of 5-HT₂R agonists and antagonists to alter acute cocaine-evoked hyperactivity with the ability of these same ligands to alter the acquisition and/or expression of cocaine sensitization. The results suggest that the 5-HT_{2A}R plays a stimulatory role in cocaine hyperactivity induced by either acute cocaine administration or challenge with cocaine 5 days following termination of the sensitization regimen, while having no influence upon the acquisition of locomotor sensitization. Conversely, the 5-HT_{2C}R appears to have an inhibitory role in cocaine hyperactivity induced by acute cocaine administration, while having little influence upon the acquisition of sensitization or cocaine-evoked hyperactivity following the sensitizing cocaine regimen. The 5-HT_{2B}R has no overt role in elicitation of cocaine-evoked hyperactivity or cocaine sensitization.

The present observations following administration of the 5-HT_{2A}R antagonist SR 46349B support and extend previous findings that the selective 5-HT_{2A}R antagonist M100907 (McMahon and Cunningham, 2001; Fletcher et al., 2002) and the non-selective 5-HT₂R antagonist ketanserin (Filip et al., 2001; McMahon and Cunningham, 2001) attenuated cocaine-evoked hyperactivity at doses of the antagonists that did not alter basal activity levels. SR 46349B (present study) and ketanserin (Filip et al., 2001) also attenuated hyperactivity induced by challenge with cocaine 5 days following termination of the sensitizing cocaine regimen. In keeping with these results, we are the first to report that the preferential 5-HT_{2A}R agonist DOI enhanced the locomotor activating effects of cocaine administered acutely, at doses of DOI that did not alter basal locomotor activity. After a sensitizing regimen of cocaine, however, DOI is no longer capable of further

enhancing hyperactivity seen upon cocaine challenge 5 days after termination of the repeated cocaine regimen. In addition, neither co-treatment with DOI nor SR 46349B during the repeated cocaine regimen effectively altered the course of sensitization, suggesting that the role for 5-HT_{2A}R in acquisition of cocaine sensitization is minimal. This may be attributed to the rapid desensitization and down-regulation of 5-HT_{2A}R that can occur following repeated administration of either 5-HT_{2A}R agonists or antagonists (for review, see Gray and Roth, 2001) and potentially cocaine (e.g., Darmani et al, 1997, but see Baumann and Rothman, 1996). Thus, although the 5-HT_{2A}R appears to be integral for the enhancement of hyperactivity induced by acute administration of cocaine, the functional role of the 5-HT_{2A}R appears to be altered with repeated cocaine administration.

Loss of the enhancement of cocaine-induced hyperactivity by the preferential 5-HT_{2A}R agonist DOI was observed in rats exposed to repeated cocaine administration. An alteration in the expression of the 5-HT_{2A}R or its downstream components after repeated cocaine administration may account for this observation. Repeated cocaine administration has been shown to result in short-term supersensitivity of 5-HT_{2A}R during withdrawal (Baumann and Rothmann, 1996) that appears to be associated with an increase in membrane-associated G_{q/11} protein expression, rather than a specific increase in 5-HT_{2A}R (B_{max}) expression (Carrasco et al., 2003). Since cocaine-induced 5-HT efflux is enhanced in cocaine-sensitized animals (Parsons and Justice, 1993), it is possible that the 5-HT_{2A}R is in a state of maximal stimulation following cocaine challenge in the sensitized animals. Under these circumstances, DOI may be unable to provide any additional stimulation of 5-HT_{2A}R over levels of activation induced by endogenous 5-HT which accumulates in

the synapse after cocaine administration alone (Parsons and Justice, 1993). Conversely, the high affinity 5-HT_{2A}R antagonist SR 46349B may effectively compete with 5-HT for 5-HT_{2A}R binding sites (Rinaldi-Carmona et al, 1992) and thus SR 46349B blockade of 5-HT_{2A}R would continue to exert its inhibitory effect upon hyperactivity evoked by cocaine challenge, as was observed in the present study. This hypothesis is further supported by the present observation that, in animals treated with the repeated cocaine regimen, the levels of hyperactivity induced by challenge with cocaine alone were greater than those elicited by acute administration of DOI+cocaine in animals that had not been previously exposed to cocaine. Thus, the present results suggest that adaptation of the 5-HT_{2A}R or its downstream signaling components may occur following repeated cocaine administration and that these modifications may contribute to the inability of the 5-HT_{2A}R agonist to modulate the sensitized response to cocaine.

Alternatively, it is important to note that DOI is not a selective 5-HT_{2A}R agonist (see Table 1), and thus may also act at other 5-HT₂R subtypes. Considering the opposing actions of the 5-HT_{2A}R and 5-HT_{2C}R on cocaine-evoked hyperactivity (present results, Fletcher et al., 2002; McMahon and Cunningham, 2001), simultaneous stimulation of 5-HT_{2A}R and 5-HT_{2C}R by DOI may have contributed to the lack of effect of DOI on cocaine-evoked hyperactivity following the cocaine sensitization regimen.

Administration of the selective 5-HT_{2C}R antagonists SDZ SER-082 (present study) or SB 242084 (Fletcher et al., 2002) as well as the 5-HT_{2B/2C}R antagonist SB 206553 (McCreary and Cunningham, 1999) enhanced cocaine-evoked hyperactivity, suggesting that the 5-HT_{2C}R exerts an inhibitory influence on acute cocaine-evoked hyperactivity. Interestingly, a biphasic effect of the preferential 5-HT_{2C}R agonist MK 212

on cocaine-evoked hyperactivity was observed; enhancement and suppression were elicited by a low (0.3 mg/kg) and high dose of MK 212 (2 mg/kg), respectively. The high dose of MK 212 (2 mg/kg) was also shown to significantly suppress basal locomotor activation. A reciprocal biphasic effect was previously demonstrated following administration of the 5-HT_{2B/2C}R antagonist SB 206553, with lower doses suppressing and higher doses enhancing cocaine-evoked hyperactivity (McCreary and Cunningham, 1999), however, a significant biphasic effect was not observed following administration of the selective 5-HT_{2C}R antagonist SDZ SER-082 in the present study. The biphasic nature of the response may be due to the lack of complete selectivity of either MK 212 or SB 206553 for the 5-HT_{2C}R, and in particular the lack of selectivity over the 5-HT_{2B}R. However, the inability of the selective 5-HT_{2B}R agonists and antagonists to alter cocaine-evoked hyperactivity as observed in the present and other studies (Fletcher et al., 2002) suggest that this distinction is unlikely attributable to the 5-HT_{2B}R.

A potential explanation for the biphasic effects of 5-HT_{2C}R agonists and antagonists on cocaine-induced hyperactivity is the differential influence of various populations of 5-HT_{2C}R within the DA mesolimbic pathways. In the ventral tegmental area (VTA), the origin of the DA mesolimbic pathways, 5-HT_{2C}R appear to be located on both DA and γ -aminobutyric acid (GABA) neurons (Bubar and Cunningham, 2003; Eberle-Wang et al., 1997). Systemic administration of 5-HT_{2C}R antagonists has been shown to either decrease (Blackburn et al., 2002) or increase (Di Matteo et al., 1999) the firing rate of spontaneously active VTA DA neurons, suggesting that different populations of 5-HT_{2C}R within the VTA may differentially activate VTA DA neurons (Blackburn et al., 2002). In addition, 5-HT_{2C}R are also located in the nucleus accumbens

(NAc) and prefrontal cortex (PFC), the terminal regions of the DA mesolimbic pathways. Microinfusion of 5-HT_{2C}R agonists into the NAc (Filip and Cunningham, 2002) or PFC (Filip and Cunningham, 2003) enhanced or reduced, respectively, cocaine-evoked hyperactivity, with reciprocal effects observed following antagonist administration (Filip and Cunningham, 2002; 2003). These data suggest that 5-HT_{2C}R in the NAc and PFC exert opposing influence upon cocaine-evoked hyperactivity. Thus the ability of systemically administered 5-HT_{2C}R agonists and antagonists to alter cocaine-evoked hyperactivity in a biphasic manner may be attributed to the oppositional influence of 5-HT_{2C}R populations within and/or between brain regions associated with the DA mesolimbic pathways.

In contrast to the profound effects of 5-HT_{2C}R ligands on cocaine-evoked hyperactivity following acute cocaine administration, neither MK 212 nor SDZ SER-082 effectively altered the acquisition of sensitization. The 5-HT_{2C}R antagonist SDZ SER-082 also had no effect on cocaine-evoked hyperactivity 5 days following the repeated cocaine sensitization regimen, while only the highest dose of the 5-HT_{2C}R agonist MK 212 (2 mg/kg), which suppressed basal activity alone, was effective in suppressing cocaine-evoked hyperactivity. The lack of effects of the 5-HT_{2C}R ligands on cocaine-evoked hyperactivity following the cocaine sensitization regimen suggests that repeated cocaine administration results in alterations in the contribution of 5-HT_{2C}R to cocaine-evoked hyperactivity.

The loss of effect of both the 5-HT_{2C}R agonist and antagonist following the cocaine sensitization regimen suggests that the function of these receptors or their downstream signaling components is altered significantly following repeated exposure to

cocaine. Unfortunately, to our knowledge, alterations in the sensitivity or expression of the 5-HT_{2C}R or its key signaling components subsequent to repeated cocaine administration have not yet been examined. Recent evidence from our laboratory, however, suggests that 5-HT_{2C}R sensitivity is decreased during withdrawal from a sensitizing regimen of the 5-HT/DA releaser (+)-3,4-methylenedioxymethamphetamine [(+)-MDMA; Bubar et al., 2002], thus a similar consequence may occur following repeated cocaine administration. Since the 5-HT_{2C}R appears to predominantly exert an inhibitory influence upon DA mesoaccumbens pathway activation (Di Matteo et al., 1999; De Deurwaerdere and Spampinato, 1999; Gobert et al., 2000) and cocaine-evoked hyperactivity (present study; Fletcher et al., 2002), down-regulation of the 5-HT_{2C}R following repeated cocaine administration would likely result in a decrease in 5-HT_{2C}R-mediated inhibition. This loss of 5-HT_{2C}R inhibition would be expected to enhance activation of the DA mesoaccumbens pathway and cocaine-evoked hyperactivity. This hypothesis is consistent with enhanced cocaine-evoked DA release (Vanderschuren and Kalivas, 2000) and sensitization of hyperactivity, as well as the loss of influence of the 5-HT_{2C}R ligands observed following repeated cocaine administration (present results).

In conclusion, the present results confirm previous evidence that the 5-HT_{2A}R and 5-HT_{2C}R exert opposing modulatory actions on hyperactivity evoked by acute cocaine administration, while 5-HT_{2B}R do not appear to be involved in elicitation of cocaine-evoked hyperactivity. In addition, we provide evidence that repeated cocaine administration may result in adaptations that contribute to the expression of sensitization and that alter the ability of 5-HT_{2A}R and 5-HT_{2C}R ligands to modulate cocaine-evoked hyperactivity.

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REFERENCES

- Baumann MH and Rothman RB (1998) Alterations in serotonergic responsiveness during cocaine withdrawal in rats: similarities to major depression in humans. *Biol Psychiatry* 44: 578-591.
- Baxter G, Kennett G, Blaney F and Blackburn T (1995) 5-HT₂ receptor subtypes: a family re-united? *Trends Pharmacol Sci* 16: 105-110.
- Blackburn TP, Minabe Y, Middlemiss DN, Shirayama Y, Hashimoto K and Ashby CR Jr (2002) Effect of acute and chronic administration of the selective 5-HT_{2C} receptor antagonist SB-243213 on midbrain dopamine neurons in the rat: An in vivo extracellular single cell study. *Synapse* 46: 129-139.
- Bubar MJ and Cunningham KA (2003) Serotonin_{2C} receptor distribution in the ventral tegmental area. Program No. 136.1, 643.5 Experimental Biology Abstract Search and Itinerary Builder, FASEB.
- Bubar MJ, McMahon LR, De Deurwaerdere P, Spampinato U and Cunningham KA (2003) Selective serotonin reuptake inhibitors enhance cocaine-induced locomotor activity and dopamine release in the nucleus accumbens. *Neuropharmacology* 44: 342-353.
- Bubar MJ, Shank EJ and Cunningham KA (2002) Locomotor sensitization and 5-HT_{2C} receptor hyposensitivity following repeated administration of (+)-3,4-methylenedioxymethamphetamine [(+)-MDMA]. *Drug Alcohol Depend* 61: S22-S23.

Callahan PM and Cunningham KA (1995) Modulation of the discriminative stimulus properties of cocaine by 5-HT_{1B} and 5-HT_{2C} receptors. *J Pharmacol Exp Ther* 274: 1414-1424.

Callahan PM, de la Garza R 2nd and Cunningham KA (1997) Mediation of the discriminative stimulus properties of cocaine by mesocorticolimbic dopamine systems. *Pharmacol Biochem Behav* 57: 601-607.

Carrasco GA, Zhang Y, Damjanoska KJ, D'Souza DN, Garcia F, Battaglia G, Muma NA and Van de Kar LD (2003) A region-specific increase in Galphaq and Galpha11 proteins in brains of rats during cocaine withdrawal. *J Pharmacol Exp Ther* 307: 1012-1019.

Cunningham KA, Callahan PM and Appel JB (1986) Discriminative stimulus properties of the serotonin agonist MK 212. *Psychopharmacology* 90: 193-197.

Cunningham KA, Paris JM and Goeders NE (1992) Chronic cocaine enhances serotonin autoregulation and serotonin uptake binding. *Synapse* 11: 112-123.

Darmani NA, Shaddy J and Elder EL (1997) Prolonged deficits in presynaptic serotonin function following withdrawal from chronic cocaine exposure as revealed by 5-HTP-induced head-twitch response in mice. *J Neural Transm* 104: 1229-1247.

De Deurwaerdere P and Spampinato U (1999) Role of serotonin_{2A} and serotonin_{2C} receptor subtypes in the control of accumbal and striatal dopamine release elicited in vivo by dorsal raphe nucleus electrical stimulation. *J Neurochem* 73: 1033-1042.

- Delfs JM, Schreiber L and Kelley AE (1990) Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. *J Neurosci* 10: 303-310.
- Di Matteo V, Di Giovanni G, Di Mascio M and Esposito E (1999) SB242084, a selective serotonin 2C receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology* 38: 1195-1205.
- Duxon MS, Flanigan TP, Reavley AC, Baxter GS, Blackburn TP and Fone KCF (1997) Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. *Neuroscience* 76: 323-329.
- Eberle-Wang K, Mikeladze Z, Uryu K and Chesselet MF (1997) Pattern of expression of the serotonin_{2C} receptor messenger RNA in the basal ganglia of adult rats. *J Comp Neurol* 384:233-247.
- Filip M and 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 M and Cunningham KA (2003) Hyperlocomotive and discriminative stimulus effects of cocaine are under the control of serotonin_{2C} (5-HT_{2C}) receptors in rat prefrontal cortex. *J Pharmacol Exp Ther* 306: 734-743.
- Filip M, Nowak E and Papla I (2001) On the role of serotonin_{2A/2C} receptors in the sensitization to cocaine. *J Physiol Pharmacol* 52: 471-481.
- Fletcher PJ, Grottick AJ and Higgins GA (2002) Differential effects of the 5-HT_{2A} receptor antagonist M100907 and the 5-HT_{2C} receptor antagonist SB 242084

on cocaine-induced locomotor activity, cocaine self-administration and cocaine-induced reinstatement of responding. *Neuropsychopharmacology* 27: 576-586.

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

Gobert A, Rivet JM, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas JP, Cistarelli L, Melon C and 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.

Gray JA and Roth BL (2001) Paradoxical trafficking and regulation of 5-HT(2A) receptors by agonists and antagonists. *Brain Res Bull* 56: 441-451.

Kennett GA (1993) 5-HT_{1C} receptors and their therapeutic relevance. *Curr Opin Invest Drugs* 2: 317-362.

Kennett GA, Ainsworth K, Trail B and Blackburn TP (1997) BW 723C86, a 5-HT_{2B} receptor agonist, causes hyperphagia and reduced grooming in rats. *Neuropharmacology* 36: 233-239.

Keppel G (1973) Design and analysis: a researcher's handbook. Englewood Cliffs (NJ): Prentice-Hall.

- Koe BK (1976) Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. *J Pharmacol Exp Ther* 199: 649-661.
- McCreary AC and Cunningham KA (1999) Effects of the 5-HT_{2C/2B} antagonist SB 206553 on hyperactivity induced by cocaine. *Neuropsychopharmacology* 20: 556-564.
- McMahon LR and Cunningham KA (2001) Antagonism of 5-hydroxytryptamine_{2A} receptors attenuates the behavioral effects of cocaine in rats. *J Pharmacol Exp Ther* 297: 357-363.
- McMahon LR, Filip M and Cunningham KA (2001) Differential regulation of the mesoaccumbens circuit by serotonin 5-HT_{2A} and 5-HT_{2C} receptors. *J Neurosci* 21: 7781-7787.
- Meert TF and Janssen PAJ (1992) Ritanserin, a new therapeutic approach for drug abuse. Part 2: Effects on cocaine. *Drug Dev Res* 25: 39-53.
- Nozulak J, Kalkman HO, Floersheim P, Hoyer D, Schoeffter P and Buerki HR (1995) (+)-cis-4,5,7a,8,9,10,11,11a-octahydro-7H-10-methylindolo[1,7-bc][2,6]-naphthyridine: a 5-HT_{2C/2B} receptor antagonist with low 5-HT_{2A} receptor affinity. *J Med Chem* 38: 28-33.
- Parsons LH and Justice JB Jr (1993) Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area, and dorsal raphe nucleus following repeated cocaine administration. *J Neurochem* 61: 1611-1619.

Pettit HO, Ettenberg A, Bloom FE, and Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology* 84: 167-173.

Przegalinski E, Filip M, Papla I and Siwanowicz J (2001) Effect of serotonin (5-HT)_{1B} receptor ligands on cocaine sensitization in rats. *Behav Pharmacol* 12: 109-116.

Rinaldi-Carmona M, Congy C, Santucci V, Simiand J, Gautret B, Neliat G, Labeeuw B, Le Fur G, Soubrie P and Breliere JC (1992) Biochemical and pharmacological properties of SR46349B, a new potent and selective 5-hydroxytryptamine₂ receptor antagonist. *J Pharmacol Exp Ther* 262: 759-768.

Schmidt CJ, Fadaye GM, Sullivan CK and Taylor VL (1992) 5-HT₂ receptors exert a state-dependent regulation of dopaminergic function: studies with MDL 100,907 and the amphetamine analogue, 3,4-methylenedioxymethamphetamine. *Eur J Pharmacol* 223: 65-74.

Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S and Millan MJ (1995) 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT)_{2A} receptors: modulation by novel 5-HT_{2A/2C} antagonists, D₁ antagonists and 5-HT_{1A} agonists. *J Pharmacol Exp Ther* 273: 101-112.

Vanderschuren LJ and Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology* 151: 99-120.

White FJ and Kalivas PW (1998) Neuroadaptations involved in amphetamine and cocaine addiction. *Drug Alcohol Depend* 51: 141-153.

FOOTNOTES

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FIGURE LEGENDS

Figure 1. Basal and cocaine-evoked hyperactivity following pretreatment with the 5-

HT_{2A}R ligands. Data represent the mean horizontal distance traveled (\pm S.E.M.) summed over the 60 min recording period after injection of **[A]** vehicle (VEH) or the 5-HT_{2A}R agonist DOI (0.1, 0.3 or 1 mg/kg), or **[B]** VEH or the 5-HT_{2A}R antagonist SR 46349B (SR; 0.25, 0.5 or 1 mg/kg) followed by an injection of saline (SAL) or cocaine (COC; 10 mg/kg). Data points represent the mean of data from 8 rats. * $p < 0.05$ vs. VEH-SAL; ^ $p < 0.05$ vs. VEH-COC.

Figure 2. Basal and cocaine- evoked hyperactivity following pretreatment with the

5-HT_{2B}R ligands. Data represent the mean horizontal distance traveled (\pm S.E.M.) summed over the 60 min recording period after injection of **[A]** vehicle (VEH) or the 5-HT_{2B}R agonist BW 723C86 (BW; 3 or 10 mg/kg), or **[B]** VEH or the 5-HT_{2B}R antagonist SB 204741 (SB; 1 or 3 mg/kg) followed by an injection of saline (SAL) or cocaine (COC; 10 mg/kg). Data points represent the mean of data from 8 rats. * $p < 0.05$ vs. VEH-SAL.

Figure 3. Basal and cocaine- evoked hyperactivity following pretreatment with the

5-HT_{2C}R ligands. Data represent the mean horizontal distance traveled (\pm S.E.M.) summed over the 60 min recording period after injection of **[A]** vehicle (VEH) or the 5-HT_{2C}R agonist MK 212 (MK; 0.1, 0.3, 1 or 2 mg/kg), or **[B]** VEH or the 5-HT_{2C}R antagonist SDZ SER-082 (SDZ; 0.25, 0.5 or 1 mg/kg) followed by an injection of saline (SAL) or cocaine (COC; 10 mg/kg). Data points represent the mean of data from 8 rats. * $p < 0.05$ vs. VEH-SAL; ^ $p < 0.05$ vs. VEH-COC.

Figure 4. Challenge with 5-HT_{2A}R ligands + cocaine following the repeated cocaine sensitization regimen. Rats were treated repeatedly with saline (SAL; 1 ml/kg; open bars) or cocaine (COC; 10 mg/kg; hatched bars) once a day for 5 days. Five days following the last repeated treatment, rats were challenged with [A] the 5-HT_{2A}R agonist DOI (0, 0.1, 0.3 or 1.0 mg/kg) + cocaine (10 mg/kg), or [B] the 5-HT_{2A}R antagonist SR 46349B (0, 0.25, 0.5 or 1 mg/kg) + cocaine (10 mg/kg). Data points represent the mean horizontal distance traveled (\pm SEM) over the 1-hr recording period from 8 rats. * $p < 0.05$ vs. repeated SAL - COC challenge group, # $p < 0.05$ vs. repeated COC - COC challenge group.

Figure 5. Challenge with 5-HT_{2B}R ligands + cocaine following the repeated cocaine sensitization regimen. Rats were treated repeatedly with saline (SAL; 1 ml/kg; open bars) or cocaine (COC; 10 mg/kg; hatched bars) once a day for 5 days. Five days following the last repeated treatment, rats were challenged with [A] the 5-HT_{2B}R agonist BW 723C86 (0, 3 or 10 mg/kg) + cocaine (10 mg/kg), or [B] the 5-HT_{2B}R antagonist SB 204741 (0, 1, or 3 mg/kg) + cocaine (10 mg/kg). Data points represent the mean horizontal distance traveled (\pm SEM) over the 1-hr recording period from 8 rats. * $p < 0.05$ vs. repeated SAL - COC challenge group.

Figure 6. Challenge with 5-HT_{2C}R ligands + cocaine following the repeated cocaine sensitization regimen. Rats were treated repeatedly with saline (SAL; 1 ml/kg; open bars) or cocaine (COC; 10 mg/kg; hatched bars) once a day for 5 days. Five days following the last repeated treatment, rats were challenged with [A] the 5-HT_{2C}R agonist MK 212 (0, 0.1, 0.3, 1, or 2 mg/kg) + cocaine (10 mg/kg), or [B] the 5-HT_{2C}R antagonist SDZ SER-082 (0, 0.25, 0.5 or 1 mg/kg) + cocaine (10

mg/kg). Data points represent the mean horizontal distance traveled (\pm SEM) over the 1-hr recording period from 8 rats. * $p < 0.05$ vs. repeated SAL - COC challenge group, # $p < 0.05$ vs. repeated COC - COC challenge group.

Table 1. Affinity (K_i , nM) of ligands for 5-HT₂R subtypes

Ligand	Receptor Subtype			Reference
	5-HT _{2A} R	5-HT _{2B} R	5-HT _{2C} R	
BW 723C86	>3891	12	125	Baxter et al., 1995
DOI	50 ^a	39 ^a	15 ^a	Kennett, 1993
MK 212	15848	1258	630	Kennett, 1993
SB 204741	5011	15	>1000	Baxter et al., 1995
SR46349B	5.8 ^b	>100	120 ^b	Rinaldi-Carmona et al., 1992
SDZ SER-082	630 ^c	58	15 ^c	Nozulak et al., 1995

a EC₅₀ (nM)

b IC₅₀ (nM)

c K_D (nM)

Table 2. Effects of the 5-HT₂R ligands on the development of cocaine sensitization. Rats were treated repeatedly with vehicle (VEH)+saline (SAL), vehicle+cocaine (COC; 10 mg/kg) or a 5-HT₂R ligand+cocaine (10 mg/kg) for 5 days. Five days following termination of the repeated regimen, rats were challenged with cocaine (10 mg/kg) and locomotor activity was measured. Data points represent the mean horizontal distance traveled (cm ± SEM; 8 rats/group) in response to the cocaine challenge. * $p < 0.05$ vs. VEH/SAL.

Repeated Treatment	Distance traveled (cm ± SEM)	Repeated Treatment	Distance traveled (cm ± SEM)
VEH/SAL	971 ± 184	VEH/SAL	1317 ± 275
VEH/COC	4158 ± 527 *	VEH/COC	3967 ± 499 *
DOI 0.1/COC	4854 ± 454 *	SR 46349B 0.25/COC	4653 ± 609 *
DOI 0.3/COC	5034 ± 685 *	SR 46349B 0.5/COC	2855 ± 971
DOI 1.0/COC	4412 ± 618 *	SR 46349B 1.0/COC	3732 ± 995 *
VEH/SAL	2335 ± 581	VEH/SAL	2335 ± 851
VEH/COC	5915 ± 844 *	VEH/COC	5915 ± 844 *
BW 723C86 3.0/COC	5200 ± 858 *	SB 204741 1.0/COC	4992 ± 868 *
BW 723C86 10.0/COC	6321 ± 1012 *	SB 204741 3.0/COC	3934 ± 914
VEH/SAL	1266 ± 379	VEH/SAL	1784 ± 296
VEH/COC	4333 ± 1023 *	VEH/COC	5997 ± 788 *
MK 212 0.1/COC	3769 ± 616 *	SDZ SER-082 0.25/COC	4862 ± 1002 *
MK 212 0.3/COC	5102 ± 784 *	SDZ SER-082 0.5/COC	4780 ± 966 *
MK 212 1.0/COC	3947 ± 633 *	SDZ SER-082 1.0/COC	5746 ± 1213 *
MK 212 2.0/COC	3366 ± 729 *		











