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Injection of the 5-HT_{2C} Receptor Agonist Ro60-0175 into the Ventral Tegmental Area Reduces Cocaine-Induced Locomotor Activity and Cocaine Self-Administration

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Previously, we have shown that systemic administration of the 5-HT_{2C} receptor agonist Ro60-0175 reduces cocaine-induced locomotor activity and cocaine self-administration. Ro60-0175 also alters the activity of midbrain dopamine (DA) neurons of the ventral tegmental area (VTA), a region where 5-HT_{2C} receptors are expressed. The present experiments investigated whether microinjections of Ro60-0175 into the VTA would alter the locomotor stimulant effect of cocaine and cocaine self-administration. In the tests for locomotor activity injection of 3 and 10, but not 1 μg, Ro60-0175 into the VTA reduced the locomotor stimulation resulting from injection of 10 mg/kg cocaine. In tests of cocaine self-administration, rats were trained to lever press for intravenous infusions of 0.25 mg cocaine delivered on either a fixed ratio 5 (FR5) or a progressive ratio schedule. Intra-VTA injection of Ro60-0175 at doses of 3 and 10 μg reduced responding for cocaine on both schedules without significantly altering the latency to initiate responding or the rate of responding. A subsequent experiment determined that the suppressant effect of intra-VTA Ro60-0175 (3 μg) on responding for cocaine was prevented by pretreatment with the selective 5-HT_{2C} receptor antagonist SB242,084 (0.5 mg/kg). In a final experiment, intra-VTA injection of Ro60-0175 reduced responding for food reinforcement on the same progressive ratio schedule as used for cocaine self-administration. These results demonstrate that stimulation of 5-HT_{2C} receptors in the VTA is sufficient to attenuate the stimulant and reinforcing effects of cocaine. These effects complement electrophysiological and neurochemical findings, and indicate that 5-HT_{2C} receptors localized within the VTA modulate the activity of mesolimbic DA neurons.
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INTRODUCTION

It is well established that serotonin (5-hydroxytryptamine; 5-HT) can modulate the activity and functioning of dopaminergic neurons. This modulation of dopamine (DA) function by serotonergic processes is complex, involving both excitatory and inhibitory influences (Bankson and Cunningham, 2001; Saito *et al*, 1996 for reviews). The direction of modulation may depend in part on which of the multiple 5-HT receptor subtypes are involved (eg Fletcher *et al*, 2002). At least 14 distinct 5-HT receptors have now been cloned (Boess and Martin, 1994), the majority of which appears to play functional roles in the CNS (Barnes and Sharp, 1999; Hoyer *et al*, 2002), including modulation of dopaminergic neurotransmission. Over the past few years, neuroanatomical, electrophysiological, neurochemical, and behavioral analyses have revealed that the 5-HT_{2C} receptor subtype exerts a marked inhibitory influence over the activity of midbrain DA systems (reviewed by Di Matteo *et al*, 2002).

The 5-HT_{2C} receptor has a widespread distribution in mammalian brain tissue, and is especially abundant in dopaminergic cell body regions of the substantia nigra and ventral tegmental area (VTA) as well as in terminal projection areas of the nucleus accumbens, striatum, and prefrontal cortex (Pompeiano *et al*, 1994; Abramowski *et al*, 1995; Eberle-Wang *et al*, 1997). The moderately selective 5HT_{2C} receptor agonist Ro60-0175 (Martin *et al*, 1998) reduces the firing rate of mesolimbic DA neurons originating in the ventral tegmental area (Di Matteo *et al*, 2000), leading to a reduction in DA release in terminal regions of the nucleus accumbens and frontal cortex

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(Di Matteo *et al*, 2000; Gobert *et al*, 2000). These effects are reversed by the selective 5-HT$_{2C}$ receptor antagonist SB242,084 (Di Matteo *et al*, 2000; Gobert *et al*, 2000). Additionally, by itself, SB242,084 increases the burst-firing of dopaminergic neurons in the VTA, leading to increased release of DA in the nucleus accumbens (Di Matteo *et al*, 1999). Thus, it appears that 5-HT$_{2C}$ receptors may exert a tonic inhibitory influence over the activity of ascending DA neurons.

In order to determine whether this 5-HT$_{2C}$ receptor-mediated modulation of DA neuronal activity has a potential functional role at the behavioral level, we have investigated the effects of 5-HT$_{2C}$ receptor ligands on the behavioral effects of cocaine. We have used cocaine in this work since its stimulant action and ability to act as a positive reinforcer depend in large part on elevating DA levels in the nucleus accumbens subsequent to blockade of the DA transporter (Roberts *et al*, 1980; Pettit and Justice, 1991; Di Ciano *et al*, 1995). Consequently, the effects of cocaine depend upon the firing activity of mesolimbic DA neurons. We have previously reported that systemic administration of the moderately selective 5HT$_{2C}$ receptor agonist Ro60-0175 attenuated the locomotor stimulant effect of 15 mg/kg cocaine, and this effect was reversed by the selective 5-HT$_{2C}$ receptor antagonist SB242,084 (Grottick *et al*, 2000). Ro60-0175 also reduced cocaine self-administration and attenuated the response-reinstating effect of cocaine in animals with a prior history of cocaine self-administration (Grottick *et al*, 2000). In contrast to these effects, SB242,084 potentiated the locomotor activity induced by 10 mg/kg cocaine, increased self-administration of cocaine, and potentiated the ability of cocaine to reinstate lever pressing in rats whose self-administration behavior had been extinguished (Fletcher *et al*, 2002). These bi-directional effects of 5-HT$_{2C}$ agonists and antagonists on the behavioral effects of cocaine are consistent with the bi-directional effects of these drugs on electrophysiological and neurochemical aspects of dopaminergic activity.

Given the importance of the dopaminergic neurons originating in the VTA for mediating the stimulant and reinforcing effects of cocaine, it is plausible that 5-HT$_{2C}$ receptors located in this structure could mediate the effects of 5-HT$_{2C}$ receptor ligands on cocaine-induced behaviors. The primary purpose of the present experiments was to investigate this possibility, and to examine whether selective activation of 5-HT$_{2C}$ receptors in the VTA modified the expression of DA-dependent behaviors. Specifically, we examined the effects of injecting Ro60-0175 into the VTA on cocaine-stimulated locomotor activity and cocaine self-administration. Additionally, we determined the effects of this manipulation on the reinforcing effects of a nondrug reinforcer, food.

MATERIALS AND METHODS

Subjects

Adult male Sprague–Dawley rats (Charles River, Quebec) weighing 280–320 g at the beginning of each study were used. They were housed in clear plastic, rectangular, solid-bottomed cages; rats used for locomotor activity tests were housed in pairs, while those used in the operant studies were housed singly. The housing room was maintained on a 12 h light/dark cycle (lights on at 0800) and at a temperature of 22 $\pm$ 2°C. Animals used in locomotor activity tests had food and water available freely at all times. For rats in the operant studies, access to food was restricted as detailed below. All training and testing were conducted during the light phase. Experimental procedures and manipulations conformed to the guidelines laid down by the Canadian Council on Animal Care and were approved by the CAMH Animal Care Committee.

Surgery and Histology

The rats were anesthetized with sodium pentobarbital (Somnotol, 45–50 mg/kg i.p.). For cannula implantation, rats were placed in a stereotaxic frame with the incisor bar set at 5 mm above the interaural line. Stainless-steel guide cannulae (23 g) were implanted bilaterally, at an angle of 10° to the vertical, in the vicinity of the VTA. The cannulae were positioned 2 mm above the intended injection site, according to the coordinates: AP -3.0 mm, L $+3.4$ mm, D/V -5.9 mm relative to bregma (Pellegrino *et al*, 1979). Cannulae were anchored to the skull using jeweler's screws and dental cement. Stainless-steel (28 g) wire obdurators were used to keep the cannulae clear. At the completion of the experiments, rats were deeply anaesthetized with Somnotol and a volume of 0.5 µl fast-green dye was injected into each brain site to aid in the localization of injection sites. The brains were removed and stored in formaldehyde for at least 7 days, and then stored in 30% sucrose solution. Brains were then frozen, cut in a cryostat in 40 µm sections, and stained with cresyl violet. Approximately 10% of the total number of animals used were found to have one or both injection sites located outside the VTA. These animals were not used in the data analysis, and the group sizes given below reflect this adjustment.

For the cocaine self-administration studies, rats underwent a second surgical procedure to implant a catheter into the jugular vein. Catheters were constructed from two lengths of silastic tubing, differing in outer diameter, and connected by a small piece of heat-shrunk tubing. The smaller diameter tubing (OD, 0.025 in) was inserted into the right jugular vein. The larger diameter tubing (OD, 0.046 in) was connected to a length of 22 g stainless-steel tubing that was cemented inside a nylon bolt. This terminal end of the catheter exited between the scapulae, and was anchored there by means of sutures and a small piece of Marlex mesh. Following surgery, animals were injected with the antibiotic Penlong (1 ml/kg) to minimize the incidence of postsurgical infection. Catheters were flushed daily with 0.05–0.1 ml of a 0.9% saline solution containing 5 IU/ml heparin and 800 IU streptokinase to maintain patency. Rats were allowed a 1-week period to recover from surgery.

Locomotor Activity

Tests of locomotor activity were conducted in four clear Plexiglas activity chambers (Med Associates Inc., St Albans, VT) measuring 43 cm long, 43 cm wide, and 30 cm high. An array of 16 $\times$ 16 photodetectors, spaced 2.5 cm apart, and positioned 2.5 cm above the floor of the chamber was used

to detect locomotor activity. The software allowed a distinction to be made between repetitive interruptions of the same photobeam and interruptions of adjacent photobeams. This latter measure was used as an index of ambulatory activity.

Three groups of rats were used to investigate the effects of 1 ($n=10$), 3 ($n=11$) and 10 μg ($n=10$) Ro60-0175 injected into the VTA on the locomotor stimulation induced by 10 mg/kg cocaine HCl (BDH Inc., Toronto, Ontario). A repeated measures design was used, with each rat receiving all four possible combinations of Ro60-0175 or its vehicle (intra-VTA), and cocaine or saline (ip). Prior to any testing, all rats were first habituated to the apparatus by placing them in the activity chambers for 1 h on three consecutive days. On test days, rats were placed in the activity chamber for a 30-min habituation period. At the end of this period, rats received the appropriate treatment injected into the VTA immediately followed by ip injection of cocaine or saline. Locomotor activity was then measured for the next 90 min. In each group, the order of drug treatments was determined from a Latin square.

Ro60-0175 was injected into the VTA using a 30 g stainless-steel injector that extended 2 mm beyond the guide cannula tip, attached to a Hamilton syringe via a length of Tygon tubing. A volume of 0.5 μl was delivered over approximately 90–120 s with the needle left in place for a further 1 min to minimize reflux up the cannula shaft.

Cocaine Self-Administration

Testing was conducted in operant chambers measuring 28 cm long, 21 cm wide and 21 cm high (Med. Associates Inc., St Albans, VT, USA). Each chamber contained two response levers 4.5 cm wide and 7 cm above the floor of the chamber, and a stimulus light located 6 cm above each lever. A counterbalanced arm held a fluid swivel above the ceiling of the chamber. The swivel was attached at one end by Tygon tubing to a syringe mounted on a motor-driven syringe pump (Razel) located outside the chamber. At the other end of the swivel, a length of Tygon tubing, encased in a stainless-steel tether, connected the animal's catheter to the syringe via the swivel. Each chamber was illuminated by a houselight and housed in a sound-attenuating box equipped with a ventilating fan. The apparatus was controlled, and the data collected, by a 386-SX IBM-type computer.

Prior to surgery, rats were trained to lever press for food pellets. Rats were food restricted (approximately 18 g/day), placed in the operant chambers, and trained to press the left lever for food (45 mg Noyes pellets), according to a fixed ratio (FR) 1 schedule. Rats were allowed a maximum of 100 pellets during daily 30-min sessions. Any rats failing to obtain 100 pellets by the third day of training were placed in the operant boxes overnight and allowed 300 food pellets delivered, according to the FR1 schedule. A stainless-steel dish filled with water was also placed inside the operant chamber during this session. Thereafter, rats were placed in the chamber only during the 30-min daytime session. Once rats had earned 100 pellets on each of three consecutive days, they were considered lever-trained, and were subsequently maintained on approximately 25 g of lab chow per day.

FR5TO60s Schedule

At 6 days after surgery, rats were placed in the operant chambers for a 1 h drug self-administration session. The session began with illumination of the houselight, and 5 s later a noncontingent infusion of 0.25 mg cocaine (dissolved in 0.1 ml sterile saline) was delivered over 5 s. The infusion was accompanied by illumination of the stimulus light above the lever. This light remained illuminated for a 60-s time-out (TO) period during which responses were recorded but did not have any programmed consequences. For the remainder of the session, cocaine was available according to an FR1TO60s schedule. Over the course of the next 2 weeks, the FR value was raised to 3 and then to 5. After a further week, responding on the FR5TO60s schedule was stable and drug testing began. Each animal was tested four times following injection of vehicle, 1, 3, and 10 μg Ro60-0175. Injections were administered immediately before self-administration sessions began. As far as possible, doses were administered in counterbalanced order, with approximately equal numbers of rats receiving each dose level on each test day. Successive test days were 3–4 days apart; on intervening days, rats were run as usual. In total, 10 rats with accurate cannulae placements completed this study.

Progressive Ratio Schedule

At 1 week after catheters were implanted, rats were allowed to respond for infusions (0.1 ml during 5 s) of cocaine (0.25 mg/kg/infusion) on an FR1 schedule. Each infusion was accompanied by a stimulus light that remained on for a 20-s TO period after the infusion. Once responding was stable, a progressive ratio schedule was implemented in which the number of responses required to obtain an infusion increased for successive infusions. The progression was derived from the equation: response ratio $=(5\times e^{(0.2\times \text{infusion no.})}-5)$, and yielded response ratios of 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, etc. (Richardson and Roberts, 1996). Sessions lasted until a period of 1 h without an infusion had elapsed, or were a maximum of 5 h in length. The number of infusions earned before this breaking point was recorded. The infusion dose was held constant at 0.25 mg throughout. Testing began when break points did not vary by more than 15% on three consecutive days.

One group of rats ($n=10$) was used to test the effects of different doses of Ro60-0175 on cocaine self-administration. Rats were tested four times, following injection of vehicle, 1, 3, and 10 μg Ro60-0175 into the VTA. Tests were spaced at least 72 h apart and the order of treatments was determined from a Latin square.

A second group of rats ($n=8$) was used to examine the effects on cocaine self-administration of pretreatment with the 5-HT$_{2C}$ antagonist SB242,084 (0.5 mg/kg), or its vehicle-injected IP, prior to injection of 3 μg Ro60-0175 or its vehicle into the VTA. SB242,084 was injected 30 min prior to Ro60-0175. All rats were tested under the four treatment combinations, which were given at 72 h intervals according to a Latin square design. In all experiments, daily self-administration sessions were run as usual on the days in between drug-testing sessions.

Responding for Food

Rats were restricted to approximately 18 g food per day, given at least 2 h after the conclusion of testing, and trained to lever press for food pellets under an FR1 schedule as described above. The same progressive ratio schedule as used for cocaine self-administration was implemented; however, sessions terminated after a 20-min period had elapsed without a reinforcer delivery. Once responding was stable, one group ($n=9$) of rats was used to investigate the effects of different doses of Ro60-0175 on responding for food. Rats were tested four times, following injection of vehicle, 1, 3, and 10 μg Ro60-0175 into the VTA. A second group of rats ($n=6$) was used to examine the effects on responding for food of pretreatment with the 5-HT$_{2C}$ receptor antagonist SB242,084, or its vehicle, injected ip. 30 min prior to injection of 10 μg Ro60-0175, or its vehicle, into the VTA. In both experiments, tests were spaced at least 72 h apart and the order of treatments was determined from a Latin square.

Data Analysis

For locomotor activity studies, the dependent variable was ambulatory counts. For experiments involving responding for cocaine or food, the primary dependent variable measured was the number of cocaine infusions, or the number of food pellets earned. Additionally, the latency to respond for the first reinforcer and the mean inter-reinforcer interval was calculated. This latter measure was derived after discarding the interval to the first and last reinforcer (Depoortere *et al*, 1993). Data were analyzed by one-, two-, or three-way analysis of variance. *Post hoc* testing for locomotor activity data, and for data from the study examining the interaction between Ro60-0175 and SB242,084 on cocaine self-administration, was conducted using the LSD test. Dunnett's test for comparisons against a control mean was used to determine the effectiveness of Ro60-0175 on responding for cocaine and food.

Drugs

Ro60-0175 ((S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$) and SB242,084 (6-chloro-5-methyl-1-(2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl) indoline) were synthesized within the PRPN Chemistry department at F Hoffmann-La Roche Ltd, Basel. Ro60-0175 was dissolved in 0.9% saline and the pH was adjusted to approximately 7.0. SB242,084 was prepared in 0.9% saline solution containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid. Cocaine hydrochloride was purchased from BDH Inc., Toronto, Ontario, Canada. Cocaine was dissolved in sterile 0.9% saline. A 0.22-μm filter placed between the syringe and the drug delivery line was used to maintain sterility of the solution.

RESULTS

Injection sites within the VTA were generally found to lie within the VTA as defined by the atlas of Pellegrino *et al* (1979). Injection sites were distributed throughout the entire rostral–caudal extent of the VTA. Figure 1 shows a photomicrograph of typical cannulae placements and injection sites located within the VTA.

Locomotor Activity

The upper panels of Figure 2 show the effects of 1, 3, and 10 μg of Ro60-0175 injected into the VTA on the locomotor stimulant effect of 10 mg/kg cocaine measured across 90 min. For the group treated with 1 μg Ro60-0175, analysis of variance revealed only a significant main effect of cocaine ($F_{1,9}=33.2$, $p<0.01$). The pattern of results obtained for rats treated with 3 μg and 10 μg was similar in both cases. Analysis of variance revealed significant main effects of cocaine ($F_{1,10}=26.4$ and $F_{1,9}=42.6$, both $p<0.001$, for 3 and 10 μg, respectively) and of Ro60-0175 ($F_{1,10}=14.0$ and $F_{1,9}=17.7$, both $p<0.01$). The interaction between Ro60-0175 and cocaine was also significant in both groups ($F_{1,10}=6.78$ and $F_{1,9}=20.5$, both $p<0.03$). *Post hoc* testing confirmed that activity was increased by cocaine, and that this effect was significantly attenuated by both doses of Ro60-0175. Both doses of Ro60-0175 showed a tendency to reduce locomotor activity, but these effects were not statistically significant using the *post hoc* LSD test, involving a pooled error term derived from the overall analysis of variance. A more restricted analysis using a one-tailed *t*-test showed that the difference between rats treated with 3 μg Ro60-0175 and vehicle-treated rats was of borderline significance ($t_{10}=2.23$, $p=0.05$). However, 10 μg Ro60-0175 did not significantly reduce activity compared to vehicle-treated rats ($t_9=1.01$, $p>0.1$).

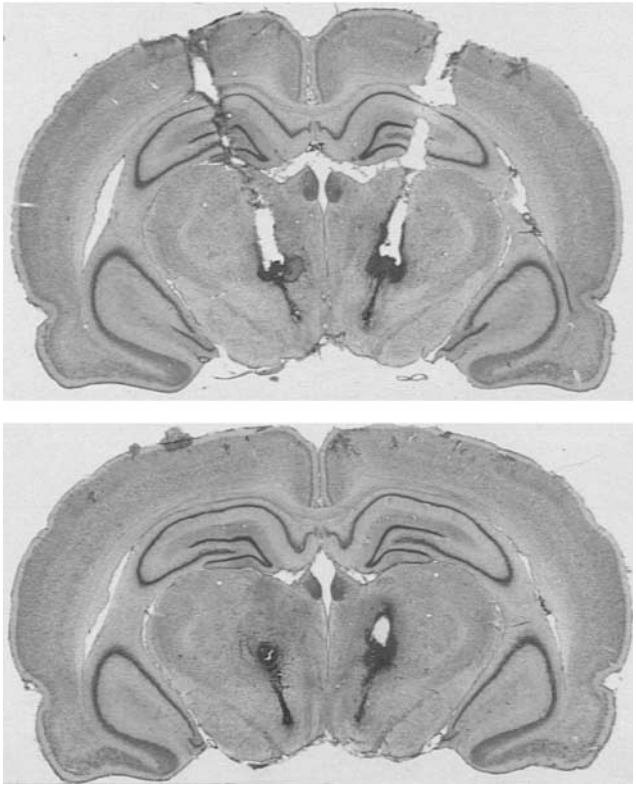


Figure 1 Photomicrograph illustrating typical cannulae placements at the level of the ventral tegmental area.

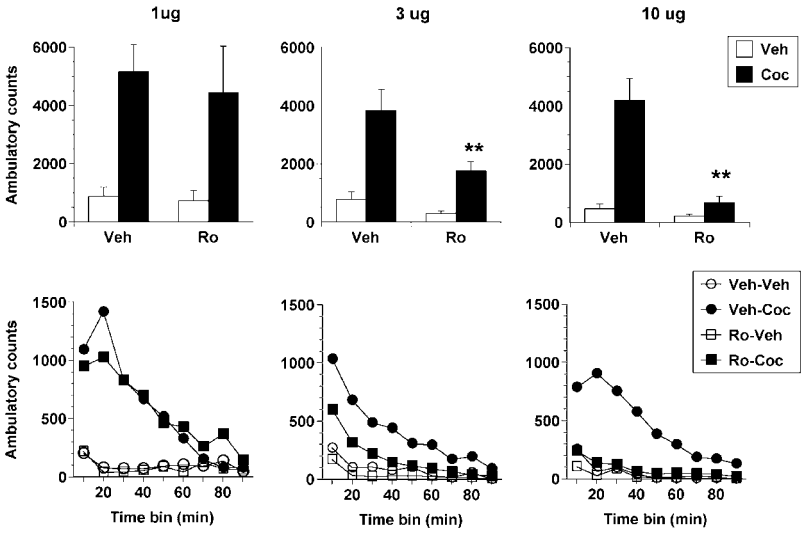


Figure 2 Effects of injecting Ro60-0175 into the VTA on locomotor activity induced by 15 mg/kg cocaine. Separate groups of rats were used to determine the effects of 1 ($n=10$), 3 ($n=11$) and 10 ($n=10$) μg Ro60-0175. The upper panels show the effects of drug treatments on the total number of ambulatory counts over the 90-min test period. The lower panels show the time course of the drug treatments in 10-min bins. *$p<0.05$; **$p<0.01$ compared to Veh-Coc treatment.

The lower panels of Figure 2 illustrate the time course of the effects of Ro60-0175 on cocaine-induced locomotion. Results of the statistical analyses mirrored those for the total activity counts. Thus, a significant interaction between Ro60-0175 and cocaine was observed for both the 3 μg ($F_{1,10}=16.61$, $p<0.01$) and 10 μg ($F_{1,9}=20.5$, $p<0.01$) groups. *Post hoc* testing showed that in the 3 μg group, vehicle–cocaine treatment significantly elevated activity counts compared to vehicle–vehicle treatment for the first 50 min of testing ($p<0.05$), and this effect was significantly attenuated by 3 μg Ro60-0175 for the first 40 min of testing ($p<0.05$). Ro60-0175 (10 μg) significantly attenuated the hyperlocomotor effect of cocaine throughout the initial 50-min test period (all $p<0.05$).

Cocaine Self-Administration

As shown in Figure 3, injecting Ro60-0175 into the VTA dose dependently reduced responding for cocaine delivered according to an FR5 schedule. Effects were significant for both the number of infusions earned ($F_{3,27}=3.39$, $p<0.03$; Figure 3a) and the number of responses made ($F_{3,27}=3.03$, $p<0.05$; Figure 3b), at doses of 3 and 10 μg. The latency to respond for the first infusion was increased by Ro60-0175 ($F_{3,27}=4.47$, $p<0.02$), but only at the highest dose; the mean (and SEM) latencies (s) for the various treatments were: vehicle, 175.7 (42.3); 1 μg, 248.3 (78.7); 3 μg, 268.4 (33.8); 10 μg, 490.5 (121.0). Following vehicle treatment, responding for cocaine showed a regular and stable pattern, as shown in Figure 4. Typically, responding occurred in quick bursts of five responses, followed by a cocaine infusion. This was followed by a fairly consistent interinfusion interval terminated by another burst of responding and the next infusion. In most animals, this pattern of responding was disrupted by 3 and 10 μg of Ro60-0175. Although there was no single pattern of responding that characterized all animals, some fairly consistent features

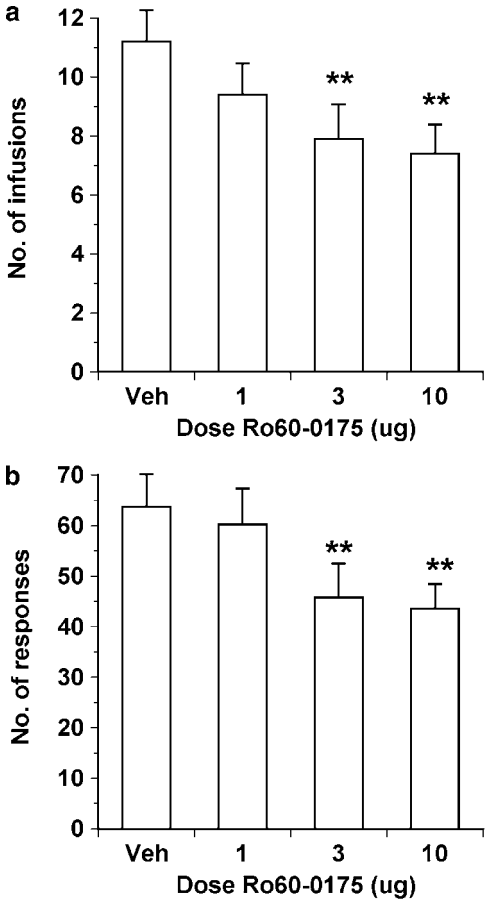


Figure 3 Effects of intra-VTA injections of 1, 3, 10 μg Ro60-0175, or its vehicle on responding for cocaine available according to an FR5 schedule. (a) Number of infusions and (b) number of responses. The bars represent the mean ± SEM values obtained from 10 rats. **$p<0.01$ compared to vehicle treatment.

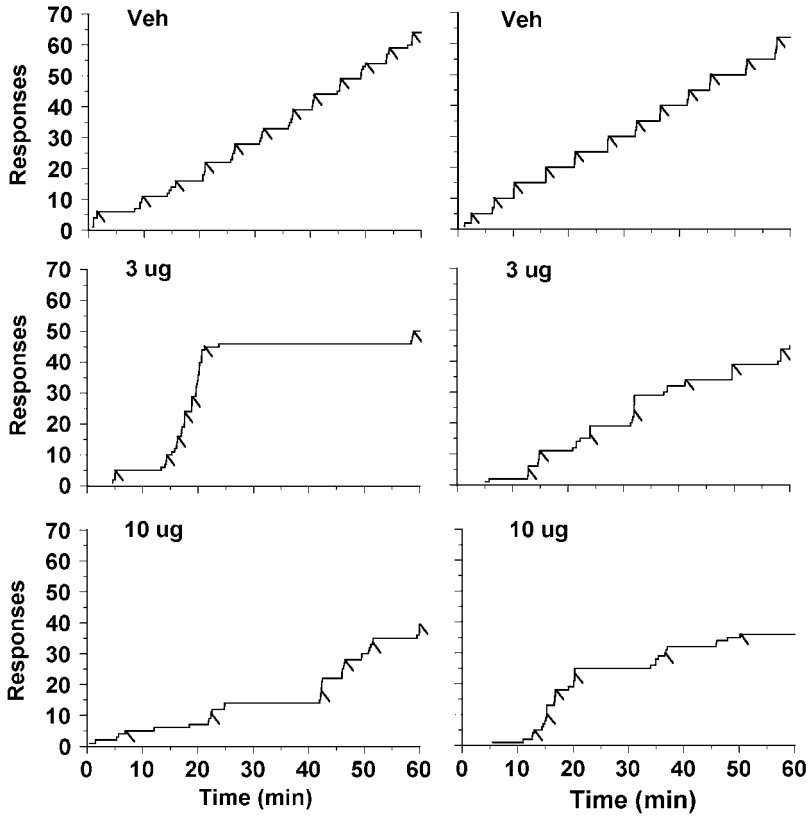


Figure 4 Examples of response records for two rats treated with vehicle, 3, and 10 μg Ro60-0175. The upper two panels illustrate that in vehicle-treated rats, responding was regular and stable. Following drug treatment, responding appeared to be irregular and erratic. Infusions are marked by hatch marks. Scales are equivalent on all graphs.

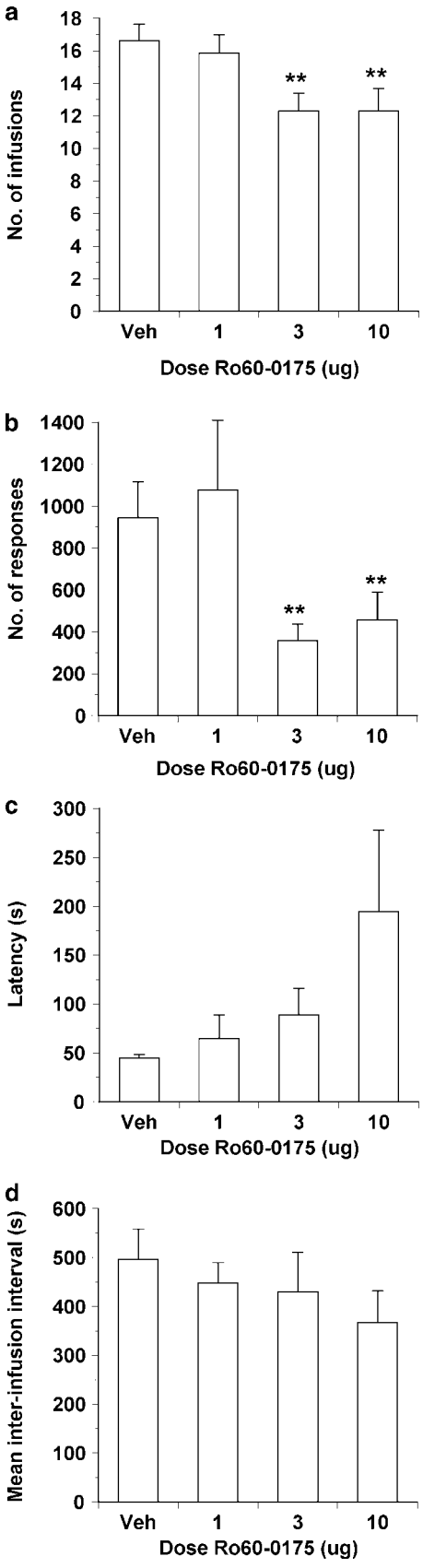
were observed. These included increased variability in the latency to begin responding or to earn the first infusion, irregularly spaced infusions across the 1 h session sometimes including relatively long intervals between infusions and occasional bursts of responding yielding several quickly earned infusions. Examples of these patterns of behavior are shown in Figure 4.

Figure 5 shows that intra-VTA injection of Ro60-0175 dose-dependently reduced the number of lever responses ($F_{3,27} = 4.34$, $p < 0.02$), and consequently the number of infusions of cocaine earned under a progressive ratio schedule ($F_{3,27} = 4.6$, $p < 0.01$). These effects were significant at the 3 and 10 μg dose levels, but not at 1 μg. Figure 5c and d shows that Ro60-0175 did not significantly alter the latency to obtain the first infusion ($F_{3,27} = 2.04$, $p > 0.1$), or the average interinfusion interval ($F_{3,27} = 1.32$, $p > 0.2$) However, the 10 μg dose of Ro60-0175 tended to increase the latency to begin responding, and thereafter to reduce the mean interinfusion interval. Three rats were found to have injection sites that were located 1–2 mm dorsolateral to the VTA. In these animals, Ro60-0175 did not appear to alter cocaine self-administration; the mean ± SEM number of infusions under the various treatment conditions were: saline, 13.0 ± 1.2; 1 μg, 14.3 ± 2.4; 3 μg, 14.7 ± 0.9; and 10 μg, 13.3 ± 1.3.

Figure 6 shows the effect of pretreatment with 0.5 mg/kg SB242084 on reduction in cocaine self-administration induced by 3 μg Ro60-017 injected into the VTA. For the measure of the number of infusions, the main effects of Ro60-0175 ($F_{1,7} = 7.02$, $p < 0.04$) and SB242,084 ($F_{1,7} = 12.92$, $p < 0.01$) were significant as was the interaction between these factors ($F_{1,7} = 8.75$, $p < 0.02$). *Post hoc* testing confirmed that the reduction in cocaine infusions induced by Ro60-0175 was completely reversed by SB242,084. Analysis of the data for number of responses revealed only a significant interaction between Ro60-0175 and SB242,084 ($F_{1,7} = 7.25$, $p < 0.04$); again SB242,084 reversed the suppressant effect of Ro60-0175.

Responding for Food

Figure 7 illustrates the effects of intra-VTA injection of Ro60-0175 on responding for food under a progressive ratio schedule. Analysis of variance revealed significant main effects of dose for both the number of reinforcers earned ($F_{3,24} = 4.19$, $p < 0.02$) and the number of responses ($F_{3,24} = 4.07$, $p < 0.02$) emitted. *Post hoc* testing confirmed that both measures were significantly reduced only by the 10 μg dose of Ro60-0175. The effects of SB242,084 (0.5 mg/kg) on the suppression of responding produced by Ro60-0175 are also shown in the lower panels of Figure 7. A significant interaction between SB242,084 and Ro60-0175 was found for the measures of number of responses ($F_{1,5} = 6.84$, $p < 0.05$) and the number of food pellets earned ($F_{1,5} = 7.04$, $p < 0.05$). The effects of Ro60-0175 to reduce responding were blocked by SB242,084.



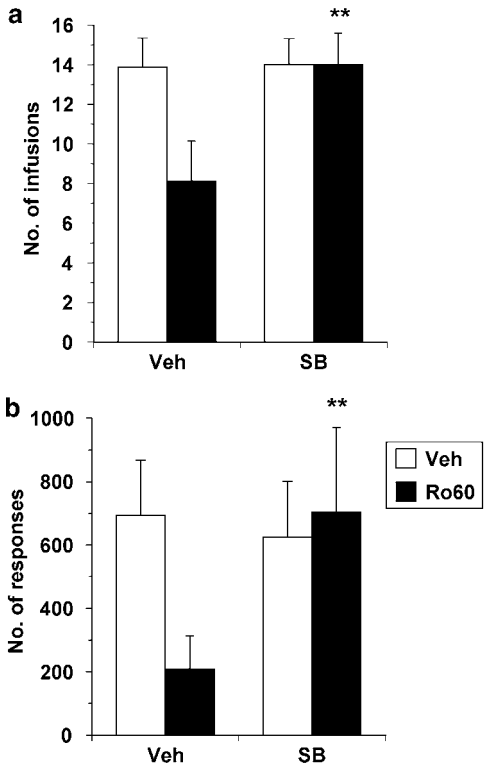


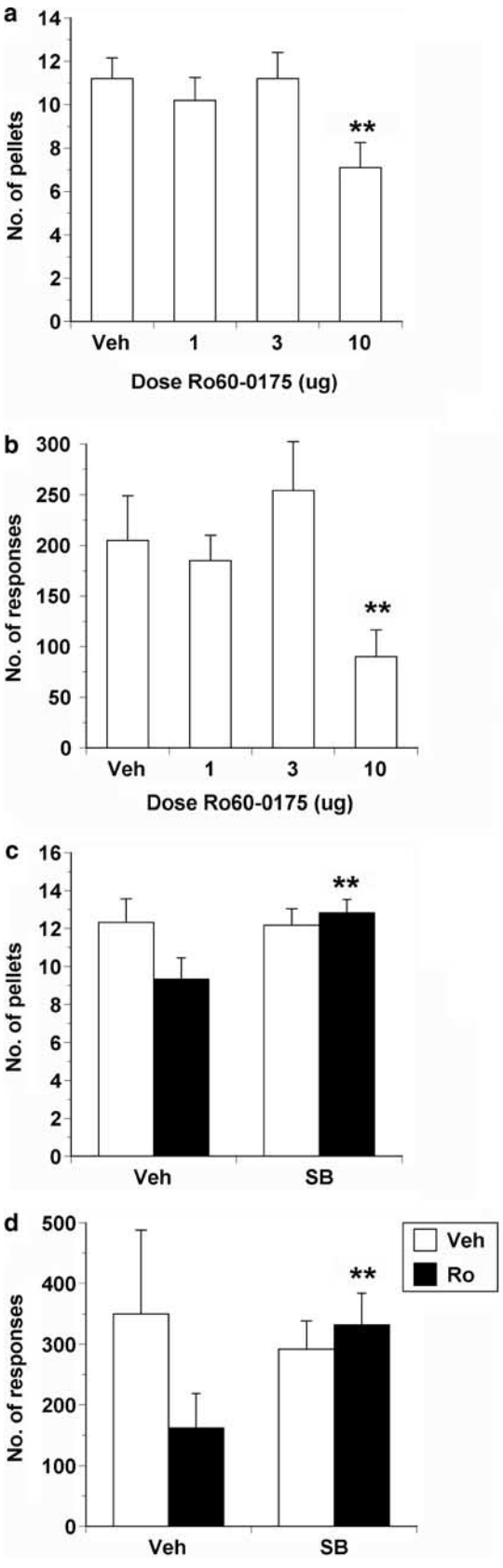
Figure 6 Effects of a combined treatment with SB242,084 (0.5 mg/kg) and 3 μg Ro60-0175 injected into the VTA on responding for cocaine delivered according to a progressive ratio schedule. The panels depict the mean ± SEM number of infusions (a) and responses (b) for eight rats. $^{**}p<0.01$ compared to vehicle treatment.

DISCUSSION

Previously, we have reported that systemic injection of Ro60-0175 attenuated a number of behavioral effects of cocaine, including locomotor activation and self-administration (Grottick *et al*, 2000). The present results show that local infusion of Ro60-0175 into the VTA is sufficient to attenuate cocaine-induced locomotion and self-administration. Responding for food was also reduced by Ro60-0175. In several animals, inaccurate cannula placements presumably resulted in delivery of Ro60-0175 outside the boundaries of the VTA. In these animals, Ro60-0175 did not modify cocaine self-administration, providing some evidence for the anatomical specificity of the VTA as a substrate for the effects of Ro60-0175. Rats receiving systemic injections of Ro60-0175 reached lower breakpoints than those reported here (Grottick *et al*, 2000). It is possible that systemically injected Ro60-0175 may act at additional brain sites to alter cocaine self-administration. Although the nucleus accumbens would seem to be a logical candidate, this seems unlikely given that local infusion of Ro60-0175

Figure 5 Effects of intra-VTA injections of 1, 3, 10 μg Ro60-0175, or its vehicle on responding for cocaine available according to a progressive ratio schedule of reinforcement. The various panels show the number of infusions (a) and responses (b), as well as the latency to obtain the first infusion (c), and the mean interval between infusions (d). The bars represent the mean ± SEM values obtained from 10 rats. $^{**}p<0.01$ compared to vehicle treatment.

into the shell region of the nucleus accumbens potentiates, rather than inhibits, cocaine-stimulated locomotion (Filip and Cunningham, 2002). Other potential regions include the prefrontal cortex and the amygdala since both regions contain 5-HT$_{2C}$ receptors (Pompeiano *et al*, 1994; Abramowski *et al*, 1995), and both regions have been implicated in various aspects of cocaine self-administration (Caine *et al*, 1995; Goeders and Smith, 1983; McGregor and Roberts, 1993, 1995; Robledo and Koob, 1993; Schenk *et al*, 1991; Weissenborn *et al*, 1997; Whitelaw *et al*, 1996). It is particularly interesting to note that selective depletion of 5-HT in the amygdala enhances responding for cocaine on a progressive ratio schedule (Loh and Roberts, 1990), an effect that is also observed after systemic treatment with a 5-HT$_{2C}$ receptor antagonist (Fletcher *et al*, 2002).



Ro60-0175 is often used as a selective agonist for 5-HT$_{2C}$ receptors and many of its behavioral actions, including its ability to attenuate cocaine-induced locomotion, are indeed mediated by 5-HT$_{2C}$ receptors (Dekeyne *et al*, 1999; Grottick *et al*, 2000, 2001; Tomkins *et al*, 2002). However, this drug also has appreciable affinity for 5-HT$_{2A}$ and particularly 5-HT$_{2B}$ receptors (Cussac *et al*, 2002). In the context of the present experiments, it seems likely that the effects of Ro60-0175 injected into the VTA result specifically from an interaction with 5-HT$_{2C}$ receptors because pretreatment with the selective 5-HT$_{2C}$ receptor antagonist SB242,084 (Kennett *et al*, 1997) blocked the effects of Ro60-0175 on cocaine self-administration, and responding for food, on the progressive ratio schedule. Previously, we have shown that SB242,084 can enhance responding for cocaine under this same progressive ratio schedule, but only at infusion doses of cocaine that are lower than the infusion dose used in the present study (Fletcher *et al*, 2002). In the present study, SB242,084 had no effect by itself on responding for cocaine or food. Thus, the blockade of the effects of Ro60-0175 by SB242,084 seems to represent a true pharmacological antagonism rather than the summation of two opposing behavioral effects. Overall, the result of this experiment shows that selective activation of 5-HT$_{2C}$ receptors within the VTA attenuates cocaine self-administration.

The fact that intra-VTA injection of Ro60-0175 reduced all of the behaviors tested raises the possibility that this manipulation exerts a nonspecific sedative-like effect. This seems unlikely for several reasons. Firstly, while Ro60-0175 tended to reduce locomotor activity in its own right, this effect was small and not statistically reliable in a dose-dependent manner. Furthermore, examination of the temporal profile of locomotor activity shows that the normal pattern of behavior was observed under Ro60-0175 treatment. Thus, activity levels were highest at the beginning of the test session, and these levels declined after the first 10 min of testing. Secondly, equivalent doses of Ro60-0175 reduced responding for cocaine under both

Figure 7 Upper panels show the effects of intra-VTA injections of 1, 3, 10 μg Ro60-0175, or its vehicle on responding for food available according to a progressive ratio schedule of reinforcement. The panels show the number of pellets earned (a) and the number of responses (b). The bars represent the mean ± SEM values obtained from nine rats. $^{**}p<0.01$ compared to vehicle treatment. The lower panels show the combined effects of 0.5 mg/kg SB242,084 (or its vehicle) and 10 μg Ro60-0175 (or its vehicle) in the VTA on the number of pellets earned (c) and number of responses (d). The bars represent the mean ± SEM values obtained from six rats. $^{**}p<0.05$ compared to vehicle–vehicle condition.

progressive ratio and FR5 schedules despite the fact that the two schedules led to markedly different response rates. Rats responding on the progressive ratio schedule were capable of responding several hundred times even under Ro60-0175 treatment; this level of responding was far in excess of that required to obtain cocaine infusions under the FR5 schedule. Finally, Ro60-0175 did not consistently alter the latency to begin self-administration, or the mean intervals between infusions, at least on the PR schedule, suggesting that there was no significant slowing of the drug-seeking behavior.

Responding for cocaine under the FR5 schedule was reduced by Ro60-0175. One interpretation of this finding is that Ro60-0175 enhances the reinforcing effectiveness of cocaine, as this type of modulation of responding is also observed with increases in cocaine dose above that used in these studies (Pettit and Justice, 1991). However, examination of response records for individual subjects showed that under treatment with Ro60-0175, the normal, regular pattern of responding for cocaine seen following the vehicle treatment was disrupted, leading to more erratic response profiles. This pattern of responding is not indicative of an increase in the reinforcing efficacy of cocaine. Rather, the pattern of responding suggests that under Ro60-0175 treatment, the effectiveness of cocaine to elicit and maintain responding was diminished (Roberts *et al*, 1980). Roberts and co-workers (eg Arnold and Roberts, 1997; Brebner *et al*, 2000a) have argued that while changes in responding maintained by FR schedules can provide a measure of the rate of drug intake, interpretations in terms of alterations in the reinforcing efficacy of the drug are not straightforward. In contrast, they have suggested that changes in responding under a progressive ratio schedule provide a relatively unambiguous index of alterations in reinforcer efficacy. Under this schedule, treatment with Ro60-0175 also reduced responding for cocaine. Taken together, the reduced responding for cocaine on the FR5 schedule that was accompanied by erratic patterns of responding and the reduced breakpoint for cocaine on the progressive ratio schedule are all consistent with the possibility that Ro60-0175 injected into the VTA reduces the reinforcing effects of cocaine.

Several nonselective global manipulations that enhance 5-HT function, including L-tryptophan (McGregor *et al*, 1993; Smith *et al*, 1986) and fluoxetine (Carroll *et al*, 1990; Richardson and Roberts, 1991) also reduce cocaine, or amphetamine, self-administration on both FR and progressive ratio schedules. One implication of the present results is that these manipulations could act to reduce cocaine self-administration via their ability to enhance 5-HT neurotransmission through 5-HT$_{2C}$ receptors localized within the VTA. Systemic injection of Ro60-0175 also reduces responding for nicotine (Grottick *et al*, 2001), ethanol (Tomkins *et al*, 2002), and food (Grottick *et al*, 2000). Similarly, drugs such as fluoxetine and fenfluramine reduce food and ethanol intake and heroin self-administration (see Higgins and Fletcher, in press for review). Clearly, serotonergic effects on motivated behavior occur with a variety of reinforcers, and are not selective for cocaine. In the present experiments, Ro60-0175 injected into the VTA also attenuated responding for food delivered according to the same progressive ratio schedule as for cocaine reinforcement. This effect occurred at 10 μg only, whereas cocaine self-administration was affected at 3 and 10 μg. While these data could indicate a differential responsiveness of cocaine *vs* food-maintained responding, it should be noted that absolute levels of responding tended to be lower in the food reinforcement *vs* the cocaine reinforcement experiments. Overall, it seems that selective 5-HT$_{2C}$ receptor stimulation in the VTA, similar to nonselective elevations in 5-HT function, has a generalized action to inhibit responding for different classes of reinforcers.

In electrophysiological studies, Ro60-0175 inhibits the firing rate of mesolimbic DA neurons. This action is likely indirect since stimulation of 5-HT$_{2C}$ receptors induces membrane depolarization and neuronal excitation (Di Giovanni *et al*, 2001; Sheldon and Aghajanian, 1991; Stanford and Lacey, 1996). Examination of the patterns of expression of 5-HT$_{2C}$ receptor mRNA indicates that 5-HT$_{2C}$ receptors are localized to GABA-ergic, and not dopaminergic neurons, in the VTA (Eberle Wang *et al*, 1997). Thus, 5-HT$_{2C}$ receptor stimulation by activating these GABA-ergic neurons leads to a GABA-mediated inhibition of the mesolimbic system. The locomotor stimulant and reinforcing effects of cocaine arise in large part from elevated synaptic levels of DA resulting from inhibition of the DA transporter (Di Ciano *et al*, 1995; Pettit and Justice, 1991; Roberts *et al*, 1980). The expression of the behavioral effects of cocaine is dependent upon the endogenous release of DA in the nucleus accumbens. Stimulation of 5-HT$_{2C}$ receptors in the VTA by lowering the basal activity of mesolimbic DA neurons (Di Matteo *et al*, 2000), perhaps indirectly via GABA-ergic neurons, could thereby result in a reduction in the expression of the behavioral effects of cocaine. The ability of Ro60-0175 to reduce responding for food could also be accounted for in terms of diminished activity of the mesolimbic DA system. Thus, blockade of DA activity either through systemic injection of DA receptor antagonists or 6-OHDA lesions of the nucleus accumbens reduces operant responding for food, especially when ratio requirements are high (Salamone and Correa, 2002). Particularly relevant here is the finding that the DA receptor antagonist alpha-flupenthixol reduces responding for food on the same progressive ratio schedule used in the present experiments (Brown *et al*, 1999).

At the present time, there is no direct evidence to support the hypothesis that the behavioral effects of 5-HT$_{2C}$ receptor stimulation are linked to altered dopaminergic function via a GABA-ergic mechanism. However, a consideration of the behavioral effects of manipulations of GABA-ergic systems provides some indirect support for this hypothesis. The effects of Ro60-0175 quite closely resemble those induced by the GABA-B agonist baclofen (Brebner *et al*, 2000a; Roberts *et al*, 1996; Shoaib *et al*, 1998) since systemic injection of both drugs result in reductions in self-administration of cocaine under both FR and progressive ratio schedules. More importantly, infusions of baclofen, like Ro60-0175, into the VTA reduce self-administration of cocaine on the progressive ratio schedule (Brebner *et al*, 2000b). Any 5-HT$_{2C}$ receptor-induced increase in GABA-ergic function is likely to be nonselective in that the release of GABA could activate both GABA-A and GABA-B receptors. Although the effects of GABA-A receptor agonists on cocaine self-administration have not been as well

documented as the effects of GABA-B receptor agonists, one report indicates a mild suppression of cocaine self-administration following infusion of muscimol into the VTA (Corrigall *et al*, 2000). In other reward-related procedures, muscimol injections into the VTA failed to alter ethanol self-administration (Hodge *et al*, 1996) or responding for intracranial self-stimulation (Willick and Kokkinidis, 1995). By itself, muscimol in the VTA stimulates locomotor activity (Oakley *et al*, 1991), an effect that is not seen with Ro60-0175. Overall then, this pattern of findings suggests that any influence of 5-HT$_{2C}$ receptor stimulation to alter dopaminergic systems via GABA indirectly may be mediated via GABA-B receptors. This hypothesis could be further tested by determining the ability of GABA-B *vs* GABA-A receptor antagonists to attenuate the effects of Ro60-0175 on cocaine-mediated behaviors.

The present experiments involved exogenous application of a 5-HT$_{2C}$ receptor agonist to the VTA, and so the results do not directly shed light on the possible role of exogenous 5-HT, acting via 5-HT$_{2C}$ receptors in modifying cocaine-induced behavioral effects. Both pharmacological blockade of the 5-HT$_{2C}$ receptor (McCreary and Cunningham, 1999; Fletcher *et al*, 2002), as well as elimination of the expression of this receptor through 5-HT$_{2C}$ receptor gene deletion (Rocha *et al*, 2002), can enhance the stimulant and reinforcing effects of cocaine. This suggests that the expression of the behavioral effects of cocaine may depend to some extent upon the endogenous tone of 5-HT acting upon this receptor. Future experiments involving selective injection of 5-HT$_{2C}$ receptor antagonists into the VTA would help to further clarify any possible role for the endogenous 5-HT$_{2C}$ receptor system in this brain region in modulating the effects of cocaine.

In summary, injecting Ro60-0175 into the VTA inhibits the expression of cocaine-induced locomotor activity and the reinforcing efficacy of intravenous infusions of cocaine, as well as food. Blockade of the effect of Ro60-0175 on cocaine self-administration, and on responding for food, by SB242,084 indicates the involvement of 5-HT$_{2C}$ receptors in these effects. While other neuroanatomical sites cannot be excluded, the combination of electrophysiological (Di Matteo *et al*, 2000, 2002), biochemical (Di Matteo *et al*, 2000, 2002; Gobert *et al*, 2000), and behavioral data (present study) provides strong evidence that the VTA is a primary locus for the suppressant effects of 5-HT$_{2C}$ agonists on DA-dependent behaviors elicited by cocaine.

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