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The 5-HT_{2C} Receptor Agonist Ro60-0175 Reduces Cocaine Self-Administration and Reinstatement Induced by the Stressor Yohimbine, and Contextual Cues

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Previously, we showed that the 5-HT_{2C} receptor agonist Ro60-0175 reduces cocaine self-administration, and the ability of cocaine to reinstate responding after extinction of drug-seeking behavior. The present experiments extended these findings further by determining whether the effects of Ro60-0175 on self-administration were sustained with repeated treatment, and whether Ro60-0175 altered reinstatement induced by the pharmacological stressor yohimbine, or by the context in which self-administration occurred. In Experiment 1, Ro60-0175 (1 mg/kg, s.c.) reduced cocaine (0.25 mg/infusion) self-administration maintained by a progressive ratio schedule. This reduction was sustained over eight daily injections. In Experiment 2, rats self-administered cocaine in daily 2 h sessions for 15 days on a FR1 schedule. Following extinction, yohimbine (1 mg/kg, i.p.) reinstated responding, and this effect was reduced dose dependently by Ro60-0175 (0.3–3 mg/kg, s.c.). In Experiment 3, rats were trained to respond for cocaine on a FR1 schedule in a distinct environmental context (A); responding was then extinguished in a different context (B). Reinstatement tests occurred in either context A or B. Responding was reinstated only when rats were tested in the original self-administration context (A). This reinstatement was reduced dose dependently by Ro60-0175. All effects of Ro60-0175 were blocked by the 5-HT_{2C} receptor antagonist SB242084. Thus, Ro60-0175, acting via 5-HT_{2C} receptors, reduces cocaine self-administration and cocaine-seeking triggered by a stressor and by drug-associated cues. The effects of Ro60-0175 do not exhibit tolerance within the 8-day test period. These results indicate that selective 5-HT_{2C} receptor agonists may be a useful pharmacological strategy for treatment of drug abuse.
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INTRODUCTION

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 (PFC) (Abramowski *et al*, 1995; Eberle-Wang *et al*, 1997; Pompeiano *et al*, 1994). The moderately selective 5HT_{2C} receptor agonist Ro60-0175 (Martin *et al*, 1998) reduces the firing rate of mesolimbic DA neurons originating in the VTA, leading to a reduction in DA release in terminal regions of the nucleus accumbens and frontal cortex (Di Matteo *et al*, 2000a, b; Gobert *et al*, 2000). These effects are reversed by the selective 5-HT_{2C} receptor antagonist SB242084 (Di Matteo *et al*, 2000a; Gobert *et al*, 2000). Additionally, acute 5-HT_{2C} receptor block with SB242084 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; Gobert *et al*, 2000). Thus, it appears that 5-HT_{2C} receptors may exert a tonic inhibitory influence over the activity of ascending DA neurons.

This bi-directional modulation of dopamine function by 5-HT_{2C} receptors is apparent at the behavioral level as well. Many of the behavioral effects of psychomotor stimulants such as cocaine involve increased activity of mesolimbic dopamine function (eg, Callahan *et al*, 1991; Kelly and Iversen, 1976; Pettit *et al*, 1984). A number of studies have now shown that 5-HT_{2C} receptor agonists attenuate cocaine-stimulated locomotor activity (Filip *et al*, 2004; Filip and Cunningham, 2003; Fletcher *et al*, 2004; Grottick *et al*, 2000). Ro60-0175 also reduced responding for intravenous infusions of cocaine under both fixed ratio (FR) and progressive ratio schedules of reinforcement (Grottick *et al*, 2000). Following extinction of self-administration behavior

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cocaine is able to reinstate responding, and this effect of cocaine was also attenuated by Ro60-0175 (Grottick *et al*, 2000). In contrast to the effects of activating the 5-HT$_{2C}$ receptor, blockade of 5-HT$_{2C}$ receptors produced the opposite profile of effects. Thus, the 5-HT$_{2C}$ receptor antagonist SB242084 enhanced cocaine-induced locomotor activity, responding for cocaine, and the response reinstating effects of cocaine (Fletcher *et al*, 2002).

On the basis of this evidence there have been a number of recent suggestions that the 5-HT$_{2C}$ receptor might be a valid target for the development of medications for treating drug abuse (Bubar and Cunningham, 2006; Di Giovanni *et al*, 2006; Higgins and Fletcher, 2003; Ji *et al*, 2006). In the present experiments, we have explored further the potential utility of a 5-HT$_{2C}$ receptor agonist for drug abuse. To date all of the available data concerning the effects of manipulating 5-HT$_{2C}$ receptor activity on drug self-administration and other aspects of drug abuse behavior have been derived from studies using acute treatment. Tolerance can develop rapidly to some of the effects of 5-HT acting drugs including 5-HT$_{2C}$ receptor agonists (Fone *et al*, 1998; Hayashi *et al*, 2005; Rowland, 1994; Wang *et al*, 1995; Yamauchi *et al*, 2004). From a clinical perspective, such a rapid loss of efficacy would not be desirable. Therefore, the first experiment investigated the ability of the 5-HT$_{2C}$ receptor agonist Ro60-0175 to alter responding for cocaine following daily treatment.

One of the most serious aspects in the treatment of drug abuse is the potential for relapse after a period of abstinence (Mendelson and Mello, 1996; O'Brien, 1997). Previously, we have shown that 5-HT$_{2C}$ receptor activation attenuates the reinstatement of cocaine-seeking behavior induced by a priming injection of cocaine (Grottick *et al*, 2000). Two other types of stimulus that elicit relapse in humans, or reinstate responding in abstinent animals, are stressors and drug-associated cues (for reviews see Bossert *et al*, 2005; Shaham *et al*, 2003). Therefore, the second objective of the work was to examine whether the ability of Ro60-0175 to attenuate reinstatement of drug-seeking behavior extended to situations in which reinstatement was initiated by a stressor, or by drug-paired cues. To this end, we first examined the effect of Ro60-0175 on response reinstatement induced by the pharmacological stressor yohimbine. Yohimbine is an α-2 adrenoceptor antagonist that induces anxiety and panic attacks in humans and anxiety-like responses in animals (Bremner *et al*, 1996a, b; Charney *et al*, 1987). Opioid-dependent patients exhibit anxiety-like responses and withdrawal symptoms following treatment with yohimbine, and these states are accompanied by drug cravings (Stine *et al*, 2002). In animals, yohimbine reinstates responding for cocaine (Lee *et al*, 2004) methamphetamine (Shepard *et al*, 2004), and alcohol (Le *et al*, 2005). Second, we examined the ability of Ro60-0175 to attenuate responding induced by contextual cues previously associated with cocaine self-administration (Crombag *et al*, 2002). Ro60-0175 has approximately 10-fold selectivity for the 5-HT$_{2C}$ over the 5-HT$_{2A}$ receptor, although it is non-selective compared to the 5-HT$_{2B}$ receptor (eg Porter *et al*, 1999). Therefore, to confirm the role of 5-HT$_{2C}$ receptors in mediating effects of Ro60-0175, we also examined whether the effects of Ro60-0175 could be reversed by the selective 5-HT$_{2C}$ receptor antagonist SB242084 (Kennett *et al*, 1997).

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, and solid-bottomed cages. The housing room was maintained on a 12 h light/dark cycle (lights off at 0800 h) and at a temperature of $22 \pm 2^{\circ}$C. Access to food was restricted as detailed below. All training and testing was conducted during the dark 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

The rats were anesthetized with ketamine and xylazine for implantation of a catheter into the right 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 (o.d = 0.025 inches) was inserted into the right jugular vein. The larger diameter tubing (o.d. = 0.046 inches) was connected to a length of 22 ga 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 post-surgical 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.

Apparatus

Testing was conducted in operant chambers measuring 28 cm long, 21 cm wide and 21 cm high (Med Associates Inc., St Albans, VT). 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 house light and housed in a sound-attenuating box equipped with a ventilating fan.

For the contextual reinstatement experiments two distinct training and testing contexts were used. Sixteen boxes were divided into two sets of eight, each set being housed in a different testing room. See Experiment 3a for further details of the two contexts.

Drugs

Ro60-0175 ((*S*)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1 : 1 $C_4H_4O_4$) was synthesized within the PRPN

Chemistry Department at F Hoffmann-La Roche Ltd, Basel. Ro60-0175 was dissolved in 0.9% saline and injected s.c. SB242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl] indoline) was synthesized in the Department of Chemistry, Vernalis Research Ltd (Wokingham, UK), and prepared in 0.9% saline solution containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid and injected by the i.p. route. Yohimbine hydrochloride (17-hydroxyyohimban-16-carboxylic acid methyl ester hydrochloride) was purchased from Sigma-Aldrich (St Louis, MO). Yohimbine was dissolved in distilled H_2O and injected by the i.p. route. Cocaine hydrochloride was purchased from Medisca, 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. All doses are expressed in terms of the free base.

Procedures

Experiment 1a: Effect of daily treatment with Ro60-0175 on cocaine self-administration. Before 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 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 3rd 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 received 100 pellets on each of three consecutive days they were considered lever-trained, and were subsequently maintained on approximately 20 g of lab chow per day. One week after catheters were implanted rats were allowed to respond for infusions (0.1 ml during 5.5 s) of cocaine (0.25 mg/infusion delivered in 0.1 ml) on a FR1 schedule. Each infusion was accompanied by a stimulus light that remained on for a 20 s time-out 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 $= [5e^{(0.2 \times \text{infusion no.})} - 5]$, 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/infusion throughout. Testing began when break points did not vary by more than 15% on three consecutive days. At this point, rats were assigned to two groups matched for daily number of cocaine infusions. One group received injections of 1 mg/kg Ro60-0175 (s.c.) 15 min before being placed in the self-administration chambers; the other groups received injections of saline. Testing was conducted on eight consecutive days. Eight rats in the saline group and eight rats in the Ro60-0175 group completed the experiment.

Experiment 1b: Interaction between SB242084 and Ro60-0175 on cocaine self-administration. A further nine rats were trained to respond for cocaine (0.25 mg/infusion) as described above. Once breaking points were stable drug testing began. Each rat was tested on four occasions spaced a minimum of 72 h apart. On these test days rats were first injected with 0.5 mg/kg SB242084 or its vehicle (i.p.), 30 min later they received an injection of 1 mg/kg Ro60-0175 (s.c.) or saline. Fifteen minutes later rats were placed in the drug self-administration chambers. The order of treatments was determined from Latin Squares with approximately equal numbers of animals being tested at each treatment level. On non-drug test days the usual self-administration session was in effect.

Experiment 2a: Effects of Ro60-0175 on reinstatement induced by yohimbine. The experimental design for this experiment, and all subsequent experiments, involved three phases: cocaine self-administration, extinction of bar-pressing, and tests for reinstatement. Eleven rats were trained to respond for cocaine (0.25 mg/infusion) delivered according to a FR1 schedule, as outlined above. Again cocaine infusions were accompanied by illumination of the stimulus light for 20 s. Self-administration sessions were 2 h in duration; 15 sessions were run on consecutive days. At the end of this period, extinction conditions were in effect. Here drug syringes were removed from the pumps so that responses on the previously active lever activated the stimulus light but no longer delivered cocaine infusions. Extinction sessions were run for 8 days at which point responding on the active lever had reached a low, stable level of <15 responses in 2 h. At this point rats were tested on five occasions separated by a minimum period of 72 h. On these occasions, rats were first given a 2 h extinction session with no drug treatments. At the end of this period, the animals received an injection of 1 mg/kg yohimbine or saline (i.p.) and returned to the home cage. Thirty minutes later rats received a second injection of Ro60-0175 or saline (s.c.); 15 min later rats were returned to the self-administration chamber for another 2 h extinction session. The five-treatment combinations that were tested were: vehicle saline, and 1 mg/kg yohimbine in combination with saline, 0.3, 1, and 3 mg/kg Ro60-0175. The order of treatments was determined from Latin Squares. On the days between tests, rats were run as normal with a 2 h extinction session. The dose of yohimbine was chosen based on a consideration of published work (Shepard *et al*, 2004) and the results of a pilot study.

Experiment 2b: Interaction between SB242084 and Ro60-0175 on reinstatement induced by yohimbine. Nine rats went through the same cocaine self-administration and extinction procedures as described for Experiment 2a. In this experiment, extinction was conducted for 12 days before reinstatement testing began. Four testing days were conducted. On each test all rats received injections of 1 mg/kg yohimbine at the end of a 2 h extinction phase and were returned to the home cage. Fifteen minutes later rats were treated with 0.5 mg/kg SB242084 or its vehicle, followed 30 min later by 1 mg/kg Ro60-0175 or saline. After a further 15 min, rats were returned to the self-administration chamber for a 2 h extinction session. All rats were

tested under all four-treatment combinations spaced at least 72 h apart; the order of treatments was determined from Latin Squares.

Experiment 3a: Effects of Ro60-0175 on reinstatement induced by contextual cues. This experiment examined the effects of Ro60-0175 on the reinstatement of cocaine-seeking induced by contextual cues. The experiment required the use of two different contexts in which the three phases of the experiment (self-administration, extinction, and reinstatement) were conducted. The experiment began with the acquisition of cocaine self-administration (15 days), followed by extinction training (22 days) and testing for reinstatement of cocaine seeking (10 days). Rats were assigned to a reinstatement group ($n=8$) or a control group ($n=8$). For rats in the reinstatement group cocaine self-administration occurred in one context (A), extinction occurred in the alternative context (B), and reinstatement testing was conducted in context A. For the control group self-administration occurred in context A, and extinction and reinstatement both occurred in context B. In one context, operant chambers had a textured Plexiglas floor insert; the chamber houselight, and the ventilating fans within the sound-attenuating chamber were both turned on. Sessions in this context began 2 h following light offset in the colony room. In the alternate context, operant chambers were located in a different area of the lab. These chambers had the standard stainless-steel rod floors; the houselight and ventilating fans were both turned off. Sessions in the alternate context began 5 h following light offset in the colony room.

For the cocaine self-administration phase rats were trained to respond for cocaine infusions (0.25 mg in 0.1 ml over 5.5 s) according to a FR1 schedule as described for Experiments 1 and 2. Cocaine infusions were accompanied by a 20 s illumination of the houselight during which time further lever presses were recorded but not reinforced. Fifteen 2 h sessions of self-administration were conducted. During the extinction phase syringes were removed from the pumps, responses on the formerly active lever activated the stimulus light but no longer delivered cocaine infusions. During the reinstatement phase each rat was tested four times beginning 15 min after treatment with 0.3, 1, and 3 mg/kg Ro60-0175 and saline. The order of treatment was determined from Latin Squares. Tests were spaced at least 72 h apart, and rats were run under their appropriate extinction conditions on intervening days.

Experiment 3b: Interaction between SB242084 and Ro60-0175 on reinstatement induced by contextual cues. This experiment used eight rats in the reinstatement group, and eight rats in the control group. The behavioral procedures for self-administration, extinction, and reinstatement were identical to those for Experiment 3a. On reinstatement days rats were tested with every combination of 0.5 mg/kg SB242084 or its vehicle and 1 mg/kg Ro60-0175 or saline. The injections were given 30 min apart, and testing began 15 min after the second injection. The order of treatment was determined from Latin Squares. Tests were spaced at least 72 h apart, and rats were run under their appropriate extinction conditions on intervening days.

Statistical Analyses

Statistical analyses were performed using Statistica version 7.1 (StatSoft Inc., Tulsa, OK). Data from Experiment 1a were analyzed using a two-way analysis of variance with Ro60-0175 as a between subjects factor and days as a within subjects factor. In Experiment 1b, a repeated measures two-way analysis of variance was used with SB242084 and Ro60-0175 as factors. Data from Experiment 2a were analyzed with one-way analysis of variance; active and inactive lever responses were analyzed separately. For Experiment 2b, a two-way analysis of variance, with Ro60-0175 and SB242084 as within-subjects factors, was used. Data from Experiment 3a were analyzed with a three-way analysis of variance with group (control and reinstatement) as a between subjects factor and dose of Ro60-0175 and lever as within subjects factors. For Experiment 3b, data for active and inactive lever responses were analyzed separately. Each analysis involved group as a between-subjects factor and Ro60-0175 and SB242084 as within subjects factors. All *post hoc* comparisons were made using the Newman–Keuls test with $\alpha=0.05$.

RESULTS

Experiment 1a: Effect of Daily Treatment with Ro60-0175 on Cocaine Self-Administration

The results of this experiment are shown in Figure 1. Animals were assigned to matched groups for drug-treatment based on baseline performance and so there were no significant effects of group assignment during baseline ($p>0.1$). During the drug-treatment phase one rat treated with Ro60-0175 showed unusually erratic responding ranging from 9 to 24 infusions; data from this animal were omitted from all of the analyses. Rats treated with Ro60-0175 responded for significantly fewer infusions than rats treated with saline ($F_{(1,14)}=11.27$, $p<0.01$). This effect was stable across the testing period; neither the main effect of days ($F_{(7,98)}=0.6$, $p>0.7$) nor the interaction between

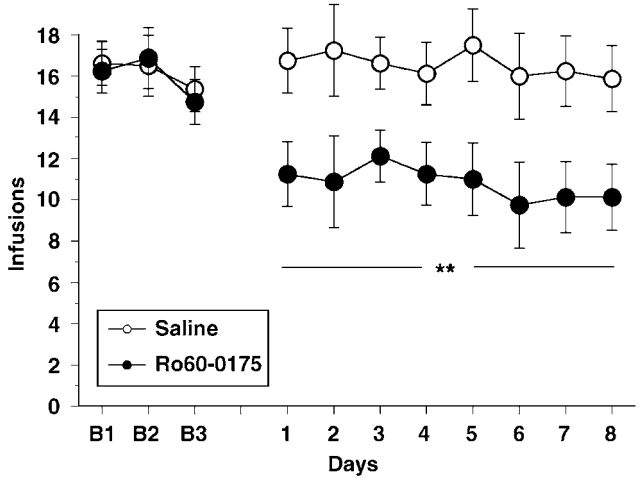

Figure 1 The effects of daily treatment with 1 mg/kg Ro60-0175 ($n=8$) or saline ($n=8$) on the number of cocaine infusions earned on a progressive ratio schedule of reinforcement. Symbols represent the mean ± SEM number of infusions for the two groups of animals. Data points at B1–B3 show the average number of cocaine infusions during the last 3 days of the baseline phase. $^{**}p<0.01$ compared to saline on all days.

Ro60-0175 and days ($F_{(7,98)} = 2.13$, $p > 0.9$) was significant. An identical pattern of results was found for active lever responses (data not shown). Averaged across days, saline-treated rats emitted 1025 ± 338 responses per session, whereas rats treated with Ro60-0175 made 319 ± 124 responses. Figure 2 shows representative cumulative response records from rats treated with saline or 1 mg/kg Ro60-0175. These clearly show that the regularity of responding seen in control animals was preserved in drug-treated animals, but that these animals reached their break-points earlier.

Experiment 1b: Interaction between SB242084 and Ro60-0175 on Responding for Cocaine

Figure 3 shows that Ro60-0175 reduced responding for cocaine and that this effect was reversed by SB242084. There were significant main effects of Ro60-0175 ($F_{(1,8)} = 50.1$, $p < 0.001$) and SB242084 ($F_{(1,8)} = 77.1$, $p < 0.0001$) and a significant interaction ($F_{(1,8)} = 23.27$, $p < 0.001$).

Experiment 2a: Effects of Ro60-0175 on Reinstatement Induced by Yohimbine

Over the course of 15 days rats self-administered an average total of 498 ± 36 infusions of cocaine. Over the last 3 days of self-administration the average daily number of infusions was 33.5 ± 3.0 infusions. As can be seen in Figure 4a, during the extinction phase responding decreased steadily over days ($F_{(7,70)} = 9.75$, $p < 0.001$). For reinstatement tests responding on the active and inactive levers were analyzed separately. For active lever responding a significant main effect of treatment was found ($F_{(4,40)} = 12.42$, $p < 0.001$). *Post hoc* testing showed that yohimbine treatment alone increased responding relative to vehicle injection, and that this response was attenuated dose dependently by Ro60-0175. A significant main effect of treatment was also found for responding on the inactive lever ($F_{(4,40)} = 5.16$, $p < 0.01$); this was entirely due to the fact that responding under yohimbine alone was slightly, but significantly higher than under all other treatment combinations ($p < 0.05$).

Experiment 2b: Interaction between SB242084 and Ro60-0175 on Reinstatement Induced by Yohimbine

Over the course of 15 days rats self-administered an average total of 539 ± 27 infusions of cocaine. Over the last 3 days of self-administration the average daily number of infusions was 33.9 ± 1.5. During extinction responding decreased steadily over days ($F_{(9,72)} = 21.34$, $p < 0.001$) (see Figure 5a). Figure 5b and c show that the effects of Ro60-0175 on yohimbine-induced reinstatement were reversed by SB242084. For responding on the previously active lever the main effects of Ro60-0175 and SB242084, as well as their interaction were all significant (smallest $F_{(1,8)} = 6.33$, $p < 0.03$). *Post hoc* comparisons confirmed that, relative to vehicle treatment, Ro60-0175 reduced

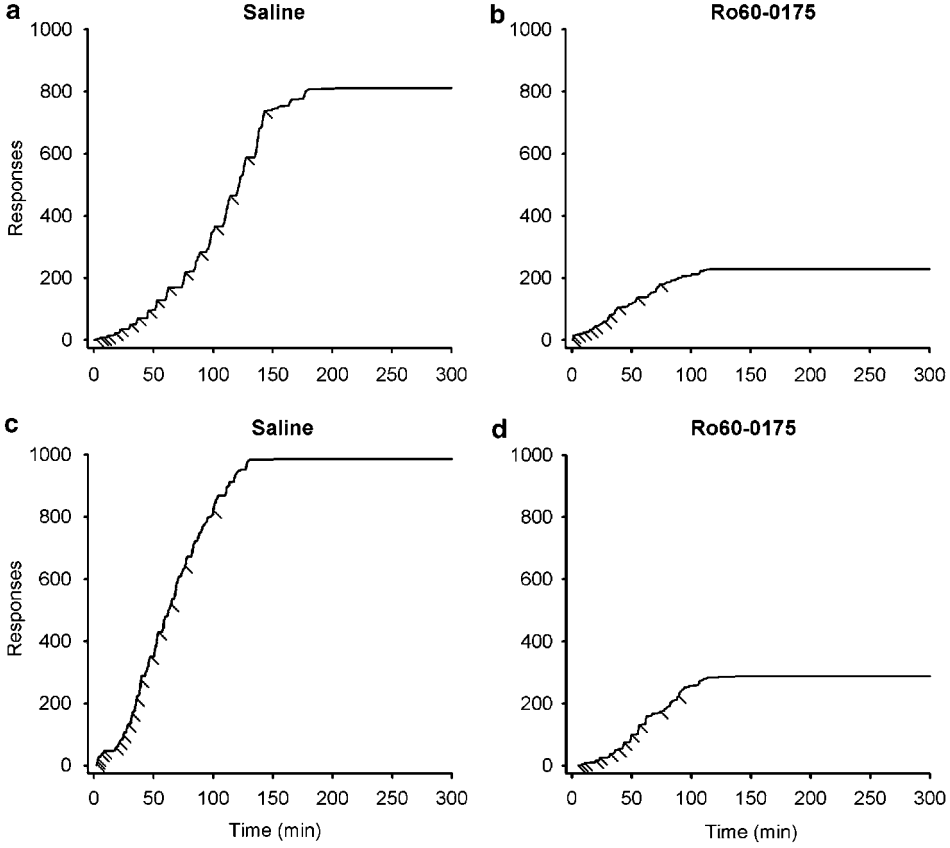

Figure 2 Representative cumulative response records for rats treated with 1 mg/kg Ro60-0175 or saline. Panels a and c illustrate that in vehicle-treated rats responding was regular and stable. Following treatment with Ro60-0175 (b and d) responding was also regular but rats reached their breaking point at an earlier time and lower ratio compared to control animals. Infusions are marked by hatch marks. Scales are equivalent on all graphs.

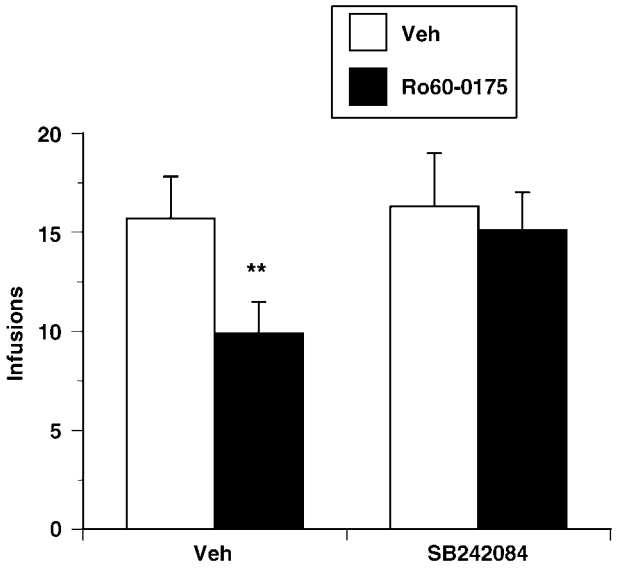

Figure 3 The effects of a combined treatment with SB242084 (0.5 mg/kg) and Ro60-0175 (1 mg/kg) on the number of cocaine infusions earned on a progressive ratio schedule. Nine rats were tested once under all treatment combinations. Bars represent the mean ± SEM number of infusions under each treatment. $^{**}p<0.01$ compared to Veh–Veh condition.

responding and that this effect was prevented by SB242084 pretreatment. For responding on the previously inactive lever there were no significant effects ($p>0.2$).

Experiment 3a: Effects of Ro60-0175 on Reinstatement Induced by Contextual Cues

During the self-administration phase rats in the control group responded for an average total of 386.8 ± 46.3 infusions, and rats in the reinstatement group responded for 344.9 ± 54.9 infusions. Average daily infusions, calculated over the last 3 days of self-administration were 29.8 ± 5.4 (control) and 30.6 ± 5.6 (reinstatement) infusions. These differences were not significant ($p>0.1$). Responding during the extinction phase is shown in Figure 6a. There was no influence of group on extinction responding ($p>0.6$). Responding was higher on the active *vs* inactive lever ($F_{(1,14)}=36.14$, $p<0.001$) and declined over sessions ($F_{(21,294)}=13.16$, $p<0.001$). Figure 6b and c show that Ro60-0175 reduced reinstatement induced by contextual cues. Overall, responding was higher in the reinstatement group than in the control group ($F_{(1,14)}=12.62$, $p<0.003$) and on the active *vs* the inactive lever ($F_{(1,14)}=23.85$, $p<0.0001$). The overall three-way interaction was significant ($F_{(3,42)}=10.00$, $p<0.001$). Tests of simple interactions showed that the interaction between group and dose of Ro60-0175 was significant for responses on the active lever ($F_{(3,42)}=10.09$, $p<0.001$) but not the inactive lever ($p>0.3$). Tests of simple main effects then showed that the main effect of dose was significant only in the reinstatement group ($F_{(3,21)}=13.09$, $p<0.001$) and not the control group ($p>0.3$). *Post hoc* tests showed that all three doses of Ro60-0175 significantly reduced responding on the active lever in the reinstatement group.

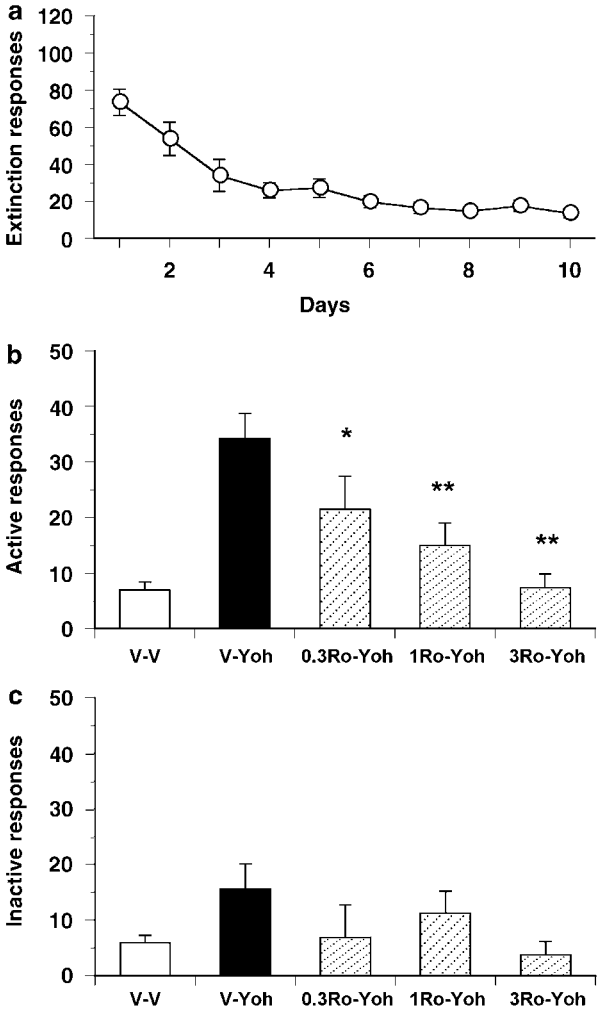

Figure 4 The effects of different doses of Ro60-0175 on reinstatement of responding induced by yohimbine (1 mg/kg). Panel a shows that during extinction responding rapidly decreased over consecutive sessions. Panel b shows the mean number of responses on the previously active lever following drug treatment. Panel c shows the mean number of responses on the previously inactive lever following drug treatment. For reinstatement tests, rats were first given a 2 h extinction session with no drug treatments. They then received an injection of 1 mg/kg yohimbine or saline and were brought to the home cage. Thirty minutes later rats received an injection of Ro60-0175 or saline; 15 min later rats were returned to the self-administration chamber for a 2 h extinction session. Bars represent mean ± SEM number of responses from 11 rats tested once under each treatment condition. $^{*}p<0.05$; $^{**}p<0.01$ compared to Veh–Yoh condition.

Experiment 3b: Interaction between SB242084 and Ro60-0175 on Reinstatement Induced by Contextual Cues

During the self-administration phase rats in the control group responded for an average total of 391.0 ± 99.8 infusions, and rats in the reinstatement group responded for 459.3 ± 66.5 infusions. Average daily infusions for the last 3 days of self-administration were 31.5 ± 7.8 (control) and 35.6 ± 5.6 (reinstatement) infusions. These differences were not significant ($p>0.1$). Responding during the extinction phase is shown in Figure 7a. There was no influence of group on extinction responding ($p>0.6$). Responding was higher on the previously active *vs* inactive

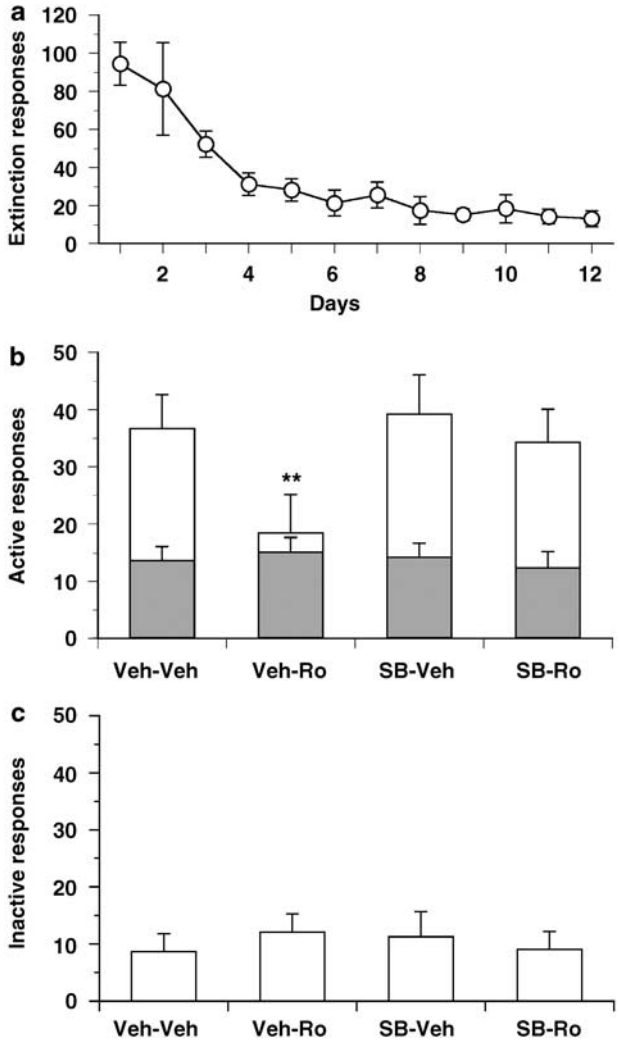

Figure 5 The effects of combining SB242084 (0.5 mg/kg) and Ro60-0175 (1 mg/kg) on reinstatement of responding induced by yohimbine (1 mg/kg). Panel a shows that during extinction responding rapidly decreased over consecutive sessions. Panel b shows the mean number of responses on the previously active lever following drug treatments (open bar). The shaded portion of the bar shows the mean (±SEM) level of responding on the drug-free extinction session conducted on the day before drug testing. Panel (c) shows the mean number of responses on the previously inactive lever following drug treatments. The average number of inactive lever responses on the drug-free extinction session conducted on the day before drug testing was less than 10 responses; these data are not shown on the figure for reasons of clarity. On each reinstatement test, rats received injections of 1 mg/kg yohimbine at the end of a 2 h extinction phase and were returned to the home cage. Fifteen minutes later rats were treated with 0.5 mg/kg SB242084 or its vehicle, followed 30 min later by 1 mg/kg Ro60-0175 or saline. Fifteen minutes after that, rats were returned to the self-administration chamber for a 2 h extinction session. Bars represent mean ± SEM number of responses from nine rats tested once under each treatment condition. $^{**}p<0.01$ compared to Veh–Veh condition.

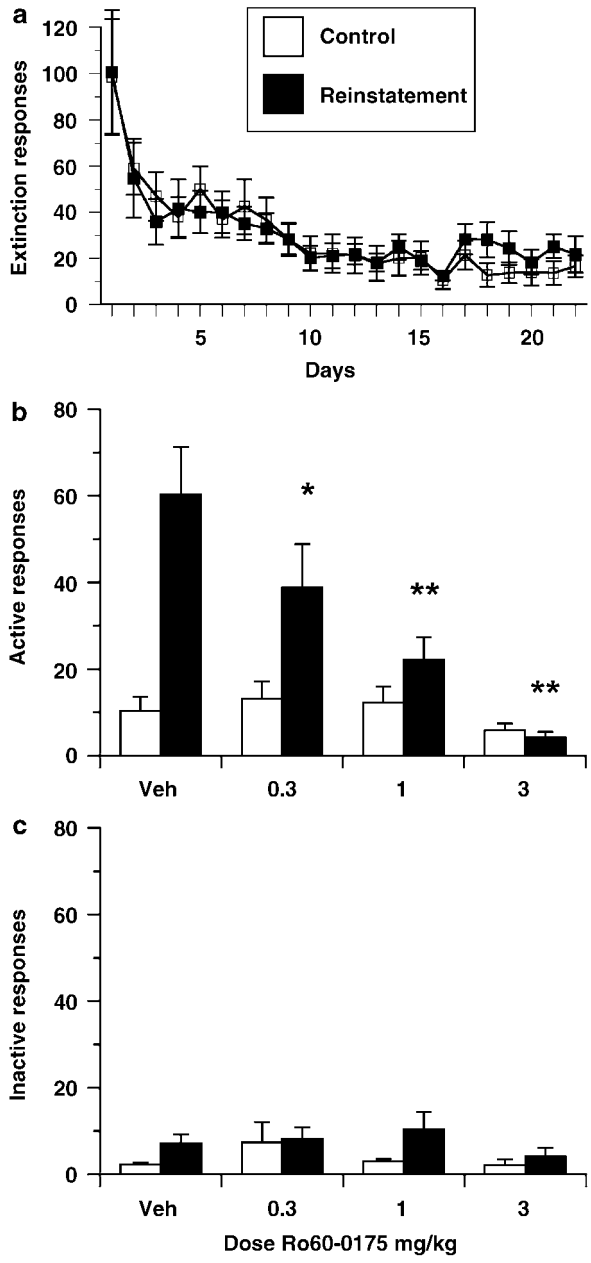

Figure 6 The effects of Ro60-0175 on reinstatement induced by the self-administration context. Panel a shows that during extinction responding decreased over consecutive sessions, and that there were no differences between the control ($n=8$) and reinstatement ($n=8$) groups. Panel b shows the mean ± SEM number of responses on the previously active lever for the reinstatement and control groups following drug treatments. Panel c shows the mean ± SEM number of responses on the previously inactive lever for the reinstatement and control groups following drug treatments. The control group was tested in the same context as for extinction training; the reinstatement group was tested in the original self-administration context. All animals were tested once at each dose level of Ro60-0175. $^{*}p<0.05$; $^{**}p<0.01$ compared to Veh condition.

lever ($F_{(1,14)}=17.32$, $p<0.001$) and declined over sessions ($F_{(21,294)}=12.60$, $p<0.001$). Figure 7b and c show that the ability of Ro60-0175 to reduce reinstatement induced by contextual cues was reversed by SB242084. For active lever responses there was an overall significant main effect of group ($F_{(1,14)}=5.60$, $p<0.03$) with responding being higher in the reinstatement group than the control group. The main effects of Ro60-0175 ($F_{(1,14)}=11.81$, $p<0.01$) and SB242084 ($F_{(1,14)}=4.62$, $p<0.05$) were significant, but the interactions involving these two factors were not significant ($p>0.2$). *Post hoc* comparisons showed that Ro60-0175 reduced responding compared to vehicle in the reinstatement group, and that this effect was prevented by pretreatment with SB242084. For responding on the inactive lever, there were no significant main effects or interactions (all $p>0.08$).

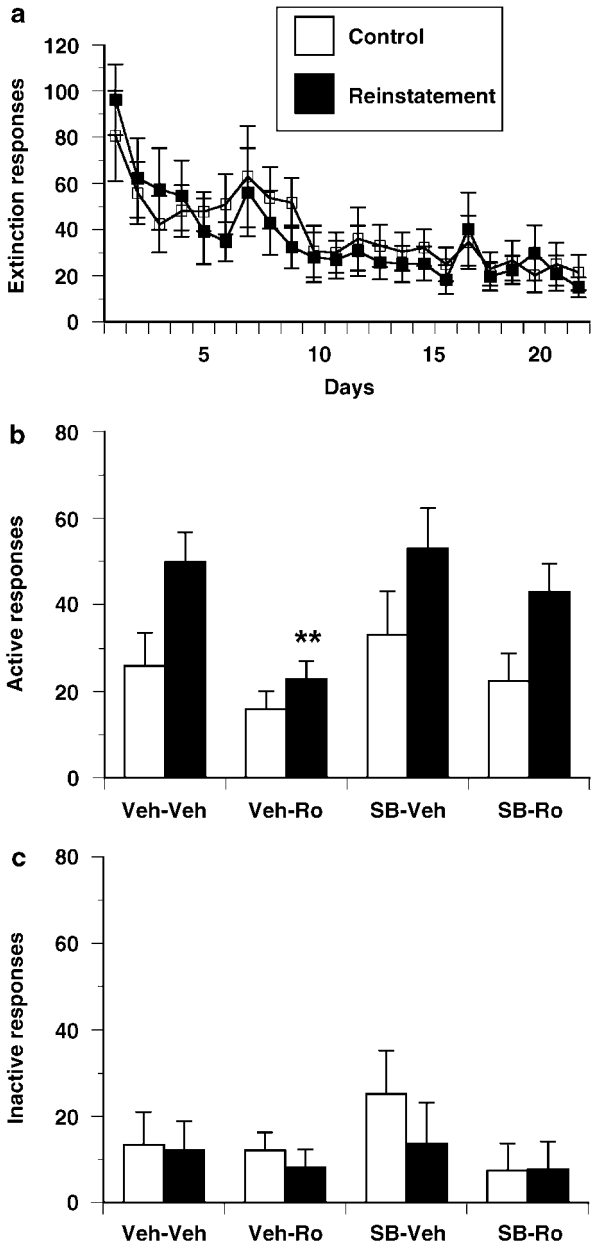

Figure 7 The effects of 1 mg/kg Ro60-0175 (or vehicle) following pretreatment with 0.5 mg/kg SB242084 (or vehicle) on reinstatement induced by the self-administration context. Panel a shows that during extinction conditions, in which bar presses were not reinforced, responding rapidly decreased over consecutive sessions and that there were no differences between the control ($n = 8$) and reinstatement ($n = 8$) groups. Panel b shows the mean ± SEM number of responses on the previously active lever for the reinstatement and control groups following drug treatments. Panel c shows the mean ± SEM number of responses on the previously inactive lever for the reinstatement and control groups following drug treatments. The control group was tested in the same context as for extinction training; the reinstatement group was tested in the original self-administration context. All animals were tested once following each combination of Ro60-0175 (or vehicle) and SB242084 (or vehicle). $^{**}p < 0.01$ compared to Veh–Veh condition.

DISCUSSION

Three main findings emerged from these experiments. First, we confirmed that the 5-HT$_{2C}$ receptor agonist Ro60-0175 reduces cocaine self-administration and found that this effect is sustained with daily injections over a period of 8 days. Second, Ro60-0175 attenuated the reinstatement of responding for cocaine, elicited by the pharmacological stressor yohimbine as well as by contextual cues that had previously been associated with self-administered cocaine. Third, the effects of Ro60-0175 were blocked by the highly selective 5-HT$_{2C}$ receptor antagonist SB242084, confirming that Ro60-0175 reduces cocaine self-administration and reinstatement of responding through stimulation of 5-HT$_{2C}$ receptors.

Studies *in vitro* have demonstrated that the 5-HT$_{2C}$ receptor undergoes rapid desensitization and internalization following agonist activation (Berg *et al*, 2001), suggesting a potential for tolerance development to the effects of 5-HT$_{2C}$ receptor agonists. The results of the first study in this report show that the ability of Ro60-0175 to reduce responding for cocaine was sustained over eight daily injections. This demonstrates that any 5-HT$_{2C}$ receptor desensitization that might be induced by Ro60-0175 is not sufficient to produce tolerance to the effect of Ro60-0175 to reduce responding for cocaine. These results are in agreement with other reports showing a lack of tolerance to the anorectic actions of Ro60-0175 (Hayashi *et al*, 2005; Vickers *et al*, 2000). However, there are reports of rapid tolerance, developing within 7 days, to the anorectic effects of the 5-HT$_{2C}$ receptor agonists m-CPP and YM 438 (Hayashi *et al*, 2005). The reasons why tolerance develops to the effects of some 5-HT$_{2C}$ receptor agonists but not others are not known. It may be that any variations in tolerance profiles between these 5-HT$_{2C}$ receptor agonists relate to how these compounds differentially activate distinct intracellular signalling pathways coupled to 5-HT$_{2C}$ receptors (eg Berg *et al*, 2001; Stout *et al*, 2002), a phenomenon termed functional selectivity (Urban *et al*, 2007).

In addition to reducing responding for cocaine, and to inhibiting reinstatement, Ro60-0175 reduces other behaviors including feeding (Hewitt *et al*, 2002) operant responding for food (Grottick *et al*, 2000) and for nicotine (Grottick *et al*, 2001). Therefore, it needs to be considered that this profile simply reflects a broad, non-specific impairment of behavior. This seems unlikely for several reasons. First, although Ro60-0175 can reduce motor capacity, as assessed by rotarod performance (Grottick *et al*, 2000), this is not seen at the doses of 0.3–1 mg/kg that inhibit responding for cocaine, and reduce reinstatement. Second, in the present experiments, Ro60-0175 had no significant effects on responding on the inactive lever in the self-administration study or both reinstatement studies. Finally, in the cocaine self-administration experiment rats treated with Ro60-0175 still completed several hundred responses showing that their ability to perform the task was not impaired. Responding on a PR schedule generally follows a consistent pattern. Infusions are taken in a reasonably regular fashion; each is generally followed by a pause in responding and preceded by a burst of responding. As shown by the representative cumulative records, the general structure of behavior was not obviously affected by Ro60-0175, other than the earlier termination of responding. Similarly, observational studies have shown that Ro60-0175 reduces food intake without disrupting the temporal

patterning of feeding-related behaviors (Hewitt *et al*, 2002). Overall, these data suggest that treatment with Ro60-0175 produces a blunted motivational state that results in reduced responding for drug and non-drug reinforcers.

Consistent with previous work (Lee *et al*, 2004; Le *et al*, 2005; Shepard *et al*, 2004) the results of Experiment 2 show that the pharmacological stressor yohimbine reinstated responding following extinction of self-administration. This response–reinstating effect of yohimbine was blocked dose dependently by Ro60-0175. Stress-induced reinstatement has been studied most frequently using footshock. This work has shown that the circuitry involved in mediating reinstatement induced by footshock overlaps substantially with that involved in mediating reinstatement induced by drug-associated cues and by drug priming (Shaham *et al*, 2003). Thus, regions such as the VTA, the PFC and the nucleus accumbens are all part of the circuitry involved in these different types of reinstatement (Bossert *et al*, 2006, 2004; Capriles *et al*, 2003; Fuchs *et al*, 2005; McFarland *et al*, 2004; McFarland and Kalivas, 2001). Local infusion of a D1 receptor antagonist into the PFC blocked footshock-induced reinstatement suggesting that such reinstatement could result from enhanced dopaminergic activity in the PFC (Capriles *et al*, 2003). Yohimbine activates a number of brain regions that are also activated by footshock, including the nucleus accumbens, the VTA and the PFC (Funk *et al*, 2006; Singewald *et al*, 2003). This latter finding, coupled with the effectiveness of dopamine receptor blockade in the PFC to reverse footshock-induced reinstatement, is intriguing in light of the fact that Ro60-0175 reduces the stress-induced rise in extracellular dopamine in the PFC (Pozzi *et al*, 2002). That effect is mediated at the level of DA cell bodies rather than in the PFC itself, since it was induced by local infusion of Ro60-0175 into the VTA but not the PFC. Overall, a possible hypothesis for the mechanism underlying the effect of Ro60-0175 to reduce yohimbine (stress)-induced reinstatement is that it results from inhibition of mesocorticolimbic DA activity.

A further brain region that is involved in mediating footshock-induced reinstatement, and which is also activated by yohimbine (Funk *et al*, 2006), is the bed nucleus of the stria terminalis (BNST) (Erb *et al*, 2001). The fact that this structure appears to have high density of 5-HT$_{2C}$ receptors (Clemett *et al*, 2000) suggests that the BNST is an alternative site where 5-HT$_{2C}$ receptor activation could alter the functioning of circuitry involved in mediating stress-induced reinstatement of drug-seeking behavior.

Another class of stimulus that can trigger reinstatement of drug-seeking behavior is drug-associated cues. In keeping with previous reports (Bossert *et al*, 2006, 2004; Crombag *et al*, 2002), the present experiments showed that the context in which cocaine was initially self-administered was a strong stimulus for reinstating responding that had been extinguished in a different context. Thus, under control conditions rats that were returned to the self-administration context, after extinction in a different context, showed an increase in responding compared to rats that remained in the extinction context. This reinstatement response was reduced by previous treatment with Ro60-0175, at a dose as low as 0.3 mg/kg. Interestingly this dose is lower than that typically required to reduce cocaine self-administration, locomotor activity, and especially cocaine-induced reinstatement (Grottick *et al*, 2000). The 5-HT releaser fenfluramine reduced reinstatement of responding elicited by response contingent presentations of discrete cues that had been paired with cocaine (Burmeister *et al*, 2004). That effect was blocked by SB242084 providing further evidence that stimulation of 5-HT$_{2C}$ receptors inhibits cue-induced reinstatement.

While the neurochemical substrates underlying contextual cue-induced reinstatement are not fully understood, the dopamine D1 receptor antagonist SCH23390 and the D2 receptor antagonist raclopride both inhibited context-induced responding in animals with a history of cocaine self-administration (Crombag *et al*, 2002). This implies a role for dopamine in mediating this form of reinstatement. In terms of neuroanatomical substrates, both the VTA and the nucleus accumbens appear to be important elements of the circuitry mediating this behavior since microinfusion of the group II metabotropic glutamate receptor agonist LY379268 into these areas reduced context-induced reinstatement of heroin seeking (Bossert *et al*, 2004, 2006). Previous work with 5-HT$_{2C}$ receptor agonists including Ro60-0175 indicates that 5-HT$_{2C}$ receptor activation reduces the activity of the mesolimbic dopamine system (Di Matteo *et al*, 2000a, b; Gobert *et al*, 2000) probably via an action at the VTA (Fletcher *et al*, 2004). Thus, a logical candidate mechanism for the effects of Ro60-0175 on context-induced reinstatement is that this response results from dampened mesolimbic DA activity. Other sites, such as the PFC, may be involved as well. Inactivation of this area attenuates reinstatement induced by contextual cues (Fuchs *et al*, 2005), and the locomotor stimulant, and discriminative stimulus properties of cocaine are reduced by activation of 5-HT$_{2C}$ receptors in the PFC (Filip and Cunningham, 2003), suggesting a functional role for these cortical receptors.

It appears that there are several points of entry in the underlying mediating circuitry for 5-HT$_{2C}$ receptor activation to inhibit reinstatement of drug seeking. Although the mechanisms that are involved are not known at this point, interactions with mesolimbic and mesocortical dopamine systems appear to be obvious candidates. Future studies involving local infusions of 5-HT$_{2C}$ receptor agonists into sites such as the VTA and PFC would elucidate sites in the brain where 5-HT$_{2C}$ agonists act to alter reinstatement.

Agonists of the 5-HT$_{2C}$ receptor reduce food intake, weight gain, and several aspects of drug-taking behavior in laboratory animals (Grottick *et al*, 2000; Higgins and Fletcher, 2003). The cannabinoid CB1 receptor antagonist rimonabant similarly reduces feeding and behaviors related to drug abuse (Tucci *et al*, 2006). Peripherally localized peptide hormones such as leptin and ghrelin are critical for the maintenance of metabolic and energy homeostasis. Recently, they have been shown also to have a modulatory effect on VTA cell firing, and to influence central reward pathways (Abizaid *et al*, 2006; Fulton *et al*, 2006; Hommel *et al*, 2006). Thus, there is evidence that several different neurotransmitters and peptides affect both feeding and reward-related behaviors. Indeed, Volkow and Wise (2005) have pointed out commonalities in the neurobiological and behavioral processes contributing to obesity and substance abuse (Volkow and Wise, 2005). They have suggested further that treatment strategies, including medications, used in the treatment of one disorder might fruitfully be

applied to treatment of the other. Several 5-HT$_{2C}$ receptor agonists are being developed as potential anti-obesity agents (Miller, 2005; Nilsson, 2006), with early results suggesting promising clinical efficacy of lorcaserin in the treatment of obesity (Jandacek, 2005). The present data suggest that the anti-obesity effects of 5-HT$_{2C}$ agonists may be due to their effect on dopaminergic reward mechanisms; in addition to those hypothalamic circuits that are more directly linked to the physiological control of aspects of feeding behavior such as satiety and energy homeostasis (Heisler *et al*, 2002). Finally, the findings that 5-HT$_{2C}$ receptor stimulation reduces drug-taking and reinstatement of drug-seeking behaviors without the development of tolerance, provide strong evidence that selective 5-HT$_{2C}$ receptor agonists might be a useful treatment for aspects of drug abuse.

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DISCLOSURE/CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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