Studies to Investigate the Role of 5-HT<sub>2C</sub> Receptors on Cocaine- and Food-Maintained Behavior<sup>1</sup>

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ABSTRACT

The present series of studies were designed to investigate the 5-HT<sub>2C</sub> receptor agonist Ro 60-0175 on cocaine- and food-maintained behavior in the rat. Ro 60-0175 (0.1–3 mg/kg, s.c.) reduced cocaine (15 mg/kg, i.p.)-induced hyperactivity. This inhibitory effect of Ro 60-0175 (1 mg/kg, s.c.) was completely blocked by pretreatment with the selective 5-HT<sub>2C</sub> antagonist SB 242,084 (0.5 mg/kg, i.p.). In further studies, Ro 60-0175 (1–3 mg/kg, s.c.) reduced responding for both food (45-mg Noyes pellet) and cocaine (0.25 mg/infusion) maintained under identical schedules of reinforcement (fixed ratio 5, time out 1 min, 60-min duration). The effect on food-maintained responding was blocked by SB 242,084 (0.5 mg/kg, i.p.). Ro 60-0175 (0.3–3 mg/kg, s.c.) also reduced the breakpoint for cocaine self-administration under a progressive ratio schedule of reinforcement. After a period of extinction training, where cocaine solution was substituted with saline, an acute priming injection of cocaine (15 mg/kg, i.p.) but not Ro 60-0175 (1 mg/kg, s.c.) reinstated cocaine responding. In this model of relapse, Ro 60-0175 (1–3 mg/kg, s.c.) pretreatment attenuated the priming effect of acute cocaine injection. In a final series of studies to examine the cataleptogenic properties of Ro 60-0175, very mild indices of catalepsy were observed at the 3 mg/kg dose only. These catalepsy scores were significantly lower than that produced by haloperidol (0.5 mg/kg, s.c.). In further tests of motor function using the Rotarod, deficits were again seen at the 3 mg/kg dose, but not at lower doses. Taken together, these studies suggest that, in addition to reducing food intake, 5-HT<sub>2C</sub> receptor agonists reduce cocaine-reinforced behavior. This would be consistent with electrophysiological and biochemical evidence suggesting an important modulatory influence of 5-HT<sub>2C</sub> receptor activation on mesolimbic dopamine function.

It has long been established that drugs which enhance serotonin (5-HT) function, notably the SSRIs, and the 5-HT releaser and reuptake inhibitor dexfenfluramine, reduce food intake (Blundell, 1984). However, these agents also reduce the self-administration of a variety of drug reinforcers, including cocaine (Carroll et al., 1990; Richardson and Roberts, 1991). Consistent with this observation, we have previously reported that dexfenfluramine reduces both heroin and alcohol self-administration at similar doses to those that reduce palatability-induced feeding (Fletcher, 1988; Higgins et al., 1992, 1994). Antagonist studies, albeit limited due to available compounds at the time, suggested that these effects of dexfenfluramine were mediated through central 5-HT2 receptors. Thus the 5-HT1/2 antagonist metergoline, and the 5-HT2 receptor antagonist ritanserin attenuated the depressant effects of dexfenfluramine on heroin self-administration; the peripheral 5-HT1/2 antagonist xylamidine, and the 5-HT3 antagonist ondansetron, being ineffective (Wang et al., 1995).

Over the last decade, our understanding of central 5-HT systems in terms of receptor pharmacology, distribution, and function has progressed considerably. At least 14 distinct 5-HT receptors have now been cloned (Boess and Martin, 1994), the majority of which appear to play functional roles in the central nervous system [see Barnes and Sharp (1999) for recent review]. In terms of the 5-HT2 receptor subclass, three subtypes have been identified, 2A–2C (Baxter et al., 1995; Barnes and Sharp, 1999). Of particular relevance to the effects of dexfenfluramine seems to be the 5-HT<sub>2C</sub> receptor, because the anorectic effects of dexfenfluramine are attenuated in a 5-HT<sub>2C</sub> receptor knockout mouse line (Tecott et al.,

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); Ro 60-0175, (S)-2-[(chloro-5-fluoro-indol-1-yl)-1-methyllethylamine 1:1 C,H,O; SB 242,084, 6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxo)pyrid-5-yl carbomyl] indoline; FR5TO1-min, fixed ratio (5), time-out 1 min; SSRI, serotinin-selective reuptake inhibitor; PR, progressive ratio; VTA, ventral tegmental area; DA, dopamine; WT, wild type; PVN, paraventricular nucleus; NA, noradrenaline; GABA, g-aminobutyric acid.
1995; Vickers et al., 1999). Also, preliminary studies using recently described subtype-selective 5-HT receptor antagonists support a role for the 5-HT$_{2C}$ receptor in denfenfluramine-induced anorexia (Hartley et al., 1995).

Originally identified in pig choroid plexus (Pazos et al., 1984), a much more widespread distribution of the 5-HT$_{2C}$ receptor has since been described in mammalian brain tissue. Mapping studies have revealed the presence of 5-HT$_{2C}$ receptor mRNA and protein in a variety of forebrain structures, as well as monoaminergic cell body areas such as the locus ceruleus, substantia nigra (A9), and ventral tegmental area (VTA, A10) (Pompeiano et al., 1994; Abramowski et al., 1995; Eberle-Wang et al., 1997). The studies of Eberle-Wang et al. (1997) demonstrated the presence of 5-HT$_{2C}$ mRNA within inhibitory GABAergic interneurons making direct synaptic contact with A9 and A10 dopaminergic cell bodies. Electrophysiological studies reveal that activation of 5-HT$_{2C}$ receptors within the VTA inhibit dopaminergic cell body firing, likely through an enhancement of GABA function (Prisco et al., 1994; Di Matteo et al., 1999; Esposito et al., 1999). Moreover, systemic injection of the 5-HT$_{2C}$ agonist, Ro 60-0175 (Martin et al., 1998) reduces extracellular dopamine (DA) levels in the nucleus accumbens and frontal cortex (Millan et al., 1998; Di Matteo et al., 1999). Conversely, the selective 5-HT$_{2C}$ receptor antagonist, SB 242,084 (Kennett et al., 1997) produces the opposite effect to Ro 60-0175 pretreatment, i.e., increase VTA cell firing and accumbens/frontal cortical DA release (Millan et al., 1998; Di Matteo et al., 1999). This implies a degree of endogenous serotonergic tone to this pathway, probably derived via serotonergic inputs originating from the midbrain raphe (Prisco et al., 1994).

Given the potential contribution of 5-HT$_{2C}$ receptors to the behavioral effects of nonspecific 5-HT ligands described above, it was the purpose of the present studies to investigate the effects of the 5-HT$_{2C}$ agonist Ro 60-0175 on food- and drug-motivated behavior. Cocaine was selected as the drug reinforcer, given the involvement of 5-HT$_{2C}$ receptors in the control of mesolimbic DA tone, and numerous studies having demonstrated the critical role of this pathway in the maintenance of cocaine self-administration and hyperactivity (Koob, 1992). In addition to cocaine self-administration and hyperactivity, the effect of Ro 60-0175 in a model of cocaine relapse, namely, reinstatement of self-administration behavior after extinction by a priming injection of cocaine (De Wit and Stewart, 1981; Markou et al., 1993) was also examined. Finally, although Ro 60-0175 has been described as a selective 5-HT$_{2C}$ agonist, and in-vivo produces behavioral effects that appear to be mediated by this subtype (Millan et al., 1997; Martin et al., 1998; Dekeyne et al., 1999), it also has significant affinity and efficacy at both 5-HT$_{2A}$ and 5-HT$_{2B}$ receptors (Martin et al., 1998; Porter et al., 1999). Therefore we have included the selective 5-HT$_{2C}$ receptor antagonist SB 242,084 (Kennett et al., 1997) in some of these studies. Two schedules of cocaine self-administration were used, responding under a fixed ratio (5), time out 1-min (FR5TO1) schedule and a progressive ratio schedule. It has been argued that, used in tandem, these schedules enable firmer conclusions to be drawn as to the nature of any drug effects on self-administration behavior (Hubner and Moreton, 1991; Markou et al., 1993).

Materials and Methods

Experiments 1, 2, and 5 were conducted at Hoffmann-La Roche, Basel, Switzerland, while experiments 3 and 4 were conducted at the Center for Addiction and Mental Health, Clarke Division, Toronto, Canada. In each case, all experiments complied with the appropriate local and national guidelines relating to animal experimentation.

Animals and Housing. Adult male Sprague-Dawley rats were used in all studies (sources: Roche rats, RCC Ltd., Fullinsdorf, Switzerland; CAMH rats, Charles River, St. Constant, Quebec, Canada). The animals weighed approximately 280 to 340 g at the time of surgery and testing and were housed either individually (self-administration studies) or in groups of four (all other studies) in polycarbonate cages with sawdust bedding. Water was freely available; food availability varied as described below. The housing room was maintained at a constant temperature of 22 ± 2°C, under a 12-h light/dark cycle (lights on: 6:00 AM to 6:00 PM, Roche; 8:00 AM to 8:00 PM, CAMH). All testing was conducted under the light phase of the animal’s light/dark cycle.

Locomotor Activity Testing. Rats were first handled, given sham injections and habituated to the test apparatus (36 × 24 × 19 cm; Benwick Electronics, UK) for three daily 2-h sessions before the experiments were started. A repeated measures design was used for all activity studies, which were of 90-min duration. A washout period of 2 to 3 days was used between each treatment cycle. Total activity counts for the session was the dependent measure.

Food-Maintained Responding. Rats were trained to lever press for food (45-mg Noyes pellet) in eight operant chambers measuring 28 cm long, 31 cm wide, and 31 cm high (MED Associates Inc., St. Albans, VT). Each chamber contained a food pellet dispenser, 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, although this was not used in the present study. Each chamber was illuminated by a house light and housed in a sound-attenuating box equipped with a ventilating fan. The apparatus was controlled by Kestrel software (Conclusive Solutions Ltd., Harlow, UK), and the data were collected by a 486-SX IBM-type computer.

Cocaine Self-Administration Procedures. Rats were anesthetized with 45 to 50 mg/kg sodium pentobarbital. A catheter constructed of tubing connected by a small piece of heat-shrink tubing was surgically implanted in the right jugular vein. The terminal end of the catheter consisted of a 22-gauge guide cannula (Plastics One, Roanoke, VA) and was anchored subcutaneously between the scapulae with a small piece of mesh. This arrangement allowed the catheter to be quickly attached and detached from the drug delivery line by means of a small plastic nut cemented to the end of a stainless steel tether encasing the drug delivery line.

Testing was conducted in 16 operant chambers measuring 28 cm long, 21 cm wide, and 21 cm high (MED Associates Inc.). Each chamber contained a food pellet dispenser, 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. This swivel was attached at one end by tubing to a syringe mounted on a motor driven syringe pump located outside the chamber. At the other end of the swivel was a line by means of a small plastic nut cemented to the end of a stainless steel tether encasing the drug delivery line. The apparatus was controlled by Kestrel software (Conclusive Solutions Ltd., Harlow, UK), and the data were collected by a 486-SX IBM-type computer.

Drugs and Injections. Ro 60-0175 ([S]-2-(chloro-5-fluoroindol-1-yl)-1-methylethylamine 1:1 C$_4$H$_4$O$_4$) and SB 242,084 (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. Haloperidol was prepared to final concentration in 0.9% saline from Haldol ampoules (5 mg/ml; Janssen-Cilag, Beerse, Belgium). Ro 60-0175 was dissolved in 0.9% saline.
before s.c. injection. SB 242,084 was prepared in 0.9% saline solution containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid. SB 242,084 was injected by the i.p. route. Cocaine hydrochloride was supplied either through the PRPN Chemistry Department (Roche) or BDH Inc. Toronto, Ontario, Canada (CAMH). All drug doses are expressed as that of the base. Ro 60-0175 was administered 15 min before the appropriate test, and a 30-min pretreatment time was used for SB 242,084.

**Statistical Analysis.** Data were analyzed by one- or two-way repeated measures ANOVA using Statistica software (Statsoft Inc., Tulsa, OK). Post hoc comparisons were carried out with Newman-Keuls test. For the cocaine self-administration study involving the PR schedule interinfusion intervals were recorded. The latency to respond for the first infusion and the mean interinfusion interval were analyzed by one-way analysis of variance. The mean interinfusion interval was calculated after excluding both the first and last intervals (Depoortere et al., 1993). Two rats failed to respond at all for cocaine after 3 mg/kg Ro 60-0175, therefore data for this high dose were excluded from the overall analysis. Catalepsy and Rotarod (Ugo Basile, Biological Research Apparatus, Varese, Italy) scores were analyzed using the Kruskall-Wallis test for nonparametric data, with post hoc comparisons by Mann-Whitney U test. In all cases the accepted level of significance was P < .05.

**Experiments 1a and 1b: Effect of Ro 60-0175 and SB 242,084 on Cocaine-Induced Increase in Locomotor Activity.** In the first part of this study (experiment 1a), 12 rats were treated with either saline vehicle or cocaine (15 mg/kg, i.p.), or cocaine and Ro 60-0175 (0.1–3 mg/kg, s.c.). A total of six treatment levels were used, and all animals received each treatment in a randomized sequence. The doses of Ro 60-0175 were selected on the basis of preliminary experiments and on the published literature, which showed induction of 5-HT<sub>2C</sub>-mediated behaviors within the dose range used in the present studies (Millan et al., 1997; Martin et al., 1998; Dekeyne et al., 1999). Ro 60-0175 was administered 15 min, and cocaine 5 min, before the onset of behavioral testing.

In the second part to this study (experiment 1b), an additional group of 12 rats was used. After the appropriate habituation and handling procedures, the rats entered a study consisting of four treatment cycles: 0 or 0.5 mg/kg SB 242,084 (i.p.) followed 15 min later with 0 or 1 mg/kg Ro 60-0175 (s.c.), 15 min before test onset. Cocaine (15 mg/kg, i.p.) was administered at each cycle 5 min before test onset. A vehicle-only treatment cycle was included before, and after, this study. This was to assess baseline activity and check whether conditioning had developed to the point of cocaine-induced hyperactivity.

**Experiments 2a and 2b: Effect of Ro 60-0175 and SB 242,084 on Responding for Food under an FR5TO1-min Reinforcement Schedule.** Rats (N = 8) were trained to lever press for food according to an FR1 schedule, and over a 10-day period, the schedule requirements were increased to FR5 with a 1-min time-out period. During the time-out the house light was extinguished, and lever presses were recorded but had no programmed consequences, i.e., FR5TO1 min. The total session duration was 60 min and was closely matched to the cocaine self-administration schedule used in experiment 3a. Once the animals had reached asymptotic levels of performance, based on the number of active lever presses and food rewards received not varying by more than 10% for 3 consecutive days, drug testing began.

For experiment 2a, all rats received vehicle or Ro 60-0175 (0.3–3 mg/kg, s.c.) according to a randomized design. A 2- to 3-day interval was used between each cycle, during which the animals continued to be run daily in the operant test chambers. At the completion of this study, the animals had a 3-month experiment free period, during which they received no drug treatment and were occasionally run in the test chambers. After this interval, the animals were returned to daily test sessions, and after a week to re-establish baseline they entered experiment 2b. A four-treatment cycle was used for this study, the animals receiving either 0 or 0.5 mg/kg SB 242,084 i.p., followed 15 min later with either 0 or 1 mg/kg Ro 60-0175 s.c. Again all animals received each treatment in randomized sequence. During the course of these experiments the animals were maintained at approximately 85% of their free feeding weight by a 45-min access to food at the end of the day.

**Experiment 3a: Effect of Ro 60-0175 on Responding for Cocaine under an FR5TO1-min Reinforcement Schedule.** Before surgery, rats were first trained to lever press for food (45-mg Noyes pellets). Rats were food-restricted (15 g per day) and placed in the operant chambers where each response on the left lever delivered food according to an FR1 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 chambers overnight and allowed 300 food pellets delivered according to the FR1 schedule. A water dish was also placed inside the operant chamber during this session. Thereafter, rats were placed in the chamber only during the 30-min session during the daytime. Using this procedure, all rats were responding for 100 pellets within 7 days. Thereafter, rats were maintained on 20 g of food per day. The animals were then implanted with i.v. cannulae as described above. Six 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 house light and 5 s later a noncontingent infusion of 0.25 mg of cocaine (dissolved in 0.1 ml of 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 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 FR1 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 FR5 schedule was stable and drug testing began. Each animal was tested four times after injection (s.c.) of 0, 0.3, 1, and 3 mg/kg Ro 60-0175. Injections were administered 15 min 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 to 4 days apart. Ten rats completed this study.

**Experiment 3b: Effect of Ro 60-0175 and i.p. Cocaine on Reinstate ment of Responding for Cocaine.** After completion of experiment 3a, rats were placed into extinction, whereby in daily 3-h sessions every response on the left lever delivered a saline infusion plus the light stimulus. After approximately 2 weeks all rats were making less than 15 responses per session. At this point the effects of 1 mg/kg Ro 60-0175 on the response reinstating effects of cocaine (15 mg/kg, i.p.) were examined. On each of four test days, rats were first given an extinction session lasting 2 h. Immediately at the end of this session rats were injected (s.c.) with either 0 or 1 mg/kg Ro 60-0175, followed 15 min later by 0 or 15 mg/kg cocaine (i.p.). Responding was then measured for a further 2 h. Each animal was tested once under each of the four drug combinations. Treatments were pseudorandomized across subjects, and on intervening days the usual 3-h extinction tests were conducted.

**Experiment 4a: Effect of Ro 60-0175 on Responding for Cocaine under a Progressive Ratio Schedule.** Before surgery, rats were trained to press the left lever for food (45-mg Noyes pellets) according to an FR1 schedule as described for experiment 3a. At this point, catheters were implanted and the rats allowed to respond for infusions (0.1 ml during approximately 4 s) of cocaine (0.25 mg per infusion) on an FR1 schedule. Each infusion was signaled by a stimulus light, which remained on for a 20-s time-out period after the infusion. Once responding was stable, a progressive ratio (PR) 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 × e<sup>(−0.200 min<sup>−1</sup> × target + 5</sup>−0.200 min<sup>−1</sup> × target + 5</sup>−0.200 min<sup>−1</sup> × target + 5</sup>−0.200 min<sup>−1</sup> × target + 5</sup>−0.200 min<sup>−1</sup> × target + 5</sup>−0.200 min<sup>−1</sup> × target + 5</sup>}, and yielded response ratios of 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, etc. (e.g., Loh and Roberts, 1990).

Sessions lasted until a period of 1 h without an infusion elapsed or
were a maximum of 5 h in length. The number of infusions earned before this breaking point was recorded. After 12 days on this schedule, when responding was stable, drug testing began.

Each animal was tested four times after injection (s.c.) of 0, 0.3, 1, and 3 mg/kg Ro 60-0175. Injections were administered 15 min 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 to 4 days apart. Eleven rats completed this study.

**Experiment 4b: Effect of Ro 60-0175 against Cocaine-Induced Reinstatement of Responding.** After completion of experiment 4a, the rats were placed into extinction, whereby in daily 3-h sessions every response on the left lever delivered a saline infusion plus the light stimulus. During the course of this phase of the study, two animals’ catheters became blocked and so infusion pumps were turned off during all subsequent sessions for these rats. After approximately 2 weeks all rats were making less than 15 responses per session. At this point the effects of Ro 60-0175 on the response reinstating effects of cocaine (15 mg/kg, i.p.) were examined. On each of 4 test days, rats were first given an extinction session lasting 2 h. Immediately at the end of this session rats were injected (s.c.) with 0, 0.3, 1, and 3 mg/kg Ro 60-0175, followed 15 min later by cocaine. Responding was then measured for a further 2 h. Doses of Ro 60–0175 were administered in counterbalanced order 48 h apart. On the intervening days the usual 3-h extinction tests were conducted.

**Experiment 5: Effect of Ro 60-0175 in Tests of Catalepsy and Rotarod Performance.** Rats were first trained to remain for 2 min on a Rotarod traveling at a constant speed of 16 rpm. The following day, the animals were randomized into five groups of N = 6–8 per group and treated with 0, 0.3, 1, 3 mg/kg, s.c. Ro 60-0175. Haloperidol (0.5 mg/kg, s.c.) was also included as a positive control. Thirty minutes after injection each animal was assessed for catalepsy using two methods. First, the animal’s forelimbs were placed on a horizontal wire 10 cm above ground level, the time (in seconds) for the animal to remove both forepaws being recorded. Second, the rats were placed on an inclined plane (60° from vertical), and the time (seconds) for the animal to move both forepaws was measured. The animals were then tested on a Rotarod using two speeds of 8 and 16 cm/s. Time elapsed before subjects fell of the Rotarod was measured, with a 300-s cut-off time used throughout. The highest score from two trials at each speed was recorded. These experiments were conducted with the experimenter unaware of the identity of drug pretreatment.

**Results**

**Experiments 1a and 1b: Effect of Ro 60-0175 and SB 242,084 on Cocaine-Induced Increase in Locomotor Activity.** Pretreatment with Ro 60-0175 (0.1–3 mg/kg) produced a dose-related inhibition of a cocaine-induced hyperactivity (Fig. 1A). Post hoc analysis after significant one-way ANOVA (F(5,55) = 24.6, P < .01) revealed that cocaine produced a significant increase in total locomotor counts recorded over the 90-min test session compared with vehicle-pretreated controls. Furthermore, Ro 60-0175 pretreatment significantly attenuated the cocaine-mediated hyperactivity in a dose-related fashion.

In experiment 1b, comparison of vehicle only counts recorded at the start and finish of this experiment failed to show any difference (t(11) = 0.4, P = .7), indicating that no significant conditioning to cocaine-induced hyperactivity developed during the course of this study. Analysis of the Ro/SB/cocaine interaction study revealed a main effect of Ro 60-0175 (F(1,11) = 24.3, P < .01), SB 242,084 (F(1,11) = 15.0, P < .01), and a significant Ro 60-0175 × SB 242,084 interaction (F(1,11) = 12.9, P < .01). Thus the inhibition of cocaine hyperactivity produced by Ro 60-0175 (1 mg/kg) was completely blocked by SB 242,084 (0.5 mg/kg) pretreatment. SB 242,084 alone produced a small but nonsignificant (P = .1) potentiation of the cocaine-induced hyperactivity (see Fig. 1B).

**Experiments 2a and 2b: Effect of Ro 60-0175 and SB 242,084 on Responding for Food under an FR5TO1-min Reinforcement Schedule.** The results from experiment 2a are presented in Fig. 2. Ro 60-0175 (0.3–3 mg/kg) produced a dose-related suppression of food-maintained responding under an FR5TO1-min schedule (i.e., total number of rewards F(3,18) = 21.7, P < .01). Significant reductions were recorded at the 1 and 3 mg/kg doses compared with vehicle control (see Fig. 2A). The effect of Ro 60-0175 on the total number of lever presses recorded during the active and time-out session phase is presented in Table 1. A main effect of drug was seen on each phase (i.e., F(3,18) > 8, P < .01), reflecting that Ro 60-0175 pretreatment reduced responding to a similar extent under both the active and time-out period. In experiment 2b, again a significant main effect of Ro 60-0175 was recorded (F(1,7) = 7.5, P < .05) and a significant Ro/SB interaction (F(1,7) = 9.0, P < .05), reflecting the fact that SB 242,084 (0.5 mg/kg) pretreatment blocked the suppressant effect of Ro 60-0175 on responding (see Fig. 2C).

**Experiment 3a: Effect of Ro 60-0175 on Responding for Cocaine under an FR5TO1-min Reinforcement Schedule.** In a manner similar to that recorded for food, under an identical reinforcement schedule Ro 60-0175 (0.3–3 mg/kg) produced a dose-related reduction in responding for i.v. cocaine (F(3,27) = 14.8, P < .01) (Fig. 2B). Significant reductions were noted at the 1 and 3 mg/kg doses compared with vehicle control. Temporal analysis of the distribution of cocaine infusions taken over the 60-min session failed to
reveal a significant treatment \times \text{time bin interaction} (F(6,54) = 1.3, \text{N.S.}), thus Ro 60-0175 pretreatment (1–3 mg/kg) reduced cocaine self-administration similarly across each time bin (see Table 2). Analysis of the total number of lever presses recorded during the test session revealed a main effect of drug on responding during the active period (F(3,27) = 14.8, P < .01), but not the time-out (F(3,27) = 1.5, N.S.) phase (see Table 1). Comparison with response output under the food maintained schedule (experiment 2a), indicated that even though significant reductions in cocaine self-administration were recorded at the 1 mg/kg dose of Ro 60-0175, these animals were capable of a considerably higher response output. In other words, the total number of lever presses recorded over the 60-min test session at 1 mg/kg Ro 60-0175 was 432 ± 137 for food, and 66 ± 11 for cocaine.

Experiment 3b: Effect of Ro 60-0175 and i.p. Cocaine on Reinstatement of Responding for Cocaine. A cocaine dose of 15 mg/kg, i.p. was selected based on experiment 1a, to reliably reinstate responding for cocaine after a period of extinction. The major purpose of this study was to determine whether Ro 60-0175 (1 mg/kg, s.c.) could reinstate cocaine responding and, furthermore, to examine any interaction between Ro 60-0175 and i.p. cocaine on reinstated behavior. Nine rats completed this study.

The outcome from experiment 3b is presented in Fig. 3A. After a 2-week period of extinction, active responding was down to approximately 15 presses/session. Acute i.p. injection of cocaine increased responding to 79 ± 19 presses/session, from 11 ± 3 recorded after acute vehicle injection, i.e., a reliable reinstatement of cocaine responding was established. Ro 60-0175 (1 mg/kg, s.c.) pretreatment did not lead to reinstatement of responding, but did attenuate the corresponding effect of acute cocaine. Formal analysis of this experiment revealed a main effect of cocaine (F(1,8) = 16.2, P < .01), but not Ro 60-0175 (F(1,8) = 2.8, N.S.) or a cocaine \times Ro 60-0175 interaction (F(1,8) = 2.7, P = 0.1). Nonetheless, responding under cocaine/Ro 60-0175 pretreatment was significantly lower than cocaine alone (P = .05).

Experiment 4a: Effect of Ro 60-0175 on Responding for Cocaine under a Progressive Ratio Schedule. The purpose of this study was to extend the observations from experiment 3a that Ro 60-0175 could modify cocaine self-administration under a progressive ratio schedule. The results are presented in Fig. 2D. One-way ANOVA showed a significant effect of Ro 60-0175 (F(3,30) = 15.2, P < .01), with all doses significantly reducing the breakpoint compared with vehicle pretreatment. As shown in Table 3 the latency to earn the first cocaine infusion of the session did not significantly differ between the vehicle and the 0.3 and 1 mg/kg Ro 60-0175 treatments (F(2,20) = 0.96, N.S.). Similarly the mean interinfusion interval was not significantly altered by Ro 60-0175 (F(2,20) = 0.97, N.S.).

Experiment 4b: Effect of Ro 60-0175 against Cocaine-Induced Reinstatement of Responding. Following the observation from experiment 3b that Ro 60-0175 reduced cocaine-induced reinstatement of responding, the purpose of this study was to examine the effect of multiple Ro 60-0175 doses on this behavior. Fifteen rats entered and 13 rats completed this study, 1 rat being removed from the final analysis because its level of responding after acute cocaine challenge was 24-fold higher than the group mean. Another rat gave no responses at all after cocaine challenge, hence it too was eliminated from the study.

After a period of extinction training, baseline responding for saline infusions was around 7 to 9 responses/session (see Fig. 3B). As in experiment 3b, acute cocaine (15 mg/kg, i.p.) challenge increased responding to 175 ± 45 responses/session. This response level was greater than that recorded in experiment 3b after cocaine challenge (79 ± 19 responses) and may be due to the higher response rates the animals previously acquired for cocaine under the PR compared to the FR5TO1-min schedule. Nonetheless, a significant ANOVA was recorded (F(3,36) = 3.2, P < .05) with Ro 60-0175 pretreatment attenuating this cocaine-induced increase in responding, but only at the high dose of 3 mg/kg.

Experiment 5: Effect of Ro 60-0175 in Tests of Catalepsy and Rotarod Performance. During the course of experiments 1 to 4 it became apparent that Ro 60-0175 had a generally suppressant effect on behavior. The purpose of this experiment was to compare the profile of Ro 60-0175 with a classical neuroleptic, haloperidol, in tests of catalepsy and motor coordination. The results are presented in Fig. 4. In each catalepsy test a significant main effect of treatment was recorded (Kruskall-Wallis: H > 17.5, P < .01), with haloper-
idol (0.5 mg/kg, s.c.) producing a significant catalepsy score. Ro 60-0175 (3 mg/kg, s.c.) also produced a significant effect, although the magnitude of change was significantly less than haloperidol, e.g., grid test [median scores—vehicle, 0 s; Ro 60-0175 (3 mg/kg), 3 s; haloperidol, 12.5 s]. Similar findings were also seen in the Rotarod test (Kruskall-Wallis: H > 14.4, P < .01), with both Ro 60-0175 (3 mg/kg) and haloperidol impairing performance. However, no effect of Ro 60-0175 was seen in either the catalepsy or Rotarod test at the lower doses of 0.3 to 1 mg/kg.

Discussion

Ro 60-0175 significantly reduced cocaine-induced hyperactivity. The doses that produced this effect (0.1–3 mg/kg) are within the range at which other 5-HT2C-mediated behaviors have been reported after Ro 60-0175 pretreatment (Millan et al., 1997; Martin et al., 1998; Dekeyne et al., 1999). SB 242,084 (0.5 mg/kg) completely blocked this effect, further supporting the involvement of the 5-HT2C receptor. McCreary and Cunningham (1999) recently reported that the 5-HT2C antagonist SB 206,553 seemed to have a biphasic effect against cocaine hyperactivity, with low doses (1–2 mg/kg) attenuating, and a higher dose (4 mg/kg) potentiating this effect of cocaine. Since cocaine inhibits 5-HT reuptake with similar potency to DA/noradrenaline (NA) reuptake, one hypothesis proposed to account for the enhancement is that 5-HT2C receptor inhibition blocks an inhibitory effect of cocaine on VTA firing caused by enhanced synaptic 5-HT levels (Einhorn et al., 1988; McCreary and Cunningham, 1999). An alternative, but not necessarily a mutually exclusive hypothesis is that 5-HT2C antagonists alone have mild stimulant properties. Indeed, we have occasionally noted mild hyperactivity after SB 242,084 pretreatment at doses of 0.5 mg/kg or...
Second, a comparison of rates of responding on the FR5TO1-rate; however, drug-treated rats ceased responding earlier. Thus, self-administration behavior was initiated within a few minutes of the start of the session and proceeded at a normal rate; however, drug-treated rats ceased responding earlier. Second, a comparison of rates of responding on the FR5TO1-rate; however, drug-treated rats ceased responding earlier. Thus, self-administration behavior was initiated within a few minutes of the start of the session and proceeded at a normal rate; however, drug-treated rats ceased responding earlier.

Alternative brain sites might include the VTA or nucleus accumbens. It is generally believed that the anorectic effects of both direct and indirect 5-HT agonists are at least partially mediated through the paraventricular nucleus (PVN) of the hypothalamus (Leibowitz et al., 1987). However, the observation that lesions to the PVN do not modify the anorectic effect of dexfenfluramine (Fletcher et al., 1993) challenges this idea and suggests alternative site(s) of action. Thus, the effect of Ro 60-0175 on food-maintained responding is unlikely to involve activation of 5-HT$_{2c}$ receptors in the PVN. Alternative brain sites might include the VTA or nucleus accumbens.
acumbens, both regions containing 5-HT<sub>2C</sub> receptors (Pompeiano et al., 1994; Abramowski et al., 1995). If indeed the VTA or nucleus accumbens do represent sites of action for 5-HT<sub>2C</sub> agonists and (perhaps indirect 5-HT agonists), then this could account for the generalized effect of Ro 60-0175 on reward, given the fundamental role that the DA mesolimbic pathway has in motivated behavior (Koob, 1992). The observation that, at slightly higher doses (>3 mg/kg), Ro 60-0175 produces signs of catalepsy and Rotarod impairment may also be consistent with the suppressant effects of 5-HT<sub>2C</sub> agonists on substantia nigra cell firing (see the Introduction). Taken together, these data suggest some mesolimbic versus nigrostriatal selectivity for Ro 60-0175, which would support findings from electrophysiological studies examining the comparative role of 5-HT<sub>2C</sub> receptors in the control of these pathways (Di Matteo et al., 1999).

A final aspect to these studies was to assess the impact of Ro 60-0175 on the reinstatement of cocaine responding. Again, Ro 60-0175 suppressed this behavior, significantly reducing the reinstatement of cocaine responding elicited by a priming injection of cocaine. Le and coworkers (1999) reported that fluoxetine reduced the reinstatement of alcohol drinking produced by a priming injection of alcohol. Furthermore, stress (electroshock)-induced reinstatement of alcohol drinking was more profoundly affected by fluoxetine pre-treatment (Le et al., 1999). It would be interesting to compare stress versus drug-induced reinstatement of cocaine responding after Ro 60-0175 pretreatment. The present studies (experiments 3b and 4b) suggest that Ro 60-0175 may be less potent at reducing the reinstatement of cocaine responding than cocaine self-administration. Thus a significant effect was only noted at the 3 mg/kg dose (experiment 4b), the effect at 1 mg/kg being of borderline significance in only one of two experiments (experiment 3b). Further investigation to establish the specificity of 5-HT<sub>2C</sub> receptor activation on drug reinstatement is warranted. Stewart (1984) demonstrated that the direct injection of morphine into the VTA, a procedure that would be predicted to increase its rate of firing (Matthews and German, 1984; Johnson and North, 1992), resulted in the reinstatement of cocaine responding after extinction. It is thus conceivable that inhibition of VTA firing accounts for the effect of Ro 60-0175 seen in this report.

In summary, the present series of experiments demonstrate significant effects of the 5-HT<sub>2C</sub> agonist Ro 60-0175 on cocaine- and food-maintained behavior. In some experiments, these effects were shown to be blocked by the selective 5-HT<sub>2C</sub> antagonist SB 242,084. We conclude that 5-HT<sub>2C</sub> receptor activation reduces both food- and cocaine-maintained behavior in an equivalent fashion. Preliminary studies suggest that at similar doses, Ro 60-0175 also reduces alcohol- and nicotine-induced self-administration and hyperactivity. Thus, in common with indirect 5-HT agonists such as dexfenfluramine and fluoxetine (see the Introduction), 5-HT<sub>2C</sub> agonists are likely to reduce a wide range of reinforcing behaviors. Recent evidence suggests that, compared to WT controls, a line of 5-HT<sub>2C</sub> knockout mice show delayed satiety in a feeding paradigm in addition to enhanced sensitivity to the disruptive effects of acute cocaine injection (Tecott et al., 1995; Heyser et al., 1999). This may imply that these animals show increased responsivity to a variety of rewarding stimuli, further supporting the view that the 5-HT<sub>2C</sub> receptor represents an important site through which 5-HT can regulate forebrain DA systems and motivated behavior in general.

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