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# Serotonin 5-$HT_{2C}$ receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine☆

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**Abstract**

The serotonin 5-$HT_{2C}$ receptor (5-$HT_{2C}$R) is abundant in the nucleus accumbens (NAc) shell and is considered an important target for 5-HT to modulate the dopamine (DA) mesoaccumbens circuit, which plays a prominent role in the behavioral effects of cocaine. The present study analyzed the ability of intra-NAc shell infusions of the 5-$HT_{2C}$R agonists, MK 212 and RO 60-0175, or the 5-$HT_{2C}$R antagonist, RS 102221, to alter either spontaneous or cocaine-evoked activity as well as the discriminative stimulus properties of cocaine. In male Sprague–Dawley rats implanted with bilateral cannulae aimed at the NAc shell, locally injected MK 212 (0.05–0.5 μg/side) or RO 60-0175 (0.5–5 μg/side) did not alter spontaneous activity, but dose-dependently enhanced hyperactivity evoked by cocaine (10 mg/kg ip). In rats trained to discriminate cocaine (10 mg/kg ip) from saline (ip) in a two-lever, water-reinforced FR 20 task, intra-NAc microinfusion of MK 212 (0.05 μg/side) or RO 60-0175 (0.5 μg/side) evoked 37% or 48% cocaine lever responding, respectively. Both MK 212 (0.05 μg/side) and RO 60-0175 (0.5 μg/side) enhanced the discriminability of submaximal doses of cocaine (0.625–2.5 mg/kg). Moreover, intra-NAc infusion of RS 102221 (0.05–1.5 μg/side) dose-dependently attenuated the stimulus effects of cocaine. These data reinforce the hypothesis that 5-$HT_{2C}$R plays a role in the regulatory neurochemistry of the NAc shell that is important to the full expression of the behaviors evoked by cocaine. 

*Keywords:* 5-$HT_{2C}$ receptors; Behavior; Cocaine; Drug discrimination; MK 212; RO 60-0175; RS 102221; Serotonin

## 1. Introduction

Cocaine dependence presents serious medical, social and criminal challenges in today's world. A research priority in the quest to identify the means to combat cocaine dependence is a full elucidation of the neurobiology of cocaine. Cocaine exhibits a high affinity for dopamine (DA), serotonin (5-hydroxytryptamine; 5-HT) and norepinephrine transporters and inhibits the reuptake of these neurotransmitters into presynaptic nerve terminals (Koe, 1976). Augmented DA neurotransmission and indirect activation of DA $D_1$- and $D_2$-like receptors have been established to play a central role in the in vivo effects of cocaine (see Di Chiara, 1995; Spealman et al., 1992; Wise, 1995). In particular, the DA mesoaccumbens pathway, which originates in DA cell bodies in the ventral tegmental area (VTA) and terminates in the nucleus accumbens (NAc), has been directly implicated in mediating the behavioral effects of cocaine, as well as the primary functions of reward, emotion and motivation (Kalivas and Nemeroff, 1988). The importance of the DA mesoaccumbens pathway has been highlighted by observations that intra-NAc cocaine infusion mimics the reinforcing (McKinzie et al., 1999), discriminative stimulus (Callahan et al., 1994) and hyperlocomotive properties of systemic cocaine (Delfs et al., 1990).

In addition to the pronounced involvement of DA in its in vivo effects, cocaine also enhances 5-HT availability for interaction with potentially all brain 5-HT receptors. Manipulations of 5-HT have been shown to modulate

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the locomotor stimulant, reinforcing and discriminative stimulus effects of cocaine (for review, see Walsh and Cunningham, 1997) and the underlying mechanisms may be related to the ability of 5-HT to modulate DA neurotransmission (see below). However, the exact contribution of specific 5-HT receptors to the behavioral profile of cocaine has not yet been fully elucidated, largely due to the multiplicity of 5-HT receptors that exist in mammalian systems. Among 14 5-HT receptors currently recognized, the 5-$HT_{2C}$ receptor (5-$HT_{2C}$R) subtype has received attention as a potential therapeutic target in neuropsychiatric conditions, such as psychosis, depression and anxiety (for review, see Barnes and Sharp, 1999). The mRNA and protein for 5-$HT_{2C}$R are densely expressed in the DA mesoaccumbens circuit (Clemett et al., 2000; Eberle-Wang et al., 1997), and recent neurochemical studies implicate a role for the 5-$HT_{2C}$R in the control of mesoaccumbens pathways (Bowers et al., 2000; De Deurwaerdere and Spampinato, 1999; Di Matteo et al., 2001; Lucas and Spampinato, 2000; Yan, 2000).

Pharmacological manipulations of the 5-$HT_{2C}$R have been shown to modulate cocaine-evoked behaviors in rats. For example, systemic administration of the 5-$HT_{2C/1B}$R agonist *m*-chlorophenylpiperazine, or the 5-$HT_{2C}$R agonist MK 212, reduced recognition of the cocaine state in a drug discrimination analysis (Callahan and Cunningham, 1995). The 5-$HT_{2C}$R agonist RO 60-0175 was shown to depress cocaine-induced hyperactivity and cocaine self-administration; this suppressant effect of RO 60-0175 was blocked by the 5-$HT_{2C}$R antagonist SB 242084 (Grottick et al., 2000). In contrast, the same dose of SB 242084 (0.5 mg/kg) was not an effective antagonist of cocaine-induced hyperactivity (Grottick et al., 2000), although the 5-$HT_{2C/2B}$R antagonist SB 206553 dose-dependently inhibited, or enhanced, cocaine-induced hypermotility (McCreary and Cunningham, 1999). These data suggest that the 5-$HT_{2C}$R may be a functionally important regulator of the neural substrates that control responsiveness to cocaine.

Recent intracranial microinjection studies aimed to identify the site(s) of action within the mesoaccumbens pathway for the 5-$HT_{2C}$R, relative to the 5-$HT_{2A}$R, concluded that hyperactivity induced by cocaine is regulated in a distinctly regional manner by 5-$HT_{2C}$R in the NAc and 5-$HT_{2A}$R in the VTA (McMahon et al., 2001). Upon intra-NAc (but not intra-VTA) microinfusion, the 5-$HT_{2C}$R antagonist RS 102221 blocked expression of cocaine-evoked hyperactivity and the 5-$HT_{2A}$R antagonist M100907 only antagonized this behavioral effect of cocaine upon intra-VTA (not intra-NAc) administration (McMahon et al., 2001). Interestingly, the observation that antagonism of 5-$HT_{2C}$R receptors in the shell of the NAc suppressed cocaine-evoked hyperactivity would not have been predicted based upon results of studies of 5-$HT_{2C}$R agonists administered systemically (above, Callahan and Cunningham, 1995; Grottick et al., 2000). Taken together, these data suggest that the behavioral influence of systemically administered 5-$HT_2$R agonists on cocaine-induced behaviors may represent a composite response to stimulation of 5-$HT_2$R differentially localized to multiple brain nuclei.

To extend our appreciation of the extent to which specific behaviors induced by cocaine are differentially controlled by the 5-$HT_{2C}$R located in the NAc, we analyzed the ability of intra-NAc microinfusion of a 5-$HT_{2C}$R agonist or antagonist to mimic or alter the hyperlocomotive or discriminative stimulus effects of cocaine. We chose to extend and expand our previous findings with regard to the role of NAc 5-$HT_{2C}$R in cocaine-induced hyperactivity (McMahon et al., 2001) using a drug discrimination assay, which has provided a useful model of the subjective effects of cocaine in humans (Drummond et al., 1995; Schuster and Johanson, 1988) and has been particularly useful in characterizing the neuronal sites of action and mechanisms that underlie the in vivo effects of cocaine (Callahan et al., 1994). The present study employed the selective and efficacious 5-$HT_{2C}$R agonists MK 212 (nM affinity for 5-$HT_{2C}$R and >16-fold lower affinity for other receptor sites; Kennett, 1993; Porter et al., 1999) and RO 60-0175 (nM affinity for 5-$HT_{2C}$R and >25–100-fold lower affinity for other receptors; Bös et al., 1997; Porter et al., 1999), as well as the selective antagonist RS 102221 ($K_i$=nM affinity for 5-$HT_{2C}$R and >35-fold lower affinity for other receptors; Bonhaus et al., 1997). The NAc shell was selected because a greater degree of 5-HT innervation (Brown and Molliver, 2000) and higher levels of 5-$HT_{2C}$R (Clemett et al., 2000) have been observed in the shell vs. the core; the NAc shell has also been shown to be more sensitive to cocaine than is the core (Pontieri et al., 1995).

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats ($n$ = 55; Harlan, Houston, TX) weighing 250–350 g at the beginning of the experiment were used. The rats were housed two or three per cage in standard plastic rodent cages in a colony room maintained at 21±2 °C and at 40–50% humidity under a 12-h light–dark cycle (lights on at 0700 h). Rats surgically fitted with indwelling bilateral guide cannulae were housed individually. Rats assigned to locomotor activity assays ($n$ = 36) were provided with continuous access to tap water and rodent chow except during experimental sessions. In drug discrimination assays ($n$ = 19), the amount of water each animal received was restricted to that given during daily training sessions, after test sessions (10–15 min) and on weekends (36 h). All experiments were conducted during the light phase of the light–dark cycle (between 0900 and 1400 h) and were carried out in accordance with the National Institutes of Health *Guide for the care and use of laboratory animals* and with approval from the Institutional Animal Care and Use Committee.

### 2.2. Surgery and cannulae implantation

Rats underwent surgical implantation of 26-gauge stainless steel bilateral guide cannulae (Small Parts, Miami Lakes, FL, USA). Each rat was anesthesized using an intramuscular (im) injection of 43 mg/kg ketamine, 8.6 mg/kg xylazine and 1.5 mg/kg acepromazine in physiological saline (0.9% NaCl). With the upper incisor bar of a stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA) positioned at −3.8 mm below the interaural line and using the intersection of the bregma and longitudinal sutures as the origin, the ventral surfaces of the bilateral guide cannulae were positioned 2 mm above the NAc shell (AP = +1.7 mm, ML = ±0.75 mm and DV = −6 mm; Paxinos and Watson, 1998). The guide cannulae were fastened to the skull with stainless steel screws (Small Parts) and cranioplastic cement (Plastics One, Roanoke, VA, USA) and were fitted with 28-gauge stainless steel bilateral obturators (Small Parts). Rats received a single injection of 300,000 U (im) of sodium ampicillin (locomotor activity experiments) or two injections of penicillin (10,000 U/kg im) after surgery (drug discrimination experiments), and were allowed a 1-week recovery period during which rats were handled and weighed daily. Following the initial 1-week recovery period, each rat was habituated to the brief confinement associated with intracranial microinjections by removing the 28-gauge internal obturator, gently restraining the rat for approximately 3 min and replacing the obturator. For each intra-NAc shell microinjection, the obturators were removed and two internal cannulae (Plastics One) were positioned so as to extend 2 mm below the tips of the bilateral guide cannulae. The bilateral internal cannulae were attached to two 5-μl syringes (Hamilton, Reno, NV, USA) via PE-50 tubing (Clay-Adams, Parsippany, NJ, USA). A microsyringe drive (Baby Bee; Bioanalytical Systems, West Lafayette, IN, USA) driven by a programmable controller (Bee Hive Controller; Bioanalytical Systems) delivered a volume of 0.2 μl/side at a rate of 0.1 μl/min. The injection cannulae remained in place for an additional 1 min to allow for diffusion away from the cannulae tips.

### 2.3. Drugs

All the drugs used were dissolved in sterile saline (0.9% NaCl). Cocaine HCl (National Institute of Drug Abuse, Research Triangle Park, NC, USA) was injected intraperitoneally (ip) in a volume of 1 ml/kg. MK 212 [6-chloro-2-(1-piperazinyl)pyrazine HCl; Tocris, Ballwin, MO, USA], RO 60-0175 [(*S*)-2-(6-chloro-5-fluroindol-1-yl)-1-methylethylamine fumarate; Hoffman La-Roche, Basel, Switzerland] or RS 102221 [8-[5-(2,4-dimethoxy-5-(4-trifluoromethylphenylsulfonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro- [4.5]decane-2,4-dione HCl; Tocris] was injected intracranially in a volume of 0.2 μl/side. All solutions injected centrally were adjusted to pH 7.2, except the solution of RS 102221, which was adjusted to pH 6–7; control vehicle at pH 6–7 did not alter basal or cocaine-stimulated locomotor activity or the stimulus effects of cocaine (10 mg/kg) (data not shown).

### 2.4. Measurement of locomotor activity

#### 2.4.1. Apparatus

Locomotor activity was monitored and quantified using a modified open-field activity system under low light conditions (San Diego Instruments, San Diego, CA, USA). Each clear Plexiglass chamber (40×40×40 cm) was housed within a sound-attenuating enclosure and was surrounded with a 4×4 photobeam matrix located 4 cm from the floor surface. Interruptions of the photobeams resulted in counts of activity in the peripheral and central fields of the chamber. Activity recorded in the inner 16×16 cm of the open field was counted as central activity, while the field bounded by the outer 12-cm band registered peripheral activity. Another horizontal row of 16 photobeams, located 16 cm from the floor surface, provided each chamber with a measurement of vertical activity (rearing). Separate counts of peripheral, central and vertical (rearing) activity were made by the control software (Photobeam Activity Software; San Diego Instruments) and stored for subsequent statistical evaluation. Video cameras positioned above the chambers permitted continuous observation of behavior without disruption.

#### 2.4.2. Behavioral tests and microinfusion protocols

Surgically implanted rats were habituated to the test environment for 3 h per day on each of the 2 days before the start of the experiment, and on each test day for 1 h before the administration of drugs. Using a repeated measures design and eight test sessions, one group of rats ($n = 22$) received an intra-NAc shell microinjection of either sterile saline (0.2 μl/side) or MK 212 (0.05, 0.15 or 0.5 μg/0.2 μl/side), and a second group of animals ($n = 14$) received either saline (0.2 μl/side) or RO 60-0175 (0.5, 1.5 or 5 μg/0.2 μl/side); each microinjection was immediately followed by an intraperitoneal injection of either saline (1 ml/kg) or cocaine (10 mg/kg). Measurements of locomotor activity began immediately after the systemic injection and were taken for a total of 60 min. Test sessions were conducted every 3 days, and the order of microinjections was counterbalanced for each rat. Systemic cocaine injections were given every other test and only once per week.

### 2.5. Drug discrimination experiments

#### 2.5.1. Apparatus

The procedures were conducted in commercially available, two-lever operant chambers (Model 80001; Lafayette Instrument, Lafayette, IN, USA). Each chamber was equipped with a water-filled dispenser mounted equidistant between two response levers on one wall and was housed in a light- and sound-attenuating cubicle (Model 80015; Lafay-

ette Instrument). A 28-V house light provided illumination; a blower supplied ventilation and masking noise. An interface (MedAssociates, St. Albans, VT, USA) connected the chambers to a computer, which controlled and recorded all experimental events using MedState software.

*2.5.2. Discrimination training and test protocols*

Standard, two-lever, water-reinforced drug discrimination procedures were utilized (Callahan and Cunningham, 1995; Callahan et al., 1994). Rats were injected intraperitoneally with cocaine (10 mg/kg) or saline (1 ml/kg) 15 min prior to daily (Monday–Friday) 30-min sessions ($n = 19$). During this phase, only the stimulus-appropriate (drug or saline) lever was present. Training began under a fixed ratio 1 (FR 1) schedule of water reinforcement and the FR requirement was incremented until all animals were responding reliably under an FR 20 schedule for each experimental condition. For half of the rats, left-lever responses were reinforced after cocaine administration, whereas right-lever responses were reinforced after saline administration; conditions were reversed for the remaining rats. During this phase of training, cocaine and saline were administered irregularly with the restriction that neither condition prevailed for more than three consecutive sessions. After responding stabilized, both levers were presented simultaneously during 15-min sessions. The rats were required to respond on the stimulus-appropriate (correct) lever in order to obtain water reinforcement, and there were no programmed consequences for responding on the incorrect lever. This phase of training continued until the performance of all rats attained criterion (defined as mean accuracies of at least 80% correct for 10 consecutive sessions).

When rats achieved the criterion for accuracy, test sessions were initiated and training sessions were run during the intervening days to maintain discrimination accuracy. Rats were required to maintain accuracies of at least 80% correct for the saline and cocaine maintenance sessions, which immediately preceded a test. During test sessions, animals were placed in the chambers and, upon completion of 20 responses on either lever, a single reinforcer was delivered and the house lights were turned off. The rat was removed from the chamber, returned to the colony and allowed free access to water for 10 min beginning 15–30 min after the end of each test. Sessions were terminated after 15 min if rats did not complete 20 responses on either lever.

*2.5.3. Pharmacological test and microinfusion protocols*

Several pharmacological manipulations were performed during test sessions. A systemic dose–response curve for cocaine was established before surgical implantation of cannulae; rats were tested 15 min after an injection of cocaine (0.625–10 mg/kg ip). Following recovery from surgery, discrimination training was reinstated. After several weeks, the systemic dose–response curve for cocaine was reestablished and did not differ from that established prior to surgery (data not shown); the postsurgical dose–response curve served as control in the present experiment.

In intracranial *substitution* tests, lever selection was assessed 10 min after bilateral intracranial infusion of sterile saline (0.9% NaCl; 0.2 μl/side), MK 212 (0.05 μg/side), RO 60-0175 (0.5 μg/side) or RS 102221 (1.5 μg/side) paired with a systemic injection of saline (1 ml/kg ip). Control tests were also conducted in which rats were assessed for lever selection 10 min following administration of either saline or cocaine (10 mg/kg ip), which had been immediately preceded by intracranial injection of saline (0.2 μl/side). In *combination* tests, an intracranial microinjection of MK 212 (0.05 μg/side), RO 60-0175 (0.5 μg/side) or RS 102221 (0.05–1.5 μg/side) immediately preceded an injection of cocaine (0.625–2.5 mg/kg ip), which produced < 80% cocaine-lever responding when given alone (“potentiation test”), or a dose of cocaine (5 mg/kg), which produced full (> 80%) substitution (“antagonism test”); rats were tested for lever selection 10 min later.

*2.6. Histology*

At the completion of the study, rats were overdosed with chloral hydrate (800 mg/kg ip), the brains were removed and stored in a 20% sucrose/10% formalin solution for at least 3 days before sectioning. Brain sections (50 μm) were mounted onto gelatin-coated glass slides. The brain sections were defatted, stained with cresyl violet, cleared with xylene and cover-slipped. The cannulae placements were verified using a light microscope. Only those animals whose cannulae were within the shell of the NAc were included for statistical analysis. No significant tissue damage was evident upon histological examination of sections.

*2.7. Statistical analyses*

For locomotor activity assays, data are presented as mean total activity counts (± S.E.M.) for the 60-min observation period and the dependent measures were total peripheral, central and vertical (rearing) activity observed during the 60-min test session. Because group comparisons were specifically defined prior to the start of the experiment, planned comparisons were conducted in lieu of an overall $F$ test in a multifactorial ANOVA; this statistical analysis has been supported in a number of statistical texts (e.g., Keppel, 1973). Thus, each experiment was subjected to a one-way ANOVA for repeated measures with levels of the treatment factor corresponding to the drug combinations administered to that group. Planned, pairwise comparisons of the treatment means were made with the least significant difference test (Keppel, 1973; SAS for Windows, Version 8.1), which were conducted with an experimentwise error rate of $\alpha = 0.05$.

For drug discrimination experiments, performance was expressed as the percentage of drug-appropriate responses to total responses before delivery of the first reinforcer and the

response rate was calculated as the total number of responses on either lever divided by the number of minutes taken to complete the FR 20. Only data from animals that completed the FR 20 during the test sessions were used. Student's *t* test for repeated measures was used to compare the percentage of drug-lever responding and response rate during test sessions with the corresponding values for either the previous drug session (substitution tests), or the test dose of the training drug alone (combination tests). All comparisons were made with an experimentwise Type I error rate ($\alpha$) set at 0.05.

## 3. Results

### 3.1. Histology

For each animal included in the analyses below, the injection cannulae projected bilaterally past the outer guide cannulae into the NAc shell (Fig. 1). Inspection of brain tissue revealed slight evidence of gliosis at the site of injection although surrounding tissue was generally intact.

### 3.2. Locomotor activity assays

#### 3.2.1. Intra-NAc shell microinjection of saline

Microinjection of saline into NAc shell followed by systemic injection of saline resulted in levels of activity (Figs. 2 and 3) similar to that reported following systemic saline injection tested alone under identical conditions (McCreary and Cunningham, 1999; McMahon and Cunningham, 2001). Microinjection of saline into the NAc shell followed by systemic injection of cocaine (10 mg/kg ip) resulted in significant increases in peripheral (154–184%), central (167–229%) and vertical (rearing) activity (89–125%) as compared with saline–saline control values ($P < .05$; Figs. 2 and 3); these cocaine-induced increases in activity are similar to those reported following systemic

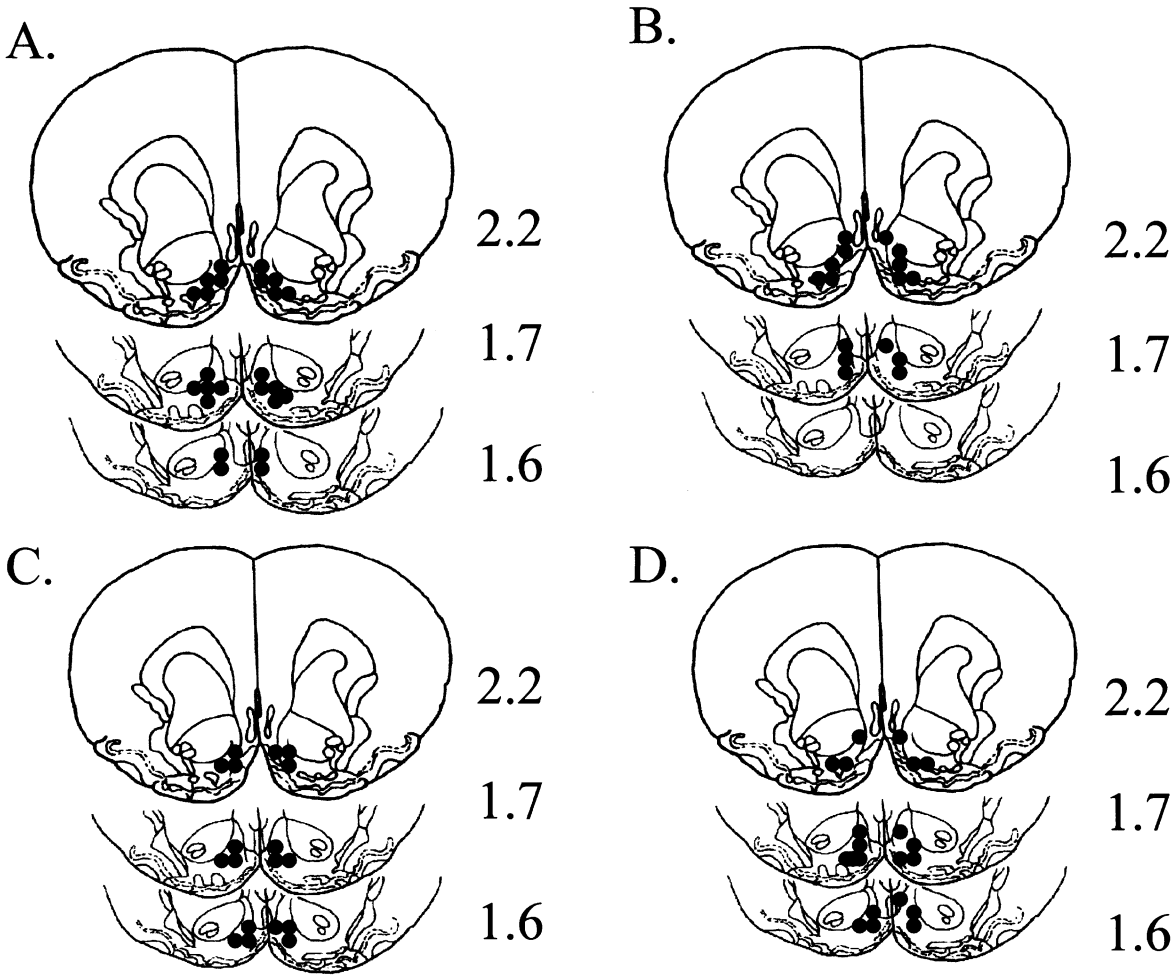


Fig. 1. Histological verification of infusion sites. The location of cannulae placements is provided for animals used for analyses in studies of basal and cocaine-stimulated locomotor activity following microinfusions of (A) MK 212 or (B) RO 60-0175 and for drug discrimination studies of (C) MK 212, RO 60-0175 or (D) RS 102221. Plates are taken from Paxinos and Watson (1998) and the numbers beside each plate correspond to millimeters from bregma. In the interest of space considerations, the placement of cannulae for 12 of 16 rats used in the analysis is shown in (A); placements in the remaining 4 of 16 rats were similarly located.

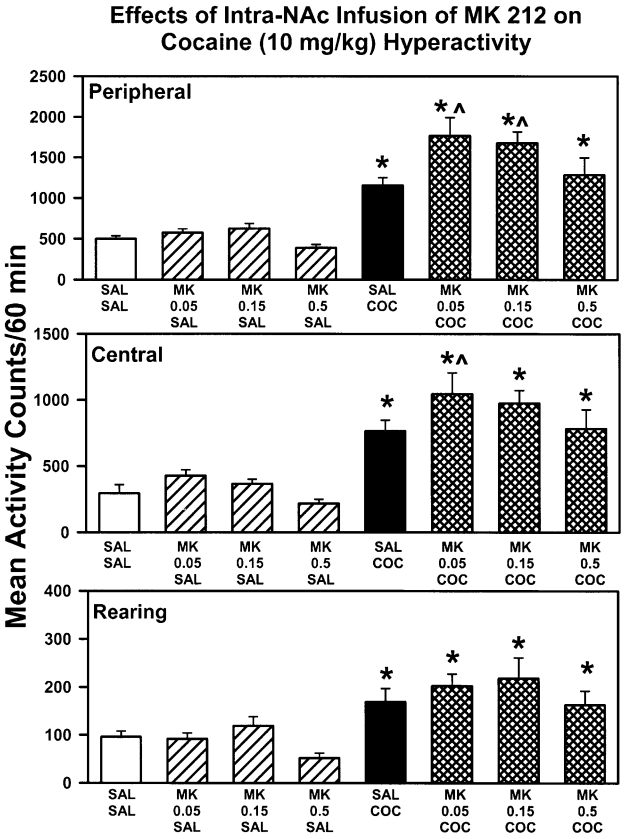


Fig. 2. Basal and cocaine-stimulated activity following intra-NAc shell microinjection of MK 212. The mean activity counts (± S.E.M.) summed over the 60-min recording period after intra-NAc shell microinjection of saline (SAL) or MK 212 (MK; 0.05, 0.15 or 0.5 μg/side), followed by an intraperitoneal injection of saline (SAL) or cocaine (COC; 10 mg/kg), are shown. Peripheral, central and rearing activities are shown on the top, middle and bottom panels, respectively. All data points represent the mean of data from 16 rats. *$P < .05$ vs. SAL–SAL; ^$P < .05$ vs. SAL–COC.

cocaine injection in rats not implanted with guide cannulae tested under identical conditions (McCreary and Cunningham, 1999; McMahon and Cunningham, 2001).

### 3.2.2. Intra-NAc shell microinjection of MK 212

Twenty-two rats received a microinjection of saline or the 5-$HT_{2C}$R agonist MK 212 (0.05, 0.15 or 0.5 μg/side), followed by systemic injection of saline or cocaine (10 mg/kg). Of these, 16 rats exhibited cannulae placements bilaterally positioned in the ventromedial portion of the NAc shell at +1.7 to +2.2 mm posterior to bregma. For these 16 rats, a main effect of treatment was observed for peripheral [$F(7,72) = 12.76$, $P < .001$], central [$F(7,72) = 10.11$, $P < .001$] and vertical (rearing) activity [$F(7,72) = 4.04$, $P < .01$]. Intra-NAc infusion of MK 212 (0.05 and 0.15 μg/side) significantly increased cocaine-evoked peripheral activity ($P < .05$; Fig. 2, top), while 0.05 μg/side MK 212 significantly enhanced central activity evoked by cocaine ($P < .05$; Fig. 2, center). Intra-NAc shell infusion of MK 212 did not alter rearing activity induced by cocaine ($P > .05$; Fig. 2, bottom). Intra-NAc shell pretreatment with MK 212 (0.05–0.5 μg/side) prior to a systemic saline injection did not alter basal locomotor activity ($P > .05$; Fig. 2).

### 3.2.3. Intra-NAc shell microinjection of RO 60-0175

Of the 14 rats originally cannulated and tested, 8 rats exhibited cannulae placements bilaterally positioned in the ventromedial portion of the NAc shell at +1.7 to +2.2 posterior to bregma. For these rats ($n = 8$), a main effect of treatment was observed for peripheral [$F(7,57) = 6.4$, $P < .001$] and central activity [$F(7,57) = 4.97$, $P < .001$], but not for rearing activity [$F(7,57) = 2.48$, $P < .38$; Fig. 3]. A dose-dependent trend toward increased peripheral and central cocaine-evoked activity was observed following intra-NAc pretreatment with RO 60-0175, and intra-NAc RO 60-0175 pretreatment significantly increased cocaine-evoked central activity at 5 μg/side ($P < .05$; Fig. 3, center). Intra-NAc shell pretreatment of RO 60-0175 (0.5–5 μg/side) prior to a systemic saline injection did not alter basal locomotor activity ($P > .05$; Fig. 3).

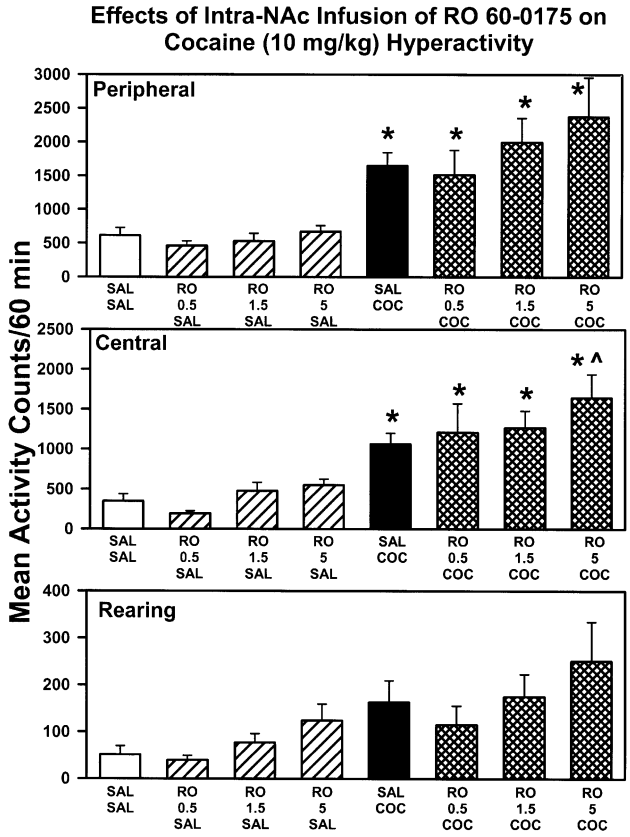


Fig. 3. Basal and cocaine-stimulated activity following intra-NAc shell microinjection of RO 60-0175. The mean activity counts (± S.E.M.) summed over the 60-min recording period after intra-NAc shell microinjection of saline (SAL) or RO 60-0175 (RO; 0.5, 1.5 or 5 μg/side), followed by an intraperitoneal injection of saline (SAL) or cocaine (COC; 10 mg/kg), are shown. Peripheral, central and rearing activities are shown on the top, middle and bottom panels, respectively. All data points represent the mean of data from eight rats. *$P < .05$ vs. SAL–SAL; ^$P < .05$ vs. SAL–COC.

### 3.3. Drug discrimination experiments

#### 3.3.1. Cocaine–saline discrimination and dose–response relationship for cocaine

Acquisition of the cocaine (10 mg/kg) vs. saline discrimination was met in an average of 28 sessions (range: 16–37). After recovery from surgery, the criterion was met in 13 sessions (range: 11–16). Administration of systemic cocaine (0.625–10 mg/kg) produced a dose-dependent increase in cocaine-appropriate responding prior to (data not shown) and after surgical implantation of cannulae (Fig. 4); no difference was observed between the pre- and postsurgical dose–response curves for cocaine (data not shown). Drug-lever responding after 0.625, 1.25 and 2.5 mg/kg cocaine was significantly different from the previous cocaine training session ($P < .05$); response rates for all test doses of cocaine did not differ from that observed during the immediately previous cocaine maintenance session ($P > .05$).

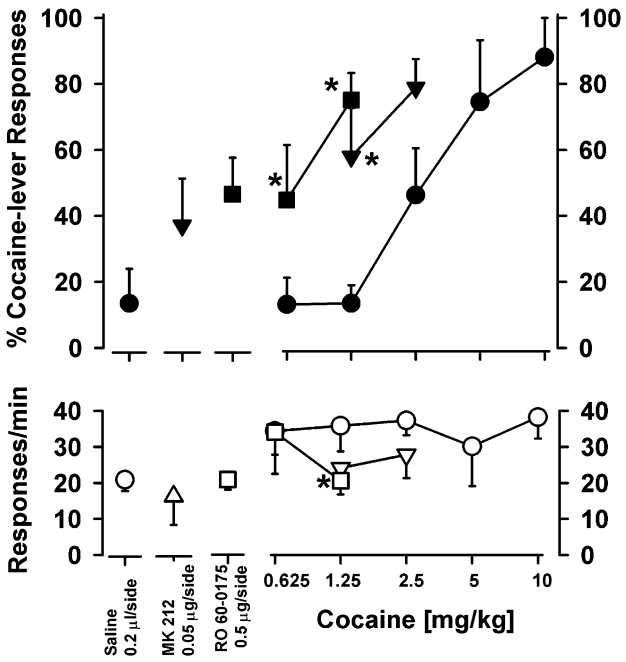


Fig. 4. Effects of intra-NAc shell microinjection of MK 212 or RO 60-0175 in rats trained to discriminate cocaine (10 mg/kg) from saline. Closed symbols (top panel) denote the mean percentage of cocaine-lever responses (± S.E.M.); open symbols (bottom panel) denote the mean number of responses per minute (± S.E.M.). Left: Performance is denoted after intra-NAc infusion of saline (0.2 µl/side; circle), MK 212 (0.05 µg/side; triangle) or RO 60-0175 (0.5 µg/side; square). Right: Performance is denoted after systemic administration of cocaine (0.625–10 mg/kg) preceded by intra-NAc shell infusion of saline (0.2 µl/side; circles) or a fixed dose of MK 212 (0.05 µg/side; triangles) or RO 60-0175 (0.5 µg/side; squares). All data points represent the means of data from 7–9/9 rats [*n*/*N*, number of rats (*n*) completing the FR 20 on either lever out of the number of rats tested (*N*)]. Asterisks (*) represent performances during test sessions that were significantly different from that observed after the appropriate dose of cocaine ($P < .05$).

Control tests were also conducted to assure that the microinjection procedure did not interfere with the discrimination between cocaine and saline. Systemic administration of saline engendered <10% drug-lever responding (data not shown), as did intra-shell NAc microinjection of saline administered prior to a systemic injection of saline (Fig. 4); response rates did not vary between the control test and the previous maintenance saline session. Intra-shell NAc microinjection of saline did not alter cocaine-lever responding seen after systemic injection of cocaine (10 mg/kg); response rates did not vary between the control test and the previous maintenance cocaine session (data not shown).

#### 3.3.2. Intra-NAc shell microinfusion of MK 212 or RO 60-0175

Of the rats originally cannulated and tested, nine rats exhibited cannulae placements bilaterally positioned in the ventromedial portion of the NAc shell at +1.7 to +2.2 posterior to bregma. In substitution tests in these animals, intra-NAc shell infusion of MK 212 (0.05 µg/side) or RO 60-0175 (0.5 µg/side) evoked 37% and 48% drug-lever responding, respectively—values that were significantly different ($P < .05$) from the previous cocaine training session; response rates were unaltered (Fig. 4).

Following pretreatment with intra-NAc shell infusion of MK 212 (0.05 µg/side), 1.25 and 2.5 mg/kg cocaine evoked 58% and 75% drug-lever responding, respectively, compared to 13% and 45% drug-lever responding with these cocaine doses following a microinjection of saline (Fig. 4). A statistically significant enhancement of drug-lever responding was observed for the combination of MK 212 (0.05 µg/side) plus 1.25 mg/kg cocaine ($P < .05$). Intra-NAc microinfusion of MK 212 (0.05 µg/side) did not alter response rates observed following either dose of systemic cocaine (Fig. 4).

A similar observation was noted following pretreatment with RO 60-0175 (0.5 µg/kg). Administration of 0.625 and 1.25 mg/kg cocaine evoked 45% and 75% drug-lever responding, respectively, following intra-NAc infusion of RO 60-0175 (0.5 µg/kg), compared to 11% and 13% drug-lever responding seen with these cocaine doses after microinjection of saline (Fig. 4). A statistically significant enhancement of drug lever responding was observed for the combination of RO 60-0175 (0.5 µg/kg) plus either dose of cocaine (0.625 and 1.25 mg/kg; $P < .05$). Intra-NAc microinfusion of RO 60-0175 administered prior to cocaine (1.25 mg/kg) resulted in a significant reduction in response rate relative to that dose of cocaine tested alone ($P < .05$; Fig. 4).

#### 3.3.3. Intra-NAc shell microinfusion of RS 102221

Of the rats originally cannulated and tested, 10 rats exhibited cannulae placements bilaterally positioned in the ventromedial portion of the NAc shell at +1.7 to +2.2 posterior to bregma. In substitution tests in these rats, RS

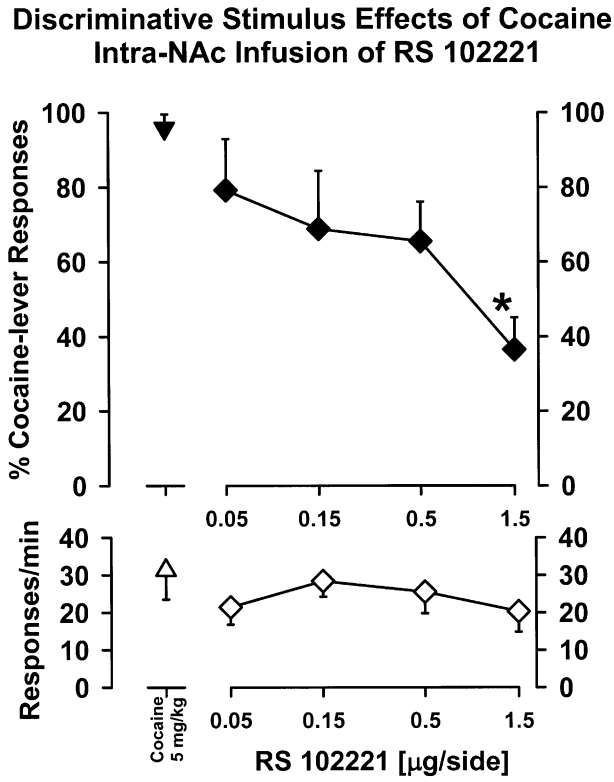


Fig. 5. Effects of intra-NAc shell microinjection of RS 102221 in rats trained to discriminate cocaine (10 mg/kg) from saline. Closed symbols (top panel) denote the mean percentage of cocaine-lever responses (±S.E.M.); open symbols (bottom panel) denote the mean number of responses/min (±S.E.M.). Left: Performance is denoted after intra-NAc infusion of saline (0.2 μl/side) prior to 5 mg/kg cocaine (triangle). Right: Performance is denoted after systemic administration of cocaine (5 mg/kg) preceded by intra-NAc shell infusion of doses of RS 102221 (0.05–1.5 μg/side). All data points represent the means of data from 8–10/10 rats. Asterisks (*) represent performances during test sessions that were significantly different from cocaine (5 mg/kg) administered alone ($P<.05$).

102221 (1.5 μg/side) evoked 2% drug-lever responding and that value was significantly different from the previous cocaine training session ($P<.05$); response rate was unaltered (data not shown). Following pretreatment with intra-shell NAc microinfusions of RS 102221 (0.05–1.5 μg/side), a dose-dependent attenuation of the discriminability of 5 mg/kg cocaine, which alone elicited ~95% drug-lever responding, was observed; pretreatment with RS 102221 did not affect response rates (Fig. 5).

## 4. Discussion

We report that local infusion of the 5-$HT_{2C}$R agonists MK 212 and RO 60-0175 into the NAc shell enhanced the hyperlocomotive effects of cocaine, extending our observation that intra-NAc shell infusion of the 5-$HT_{2C}$R antagonist RS 102221 blocked the hyperactivity induced by systemic cocaine administration (McMahon et al., 2001). Using drug discrimination techniques that model the subjective effects of cocaine, we found that local infusion of the 5-$HT_{2C}$R agonists MK 212 and RO 60-0175 into the NAc shell enhanced the discriminability of low doses of cocaine, resulting in an upward shift in the dose–response curve for cocaine. On the other hand, intra-NAc shell infusion of the selective 5-$HT_{2C}$R antagonist RS 102221 dose-dependently attenuated the recognition of the stimulus properties of cocaine. Our findings are internally consistent with the hypothesis that 5-HT efferents to the NAc shell and activation of 5-$HT_{2C}$R play an important modulatory role in the expression of the locomotor stimulant as well as the discriminative stimulus effects of cocaine, which are thought to model the subjective effects of cocaine in humans.

A number of neurotransmitter afferents to the NAc and receptors localized within the NAc have been implicated in the generation of the behavioral effects of cocaine based upon the results of intra-NAc microinfusion studies. Thus, for example, intra-NAc infusion of the DA neurotoxin 6-hydroxydopamine (Phillips et al., 1983), the DA $D_1$R-like antagonist SCH 23390 (Callahan et al., 1994), the DA $D_2$R-like antagonist sulpiride (Neisewander et al., 1995), a DA $D_5$R antisense oligonucleotide (Filip et al., 2000), the 5-$HT_3$R antagonist ondansetron (Herges and Taylor, 2000), and the glutamate antagonist L-glutamic acid diethyl ester (Pulvirenti et al., 1989), all share with the 5-$HT_{2C}$R antagonist RS 102221 (present results; McMahon et al., 2001), the ability to block some components (i.e., hyperlocomotive, discriminative stimulus, conditioned place preference and/or reinforcing effects) of the behavioral effects of cocaine (although some discrepancies do exist; Neisewander et al., 1995). Fewer assessments following intra-NAc infusion of receptor agonists have been conducted; however, the γ-aminobutyric acid (GABA) $GABA_B$R agonist baclofen (Shoaib et al., 1998), has been shown to block self-administration of cocaine and the present study indicates that the 5-$HT_{2C}$R agonists MK 212 and RO 60-0175 enhance the hyperlocomotive and stimulus effects of cocaine. Thus, it appears that the NAc is subject to a number of regulatory controls, although the pre- vs. postsynaptic localization of each of these receptors to control NAc output is not entirely clear nor is the extent to which these neurotransmitter receptors utilize distinct vs. overlapping transduction mechanisms to control NAc output.

Several considerations are necessary when assessing the mechanisms of action that underlie the influence of intra-NAc infusions of MK 212, RO 60-1075 and RS 102221 on the in vivo effects of systemically administered cocaine. The transcript for 5-$HT_{2C}$R appears to localize to medium-sized neurons (not large neurons or glial cells) that express the distribution, localization and morphology typical of striatal GABA efferent neurons (Eberle-Wang et al., 1997; Morilak et al., 1993). In vivo studies have shown that, while iontophoretic application of DA inhibited both Type I and Type II neurons in the NAc, 5-HT excited Type II

neurons in vivo (White et al., 1993)—an effect which suggests that the presence of 5-HT might serve to balance the inhibitory actions of DA in some NAc neurons. This excitatory effect of 5-HT in vivo is mirrored in in vitro slice studies of the NAc in which 5-HT was shown to depolarize 84% of neurons—an effect that was blocked by the 5-$HT_{2A/2C}$R antagonist ketanserin (North and Uchimura, 1989). If these recorded neurons and the neurons in the NAc shell, which respond to 5-$HT_{2A/2C}$R manipulation in the present study, are speculatively conceded to overlap, 5-HT acting at a 5-$HT_{2A/2C}$R would be expected to alter local neural interplay, the negative feedback regulation of VTA DA neurons by GABA neurons in the NAc and/or primary output circuits of the NAc to the extended amygdala (Heimer et al., 1991; Kalivas et al., 1993). Few experimental analyses speak directly to this hypothesis at present, although a number of studies have implicated 5-$HT_{2C}$R in the control of DA release in NAc. For example, perfusion of the 5-$HT_2$R agonist 1-(2,5-dimethoxy-4-iodo)-2-aminopropane (DOI) through a dialysis probe increased DA efflux in the NAc (Bowers et al., 2000; Lucas and Spampinato, 2000; Yan, 2000)—an effect that was reversed by coperfusion with a nonselective 5-$HT_2$R antagonist (Yan, 2000) or the 5-$HT_{2C/2B}$R antagonist SB 206553, but not by the 5-$HT_{2A}$R antagonist SR 46349B (Lucas and Spampinato, 2000). Thus, this enhancement of DA release in NAc consequent to perfusion with DOI appears to be mediated by the 5-$HT_{2C}$R. However, intra-NAc shell infusion of 5-$HT_{2C}$R agonists does not appear to alter basal locomotor activity nor mimic the stimulus effects of cocaine (present results; McMahon et al., 2001). In contrast, cocaine effectively generates hyperlocomotive (Delfs et al., 1990), stimulus (Callahan et al., 1994) and reinforcing effects of cocaine (McKinzie et al., 1999) upon local infusion into the NAc—an effect mediated at least in part through a local enhancement of DA efflux. These data suggest that increased DA release consequent to intra-NAc shell infusion of the 5-$HT_{2C}$R agonists is not of sufficient magnitude to *generate* these behaviors, but can trigger processes sufficient to *enhance* the behavioral effects of cocaine.

While the influence of intra-NAc application of 5-$HT_{2C}$R agonists on the behavioral effects of cocaine may be related to locally potentiated DA release, a complex cascade of 5-$HT_{2C}$R-generated events is required to explain this outcome. DA neurons of the VTA or substantia nigra (SN) do not contain appreciable levels of 5-$HT_{2C}$R mRNA (Eberle-Wang et al., 1997), suggesting that membranes of DA terminals in the NAc are unlikely to possess 5-$HT_{2C}$R that controls DA release presynaptically. On the other hand, the transcript for 5-$HT_{2C}$R has been colocalized to GABA neurons in VTA and SN (Eberle-Wang et al., 1997) and electrophysiological studies suggest that stimulation of 5-$HT_{2C}$R excites the activity of GABA neurons in VTA and SN (e.g., Di Giovanni et al., 2001; Liu et al., 2000). If this is the case for 5-$HT_{2C}$R in the NAc, we speculate that 5-$HT_{2C}$R stimulation might disrupt local GABA circuits within the NAc and also negative feedback loops to the VTA—actions which could contribute to the increased NAc DA efflux seen upon local perfusion with a 5-$HT_2$R agonist (Bowers et al., 2000; Lucas and Spampinato, 2000; Yan, 2000). Although we present this as a plausible possibility, a number of other prospective chemical candidates may contribute, including additional excitatory factors (e.g., acetylcholine, glutamate or nitric oxide) which could result in DA release as a consequence of local actions of 5-$HT_{2C}$R in the NAc. Nonetheless, the net effects of 5-$HT_2$R agonists on GABA and DA function in the NAc shell may underlie the ability of 5-$HT_2$R agonists to enhance cocaine-evoked behaviors upon local administration.

A key step in characterizing and understanding the influence of the NAc 5-$HT_{2C}$R on behaviors generated by systemically administered cocaine is the utilization of the most selective 5-$HT_{2C}$R ligands available. In binding studies of rodent brain tissue, the 5-$HT_{2C}$R agonists MK 212 and RO 60-0175 display >16-fold and >25–100-fold selectivity (Bös et al., 1997; Kennett, 1993; Martin et al., 1998; Porter et al., 1999), respectively, whereas the 5-$HT_{2C}$R antagonist RS 102221 displays ~35-fold selectivity for 5-$HT_{2C}$R over other receptors, including the 5-$HT_{2A}$R (Bonhaus et al., 1997). The selectivity of RS 102221, as shown in binding studies, is in keeping with in vivo observations in which RO 60-0175 did not exhibit efficacy at 5-$HT_{2A}$R to evoke head shakes (Martin et al., 1998). Furthermore, the neuropharmacological profiles of the discriminative stimulus properties of MK 212 (Cunningham et al., 1986) and RO 60-0175 (Dekeyne et al., 1999) indicate the dependence of this behavior on selective stimulation of 5-$HT_{2C}$R.

Both MK 212 and RO 60-0175 do act as potent 5-$HT_{2B}$R agonists in cells transfected with the human recombinant 5-$HT_{2B}$R (Martin et al., 1998; Porter et al., 1999). However, the preferential 5-$HT_{2B}$R agonist BW 723C86, and the 5-$HT_{2B}$R antagonist SB 204741 did not substitute for nor antagonize the stimulus effects of RO 60-0175, respectively (Dekeyne et al., 1999). Based upon the receptor affinity and behavioral profiles of MK 212, RO 60-0175 and RS 102221, the extremely low expression of the 5-$HT_{2B}$R in the rat (Duxon et al., 1997) and human brain (Kursar et al., 1994) and their absence in the NAc (Duxon et al., 1997), our data provide evidence for a role of NAc shell 5-$HT_{2C}$R over 5-$HT_{2A}$R or 5-$HT_{2B}$R in control of the hyperlocomotive and discriminative stimulus effects of cocaine.

Intra-NAc infusion of RS 102221 dose-dependently suppressed the hyperlocomotive (McMahon et al., 2001) and stimulus effects of systemically administered cocaine (present results). In contrast, RS 102221 did not alter cocaine-induced hyperactivity upon infusion into the VTA, although intra-VTA microinfusion of the selective 5-$HT_{2A}$R antagonist M100907 blocked expression of cocaine-evoked hyperactivity; M100907 was ineffective

upon microinjection into the NAc (McMahon et al., 2001). Taken together, these findings suggest that separate populations of 5-$HT_2$R resident within the VTA vs. NAc differentially function to control the mesoaccumbens pathway. Such differential regulation of this circuit may help to clarify the observed, mixed influence of systemically administered nonselective 5-$HT_2$R antagonists in previous studies of the behavioral effects of cocaine. Nonselective 5-$HT_{2A/2C}$R antagonists (e.g., ketanserin) have been reported to attenuate the hyperlocomotive (Herges and Taylor, 1998; McMahon and Cunningham, 2001; O'Neill et al., 1999) and discriminative stimulus effects of cocaine in squirrel monkeys (Schama et al., 1997) and rats (McMahon and Cunningham, 2001), but to be ineffective in other studies (Callahan and Cunningham 1995; Meert and Janssen, 1992; Peltier et al., 1994). Ketanserin, but not another 5-$HT_{2A/2C}$R antagonist ritanserin, was also reported to decrease the response rate of cocaine self-administration in squirrel monkeys (Nader and Barrett, 1990) and cocaine intake in rats (Lacosta and Roberts, 1993). Perhaps the outcome of pharmacological experiments with 5-$HT_{2A/2C}$R antagonists depends not only on the behavior measured, the receptor affinities of the ligand and its biodistribution, but also on the functional balance between 5-$HT_{2A}$R and 5-$HT_{2C}$R in the mesoaccumbens pathway and in those regions that provide important efferent control of this pathway (e.g., prefrontal cortex). Likewise, the balance between the function of 5-$HT_{2C}$R in the mesoaccumbens pathway with 5-$HT_{2C}$R located distally may also be an important contributor to the overall picture. Preliminary data from our laboratory indicate that microinfusion of a 5-$HT_{2C}$R agonist or antagonist into the rat prefrontal cortex actually reduced or enhanced, respectively, the locomotor and discriminative behaviors of cocaine (Filip and Cunningham, 2000a,b). These findings might help explain the discrepancy between systemic and intracranial application of 5-$HT_{2C}$R agonists: systemic 5-$HT_{2C}$R agonists suppressed the hyperlocomotive (Grottick et al., 2000), stimulus (Callahan and Cunningham, 1995) and reinforcing effects of cocaine (Grottick et al., 2000) in contrast to the clear directional effects of the 5-$HT_{2C}$R agonists and antagonists on cocaine-induced behaviors after local application to the NAc shell. Thus, the net effect of simultaneous activation (or blockade) of 5-$HT_{2C}$R in NAc shell and prefrontal cortex following systemic administration of a 5-$HT_{2C}$R ligand may be due to a potentially oppositional contribution of distally located 5-$HT_{2C}$R to the ultimate behavioral outcome of systemic drug studies.

In summary, both the hyperactive and discriminative stimulus effects evoked by systemic administration of cocaine were enhanced by application of 5-$HT_{2C}$R agonists into the NAc shell and blocked by antagonism of 5-$HT_{2C}$R in the NAc shell. These results extend our previous observations (McMahon et al., 2001) to include an animal model of the interoceptive effects of cocaine in humans and reinforce the hypothesis that the role of 5-$HT_{2C}$R in the regulatory neurochemistry of the NAc shell is important to the full expression of behaviors evoked by cocaine.

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