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Stimulation of 5-HT_{1B} receptors decreases cocaine- and sucrose-seeking behavior

Jazmin I. Acosta, Floren A. Boynton, Kenneth F. Kirschner, Janet L. Neisewander*

Department of Psychology, Arizona State University, Box 871104 Tempe, AZ 85287-1104, United States

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Abstract

Serotonin systems have been implicated in incentive motivation for cocaine, yet little is known about the role of 5-HT_{1B} receptors in these processes. We used the extinction/reinstatement model to examine the effects of the 5-$HT_{1B/1A}$ receptor agonist, RU24969, on reinstatement of extinguished cocaine-seeking behavior. Rats trained to self-administer cocaine subsequently underwent extinction. They were then tested twice for cue and cocaine-primed reinstatement of extinguished cocaine-seeking behavior, receiving saline pretreatment 1 day and their assigned dose of RU24969 (0.3, 1.0, 3.0 mg/kg) the other day. Rats were later trained on a schedule of sucrose reinforcement in novel chambers and then tested for effects of RU24969 on cue reinstatement of sucrose-seeking behavior and locomotion. RU24969 decreased cue and cocaine reinstatement of cocaine-seeking behavior and cue reinstatement of sucrose-seeking behavior. Locomotion was increased only at the highest RU24969 dose (3 mg/kg). A subsequent experiment demonstrated that the effects of RU24969 (1 mg/kg) on extinguished cocaine-seeking behavior were reversed by the 5-HT_{1B} antagonist GR127935 (3 mg/kg). These findings suggest that the effects of RU24969 on cue and cocaine reinstatement of cocaine-seeking behavior are 5-HT_{1B} receptor-mediated. Overall, the results suggest that stimulation of 5-HT_{1B} receptors may produce a general decrease in motivation.

Keywords: 5-HT_{1B} receptor; RU24969; GR127935; Reinstatement; Incentive motivation; Relapse

1. Introduction

Cocaine and cocaine-associated stimuli can elicit incentive motivational effects in drug abusers that can lead to craving and relapse (Ehrman et al., 1992). Incentive motivation for cocaine can be measured in animals by using the extinction/reinstatement model (de Wit and Stewart, 1981). In this model, animals are typically trained to perform an operant response with cocaine reinforcement and then undergo extinction training during which responses produce no consequences. Responding in the absence of cocaine reinforcement is referred to as cocaine-seeking behavior and is thought to provide a measure of incentive motivation for cocaine. After cocaine-seeking behavior is extinguished, responding can be reinstated by either cocaine priming, reflecting the incentive motivational effects of cocaine, or the presentation of cocaine-paired cues, reflecting the incentive motivational and conditioned reinforcing effects of the cues.

Serotonin (5-HT) plays a role in modulating cocaine-seeking behavior. Either increasing (Baker et al., 2001; Burmeister et al., 2003) or decreasing (Tran-Nguyen et al., 1999; Tran-Nguyen et al., 2001) 5-HT neurotransmission decreases cocaine-seeking behavior elicited by cocaine-associated cues but has inconsistent effects on cocaine-seeking behavior elicited by cocaine priming. These mixed findings might be attributed to the complexity of the 5-HT system since there are fourteen different 5-HT receptor subtypes that have functionally different effects (Barnes and Sharp, 1999). Serotonin receptor subtypes may be differentially affected as a result of 5-HT manipulations and may be differentially involved in cue versus cocaine reinstate-

* Corresponding author. Tel.: +1 480 965 0209; fax: +1 480 965 8544.
E-mail address: janet.neisewander@asu.edu.

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ment of cocaine-seeking behavior. For instance, previous research has shown that a 5-HT_{1A} receptor antagonist attenuates cocaine-primed reinstatement but has no effect on cue reinstatement (Burmeister et al., 2004; Schenk, 2000). Furthermore, a 5-HT_{2A} antagonist attenuates cocaine-primed reinstatement, whereas a 5-HT_{2C} antagonist enhances this behavior (Fletcher et al., 2002b). The 5-HT_{2C} antagonist also reverses the attenuation of cue reinstatement of cocaine-seeking behavior produced by the 5-HT indirect agonist, D-fenfluramine (Burmeister et al., 2004). The role of 5-HT_{1B} receptors in reinstatement of extinguished cocaine-seeking behavior has not yet been reported.

It seems likely that 5-HT_{1B} receptors are involved in cocaine-seeking behavior since recent findings suggest this receptor subtype is involved in other cocaine-induced behaviors. For instance, the 5-$HT_{1B/1A}$ receptor agonist, RU24969, and the 5-HT_{1B}-selective agonist, CP94253, partially substitute, and produce a left-ward shift in the dose–response curve, for the discriminative stimulus effects of cocaine (Callahan and Cunningham, 1997; Filip et al., 2001). Furthermore, 5-HT_{1B} agonists dose-dependently decrease cocaine self-administration on a fixed ratio schedule and increase responding on a progressive ratio schedule (Parsons et al., 1998). This pattern of findings is interpreted as an enhancement of the reinforcing effects of cocaine. Consistent with this idea, the 5-HT_{1B} agonist, CP94253, enhances cocaine-conditioned place preference (CPP) even though the drug produces conditioned place aversion when given alone (Cervo et al., 2002). Furthermore, elevated expression of 5-HT_{1B} receptors in the ventral tegmental area (VTA) shifts the cocaine-CPP dose–response curve leftward (Neumaier et al,. 2002). 5-HT_{1B} receptor knockout mice also fail to exhibit cocaine-CPP relative to their wild type counterparts (Belzung et al., 2000). In contrast, 5-HT_{1B} knockout mice exhibit enhanced susceptibility to self-administer cocaine (Rocha et al., 1998), suggesting 5-HT_{1B} receptors may inhibit psychostimulant reward. Consistent with this idea, CP93129 decreases amphetamine self-administration on a progressive ratio schedule of reinforcement (Fletcher et al., 2002a). Furthermore, RU24969 dose-dependently decreases responding on a fixed ratio 1 schedule of amphetamine reinforcement, producing equivalent decreases at each dose of amphetamine rather than a leftward shift in the amphetamine dose–response curve (Fletcher and Korth, 1999b). In addition, RU24969 elevates, and dose–dependently reduces the attenuating effects of cocaine on, intracranial self-stimulation (ICSS) thresholds, suggesting a decrease in brain stimulation reward (Harrison et al., 1999). The discrepancy among these findings may be due to differences in species, psychostimulant examined, and/or behavioral paradigm.

5-HT_{1B} receptors have also been implicated in conditioned reinforcement. Specifically, amphetamine potentiates responding maintained by conditioned reinforcers and this effect is attenuated by 5-HT_{1B} agonists (Fletcher and Korth, 1999a). This finding suggests 5-HT_{1B} receptors may play an inhibitory role in psychostimulant-potentiated conditioned reinforcement.

The present experiment examined the effects of the 5-$HT_{1B/1A}$ agonist, RU24969, on reinstatement of extinguished cocaine-seeking behavior either by response-contingent presentations of cocaine-paired cues or by cocaine priming injections. The use of the reinstatement model allowed us to examine more specifically the effects of RU24969 on the incentive motivational effects of cocaine-paired cues and cocaine priming since animals were tested in the absence of cocaine reinforcement. We also compared the effects of RU24969 on cocaine-seeking behavior to its effects on sucrose-seeking behavior and locomotion. Finally, we examined the ability of the 5-HT_{1B} antagonist, GR127935, to reverse the effects of RU24969 on cocaine-seeking behavior.

2. Methods

2.1. Animals

Male Sprague–Dawley rats weighing 225–250 g were individually housed in a climate-controlled colony with a 12-h reversed light/dark cycle (lights off at 6 AM). Housing and animal care were in adherence to the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996).

2.2. Surgery

Animals were handled daily for at least 6 days prior to surgery. Intravenous catheters were implanted into the jugular vein under anesthesia with sodium pentobarbital (50 mg/kg, IP; Sigma Chemical, St. Louis, MO) after pretreatment with atropine sulfate (10 mg/kg, IP, Sigma Chemical). Surgical procedures were performed as described in Neisewander et al. (2000). Animals recovered from surgery over a minimum of 6 days prior to self-administration training. The animals' catheters were flushed daily with 0.1 ml bacteriostatic saline containing heperin sodium (10 U/ml; Elkinns-Sinn Inc., Cherry Hill, NJ), streptokinase (0.67 mg/ml; Astra USA, Inc., Westerborough,MA), and ticarcillan disodium (66.7 mg/ml; Smithkline Beecham Pharmaceuticals, Philadelphia, PA). Proper catheter function was tested periodically by administering 0.03 ml Brevital (16.6 mg/ml; Jones Pharma Inc., St. Louis, MO), a dose that produces brief anesthetic effects only when administered intravenously.

2.3. Drugs

Cocaine hydrochloride (RTI International, Research Triangle Park, NC) was dissolved in bacteriostatic saline and filtered through a 0.2 μm filter. The 5-$HT_{1B/1A}$ agonist,

RU24969 (Tocris Cookson, Ellisville, MO), and antagonist, GR127935 (Sigma Chemical, St. Louis, MO), were dissolved in saline. GR127935 solubility required gentle heating.

2.4. Self-administration training

Self-administration training took place in operant conditioning chambers (10×28×20 cm; Med Associates, St. Albans, VT) equipped with an active lever, an inactive lever, a cue light 4 cm above the active lever, a tone generator (500 Hz, 10 dB above background noise) and a house light situated on the top center of the wall opposite the levers. To facilitate acquisition of cocaine self-administration (Carroll et al., 1981), rats were food-restricted to about 15 g of food/day beginning 2 days prior to self-administration training. Animals were trained to self-administer cocaine (0.75 mg/kg/0.1 ml, IV) for 14–26 daily, 2-h sessions which took place during their dark cycle. They were maintained on food restriction until a criterion of 7 infusions/h on a fixed-ratio (FR) 1 schedule of reinforcement was met for 2 consecutive days, after which they were given access to food ad libitum throughout the rest of self-administration, extinction, and reinstatement testing. Training then continued on the FR 1 schedule until a criterion of 7 infusions/h was met for 5 days or for a maximum of 14 days. Thereafter, rats advanced to a variable-ratio (VR) 2 and then a VR 5, remaining on the VR 5 schedule for a minimum of 5 days. Completion of each schedule resulted in the simultaneous activation of the cue light, house light, and tone followed 1 s later by a 6-s cocaine infusion. All stimuli were inactivated with the termination of the infusion except the house light, which remained activated for a 20-s timeout period, during which lever presses had no consequences.

2.5. Extinction training

Extinction training began the day after the last self-administration day and consisted of 12–21 daily 1-h sessions in the operant conditioning chamber, during which responses had no consequences. Duration of extinction was determined by the criterion of ≤20 active lever responses or an 80% decrease in response rates. As a control for effects of injection on responding, on the last day of extinction training prior to cue reinstatement testing, animals received a SC saline injection 15 min prior to the extinction session, which failed to alter their baseline extinction response rates.

2.6. Effects of RU24969 on cue reinstatement of extinguished cocaine-seeking behavior

After extinction training, rats were assigned to RU24969 dosage groups (0.3, 1.0 or 3.0 mg/kg, SC) counterbalanced for previous cocaine intake. Cue reinstatement of cocaine-seeking behavior was tested on two separate occasions using a within-subjects design in which rats were pretreated with saline prior to one test and their assigned dose of RU24969 prior to the other test, with order of pretreatment counterbalanced. The pretreatments were administered 15 min prior to the 1-h test session. The pretreatment interval and doses were selected based on previous research (Parsons et al., 1998). All cue test sessions began with an automatic delivery of the cocaine-associated cues, which consisted of the same stimulus complex that had been previously paired with cocaine infusions. Responses on the active lever during the testing sessions resulted in response-contingent presentations of the stimulus complex on a FR 1 schedule of reinforcement. We limited the number of a given type of reinstatement test to 2 per rat because cocaine-seeking behavior extinguishes across repeated tests in our model. Animals received at least four additional 1-h extinction sessions between cue reinstatement tests to restabilize baseline response rates. Baseline response rates were calculated as the average response rate of the extinction sessions prior to saline and agonist pretreatment tests. Since baseline response rates were low, reinstatement was operationally defined as at least a doubling of baseline response rates and a minimum of 10 lever presses above baseline following vehicle pretreatment.

2.7. Effects of RU24969 on cocaine-primed reinstatement of extinguished cocaine-seeking behavior

After the last cue reinstatement test, rats received at least 4 additional 1-h extinction sessions before being tested for the effects of their assigned dose of RU24969 on cocaine-primed reinstatement (i.e., the rats received the same dose of RU24969 as that given during cue reinstatement). Immediately before the extinction session prior to cocaine-primed reinstatement testing, animals received an IP injection of saline, which failed to alter baseline extinction response rates. Rats were tested twice, receiving saline pretreatment prior to one test and their assigned RU24969 pretreatment dose prior to the other test with order counterbalanced and 3–5 daily 1-h extinction session intervening between tests. The pretreatment was administered on the test days 15 min prior to the cocaine priming injection (10 mg/kg, IP). The cocaine priming dose was selected based on previous research from our laboratory indicating that it is an effective, but submaximal dose that should allow sensitivity to detect either an increase or decrease in cocaine-seeking behavior. Immediately after the cocaine priming injection, animals were placed into the operant conditioning chambers for a 1-h test session, during which responses had no consequences. Baseline responses were the average response rates on the extinction sessions prior to saline and agonist pretreatment tests.

2.8. Training and testing for cue reinstatement of sucrose-seeking behavior

After the cocaine-primed reinstatement tests, rats were food-restricted to approximately 17 g of food/day beginning

2 days prior to initial training. Animals were trained in a different set of operant conditioning chambers from those used for self-administration. These chambers were located in a different room and had a novel pine bedding beneath the floor. The active and inactive lever assignments and location of the cue light were reversed (i.e., if the right lever had been active then the left lever was now active). To limit sucrose consumption, training sessions were restricted to 30 min. Animals received 9–11 daily sessions progressing from a FR 1 to a VR 5 schedule. Completion of a reinforcement schedule simultaneously activated the house light, the cue light above the new active lever, and a tone (500 Hz, 10 dB above background noise), and the latter two stimuli oscillated on for 1 s and off for 1 s over a 7-s period. A sucrose pellet (45 mg, Noyes) was delivered 1 s after the onset of the stimuli. All stimuli were inactivated 6 s after the delivery of the reinforcer except the house light, which remained activated for a 20-s timeout period. Rats were maintained on food restriction until a criterion of 14 reinforcers on a FR 1 schedule of reinforcement was met for 2 consecutive days, after which they were given access to food ad libitum throughout the rest of the experiment. Rats then progressed to a VR 3 and then VR 5 schedule of reinforcement and all rats were on the VR 5 schedule for at least the last 5 days of training.

Extinction training began the day after the last training session and consisted of 8 daily 30-min sessions during which responses had no consequences. Subsequently, rats were tested for cue reinstatement of sucrose-seeking behavior using the same procedure and RU24969 dose assignments as described previously for cue reinstatement of cocaine-seeking behavior.

2.9. GR127935 reversal of RU24969-induced attenuation of reinstatement of extinguished cocaine-seeking behavior

A follow up experiment was conducted to examine whether the agonist effects on cocaine-seeking behavior are reversed by co-administration with an antagonist using a new cohort of animals. The animals underwent self-administration training for 14 days, followed by extinction training for 12 days using the same procedure as described previously. Rats were then assigned to GR127935 dosage groups, counterbalanced for previous cocaine intake, that received either 0.0 or 3.0 mg/kg, SC of GR127935. They were tested twice for cue reinstatement of cocaine-seeking behavior with at least 4 additional extinction sessions between tests. Subsequently, rats were given 3 additional extinction sessions and were then tested twice for cocaine-primed reinstatement with 4 extinction sessions intervening the tests. For each test type, rats were pretreated with their assigned dose of the antagonist on both test days, and 10 min after this pretreatment they received saline on one of the test days and RU24696 (1 mg/kg, SC) on the other test day with order of these conditions counterbalanced. Twenty-five minutes after the initial pretreatment injection, the 1-h test sessions commenced as described previously. The dose and pretreatment interval were selected based on previous literature (Fletcher and Korth, 1999a; Parsons et al., 1998).

2.10. Effects of RU24969 on locomotor activity

Animals with a history of cocaine self-administration from the previous experiments were assigned to one of three RU24969 dosage groups (0.3, 1.0 and 3.0 mg/kg) counterbalanced for RU24969 dose given during reinstatement tests. They were tested on consecutive days, receiving their assigned dose of RU24969 on one test day and saline on the other test day with order counterbalanced. Rats were placed into the locomotor activity chambers for a 1-h test session 15 min after the saline/RU24969 pretreatment. The chambers were made of Plexiglass (36×24×30 cm high) and equipped with two sets of photocells and light sources 25 cm apart and 4 cm above the floor and mounted at opposite ends. A computer-automated system recorded the number of times the photobeams were interrupted consecutively by the animals moving from one end of the chamber to the other.

2.11. Statistical analyses

For the dose–response experiments, response rates for cue and cocaine-primed reinstatement were analyzed using separate mixed factor 3×3 ANOVAs, with test session (baseline, saline pretreatment test, and agonist pretreatment test) as the repeated measures factor and RU24969 dosage group as the between subjects factor. Locomotion and latency to first response was analyzed using a 2×3 mixed factor ANOVA with saline and drug pretreatment as the repeated measures factor and the RU24969 dosage group as a between subjects factor. For the antagonist reversal experiment, response rates were analyzed using a mixed factor 3×2 ANOVA, with baseline, saline pretreatment test, and drug pretreatment test as the repeated measures factor and GR127935 dosage group as the between subjects factor. Post-hoc comparisons were conducted using Newman-Keuls tests. To further test the hypothesis that GR127935 reversed the effects of RU24969 on response rate and response latency, we also conducted planned comparisons between groups that received RU24969 pretreatment co-administered with the GR127935 vehicle versus RU24969 pretreatment co-administered with 3 mg/kg of GR127935. To simplify presentation, an average baseline for all groups is presented but the statistical analysis of difference from baseline was determined based on individual group data using post-hoc Newman–Keuls tests.

3. Results

All descriptive statistics given below are presented as the mean±SEM.

3.1. Reinforcer intake

The number of infusions per day during the 2-h self-administration training sessions for the two experiments averaged 25.23±1.45 and 27.93±1.54, respectively. The total number of infusions for the first experiment across the 26 self-administration sessions averaged 650.96±37.36. The total number of infusions for the second experiment across the 14 self-administration sessions averaged 386.92±22.35. The number of sucrose reinforcers per day during the 30-min sucrose training sessions averaged 26.77±1.19. The total number of sucrose reinforcers across the 9–11 days of training averaged 262.35±15.77. There were no differences in any of these measures across groups within an experiment.

3.2. Inactive lever responding during reinstatement tests

No significant group effects were found for responding on the inactive lever across test days in either experiment. The means of individual groups from all of the cocaine-seeking reinstatement tests across the 2 experiments ranged from 4.07±1.58 to 13.42±6.63. The means of individual groups from the sucrose-seeking reinstatement test days ranged from 1.20±0.58 to 3.60±1.63.

3.3. Effects of RU24969 on cue reinstatement of cocaine-seeking behavior

The effects of RU24969 on cue reinstatement of cocaine-seeking behavior are shown in Fig. 1. Animals that failed to meet the criteria of double and at least 10 responses above baseline (8 out of 28), as well as an outlier with an initial extinction response rate 18 standard deviations above the mean of all other animals in the study, were excluded from the analyses (final *n*/group=5–8). The ANOVA indicated a significant interaction between test session and RU24969 dosage group, [$F(4,34)=3.51$, $P<0.05$]. Post-hoc comparisons indicated that all dosage groups exhibited an increase in responding on the saline pretreatment test when cues were available response-contingently relative to their individual group baseline when responding had no consequences (Newman–Keuls tests, $P<0.05$), demonstrating cue reinstatement in all groups. In addition, animals receiving 0.3 mg/kg RU24969 exhibited less responding on the saline pretreatment day relative to animals receiving the 3 mg/kg RU24969 (Newman–Keuls tests, $P<0.05$). RU24969 dose-dependently decreased cue reinstatement. None of the RU24969-pretreated animals exhibited an increase in responding relative to their baseline, and animals in the two highest dose groups exhibited less responding on the RU24969 pretreatment test day relative to their saline pretreatment test day (Newman–Keuls tests, $P<0.05$). Fig. 2 shows the response patterns of a representative animal with response rates near the mean for each dosage group. Table 1 shows the mean latency to the first response on the active lever. The ANOVA indicated a significant main effect of test session [$F(1,19)=4.97$, $P<0.05$] demonstrating longer response latency on the RU24969 pretreatment test relative to the saline pretreatment test, regardless of dose.

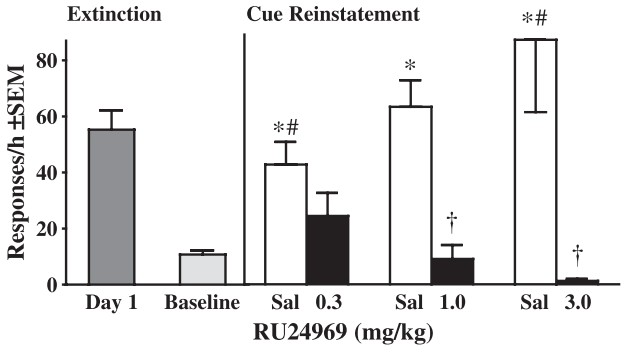

Fig. 1. Effects of RU24969 (*n* per dose=7, 8, and 5, respectively) on reinstatement of cocaine-seeking behavior by response-contingent cue presentations. To simplify presentation, extinction day 1 and baseline response rates are collapsed across all groups, however, all statistical comparisons were conducted using individual group baselines. Baselines were calculated as the mean responses during the last extinction sessions prior to each test. For reinstatement, animals were pretreated with saline 15 min prior to one test (white bars) and their assigned dose of RU24969 15 min prior to the other test (black bars) with order counterbalanced. Cues were available response-contingently during the reinstatement test sessions on a FR 1 schedule of reinforcement. The asterisks (*) represent a significant difference from extinction baseline (Newman-Keuls, $P<0.05$). The number signs (#) represent a significant difference between these designated saline groups (Newman-Keuls, $P<0.05$). The daggers (†) represent a significant difference from saline pretreatment session (Newman-Keuls, $P<0.05$).

3.4. Effects of RU24969 on cocaine-primed reinstatement of cocaine-seeking behavior

The effects of RU24969 on cocaine-primed reinstatement of cocaine-seeking behavior are shown in Fig. 3. Animals that did not meet the reinstatement criteria of double and at least 10 responses above baseline (2 out of 28) when given a cocaine prime and vehicle pretreatment were excluded from the analyses (final *n*/group=8–10). The ANOVA indicated a significant interaction between test session and RU24969 dosage group [$F(4,46)=5.63$, $P<0.001$]. Post-hoc comparisons indicated that all dosage groups exhibited an increase in responding on the saline pretreatment test when a cocaine priming injection was administered immediately before testing relative to their individual group baseline (Newman-Keuls tests, $P<0.001$) demonstrating cocaine-primed reinstatement in all groups. In addition, animals receiving 0.3 mg/kg RU24969 exhibited less responding on the saline pretreatment day relative to animals receiving the 1 mg/kg RU24969 (Newman-Keuls tests, $P<0.05$). RU24969 dose-dependently decreased cocaine-primed reinstatement. The lowest dosage group exhibited an increase in responding relative to their baseline, whereas animals in the two highest dosage groups failed to exhibit an increase relative to their

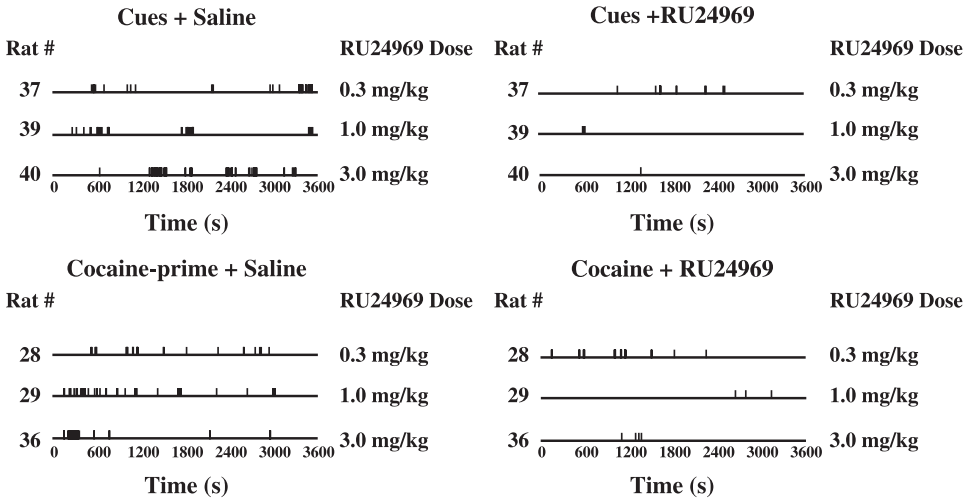

Fig. 2. Response patterns of representative animals with response rates near the mean for each RU24969 dosage group during cue and cocaine-primed reinstatement tests of cocaine-seeking behavior. Tic marks represent individual responses across time (s).

baseline and exhibited less responding on the RU24969 pretreatment test day relative to their saline pretreatment test day (Newman-Keuls tests, $P<0.001$). Fig. 2 shows the response patterns of a representative animal with response rates near the mean for each dosage group. The response latency ANOVA indicated a significant main effect of test session [$F(1,24)=7.649$, $P<0.01$], demonstrating enhanced response latency on the RU24969 pretreatment test relative to saline pretreatment test, regardless of dose (see Table 1).

3.5. Effects of RU24969 on cue reinstatement of sucrose-seeking behavior

The effects of RU24969 on cue reinstatement of sucrose-seeking behavior are shown in Fig. 4. Animals that failed to meet the criteria of double and 10 or more responses above baseline (7 out of 27) were excluded in the analyses (final n/group=5–9). The ANOVA indicated a main effect of test session, [$F(2,34)=94.22$, $P<0.001$]. Post-hoc comparisons indicated an increase in responding on the saline pretreatment test when cues were available response-contingently relative to baseline when responding had no consequences (Newman–Keuls tests, $P<0.001$), demonstrating cue reinstatement regardless of dosage group. Animals exhibited a decrease in cue reinstatement of sucrose-seeking behavior after RU24969 pretreatment relative to saline pretreatment regardless of dosage group (Newman–Keuls tests, $P<0.001$). The ANOVA of response latency indicated a significant interaction between test session and dosage group [$F(1,17)=30.30$, $P<0.05$]. Post-hoc comparisons demonstrated

Table 1
Effects of RU24969 on latency to first response (min±SEM) on the active lever

RU24969 dosage group	Pretreatment	Cue reinstatement	Cocaine-primed reinstatement	Sucrose Cue reinstatement
0.3 mg/kg	Saline	2.2±1.1	7.3±4.6	0.6±0.1
	RU24969	10.1±6.5*	12.3±4.1*	9.7±3.3†
1.0 mg/kg	Saline	3.3±1.1	9.8±7.3	1.86±0.9
	RU24969	17.1±9.4*	27.5±9.3*	31.5±12.8*
3.0 mg/kg	Saline	7.1±3.5	2.1±0.9	0.5±0.0
	RU24969	20.0±8.9*	18.5±10.7*	48.1±11.87*

The asterisk (*) represents difference from saline pretreatment, $P<0.05$. The dagger (†) represents a difference from other RU24969 dosage groups, Newman-Keuls tests, $P<0.05$.

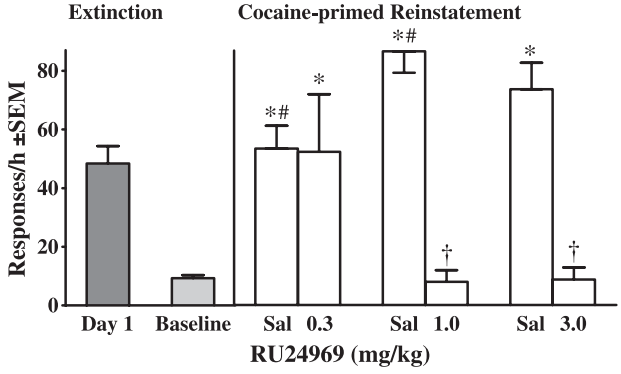

Fig. 3. Effects of RU24969 (n per dose=10, 8, and 8, respectively) on reinstatement of cocaine-seeking behavior by a cocaine priming injection (10 mg/kg, IP). To simplify presentation, extinction day 1 and baseline response rates are collapsed across all groups, however, all statistical comparisons were conducted using individual group baselines. Baselines were calculated as the mean responses during the last extinction sessions prior to each test. For reinstatement, animals were pretreated with saline 15 min prior to one test (white bars) and their assigned dose of RU24969 15 min prior to the other test (black bars) with order counterbalanced. The cocaine prime was administered immediately before testing. No cues were presented during the test sessions. The asterisks (*) represent a significant difference from extinction baseline (Newman-Keuls, $P<0.05$). The number signs (#) represent a significant difference between these designated saline groups (Newman-Keuls, $P<0.05$). The daggers (†) represent a significant difference from saline pretreatment session (Newman-Keuls, $P<0.05$).

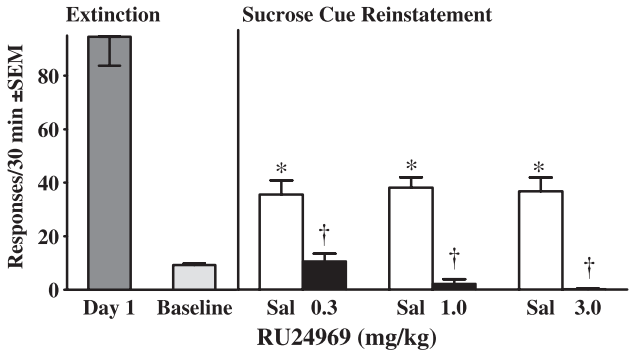

Fig. 4. Effects of RU24969 (n per dose=9, 6, and 5, respectively) on reinstatement of sucrose-seeking behavior by response-contingent cue presentations. To simplify presentation, extinction day 1 and baseline response rates are collapsed across all groups, however, all statistical comparisons were conducted using individual group baselines. Baselines were calculated as the mean responses during the last extinction sessions prior to each test. For reinstatement, animals were pretreated with saline 15 min prior to one test (white bars) and their assigned dose of RU24969 15 min prior to the other test (black bars) with order counterbalanced. Cues were available response-contingently during the reinstatement test sessions on a FR 1 schedule of reinforcement. A significant main effect of test session was obtained and post hoc analyses indicated an increase in responding from extinction baseline following saline pretreatment (Newman-Keuls, $P<0.001$) and a decrease in responding following RU24969 pretreatment relative to saline pretreatment (Newman-Keuls, $P<0.001$). The asterisks (*) represent a significant difference from extinction baseline based on the data collapsed across dosage group. The daggers (†) represent a significant decrease from saline pretreatment test session based on the data collapsed across dosage group.

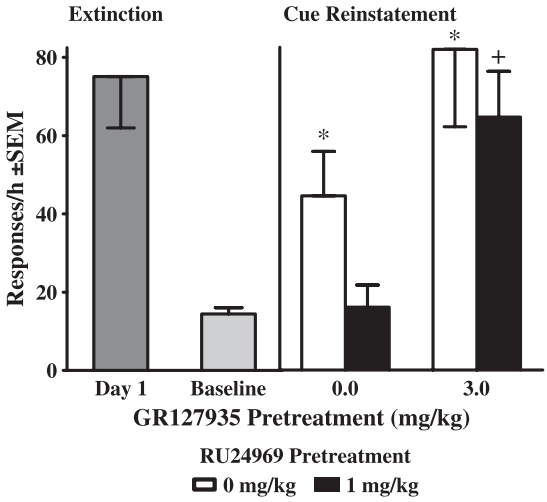

Fig. 5. GR127935 (n per dose=11 and 14, respectively) reversal of RU24969-induced attenuation of reinstatement of cocaine-seeking behavior by response-contingent cue presentations. To simplify presentation, extinction day 1 and baseline response rates are collapsed across all groups, however, all statistical comparisons were conducted using individual group baselines. Baselines were calculated as the mean responses during the extinction sessions prior to each test. Animals were first pretreated with their assigned dose of GR127935 25 min prior to testing and saline 15 min prior to one test (white bars) and RU24969 (1 mg/kg) 15 min prior to the other test (black bars). Cues were available response-contingently during the reinstatement test sessions on a FR 1 schedule of reinforcement. A significant main effect of test session was obtained and post hoc analyses based on the data collapsed across GR127935 dosage group indicated an increase in responding from extinction baseline following saline pretreatment (Newman-Keuls, $P<0.001$), as represented by the asterisks (*). The plus sign (+) represents a significant difference from the respective 0 mg/kg GR127935 dosage group (planned t-tests, $P<0.05$).

increased response latency in animals receiving the 1 and 3 mg/kg RU24969 dose pretreatments relative to saline pretreatment and relative to the 0.3 mg/kg dose of RU24969 pretreatment (Newman–Keuls tests, $P<0.05$) (see Table 1).

3.6. GR127935 reversal of RU24969 effects on reinstatement

GR127935 reversal of the RU24969-induced attenuation of cue reinstatement of cocaine-seeking behavior is shown in Fig. 5. An outlier in the 0 mg/kg GR127935 group with a RU24969 pretreatment test response rate 4 standard deviations above the mean of all other animals in this group was excluded from the analyses (final n/group=11 and 14). The ANOVA indicated a significant main effect of test session, [$F(2,46)=10.71$, $P<0.001$]. Post-hoc comparisons demonstrated an increase in responding on the saline pretreatment test when cues were available response-contingently relative to baseline when responding had no consequences regardless of group (Newman–Keuls tests, $P<0.05$), demonstrating cue reinstatement. Responding on the GR127935 pretreatment test did not differ significantly from either baseline or the saline pretreatment test when collapsed across GR12935 dosage groups. However, planned comparisons indicated that RU24696 pretreatment decreased responding in animals receiving GR127935 vehicle relative to animals receiving 3 mg/kg GR127935 $t(23)=-3.42$, $P<0.01$], suggesting that GR127935 reversed the RU24969-induced decrease in reinstatement. Table 2 shows the mean latency to first response on the active lever of animals in this experiment. Although the ANOVA failed to reveal any significant effects, planned comparisons revealed that RU24969 pretreatment increased response latency in animals receiving GR127935 vehicle relative to animals receiving 3 mg/kg GR127935 [$t(23)=2.10$, $P<0.05$], demonstrating a reversal of the effect of RU24969 on response latency.

GR127935 reversal of the RU24969-induced attenuation of cocaine-primed reinstatement of cocaine-seeking behav-

Table 2
Effects of GR127935 on RU24969-induced increases of latency to first response (min±SEM) on the active lever

GR127935 dosage group	Pretreatment	Cue reinstatement	Cocaine-primed reinstatement
0.0 mg/kg	Saline	10.50±5.6	1.66±0.4
	RU24969 (1 mg/kg)	22.64±6.5^{+}	23.45±7.6^{+}
3.0 mg/kg	Saline	11.83±5.4	3.49±1.7
	RU24969 (1 mg/kg)	6.84±4.2	3.87±2.2

The plus sign (+) represents difference from RU24969 group pretreated with 3 mg/kg GR127935, planned comparison, $P<0.05$.

ior is shown in Fig. 6. The ANOVA indicated a significant interaction between test session and GR127935 dosage group [$F(2,48)=4.13$, $P<0.05$]. Post-hoc comparisons demonstrated an increase in responding for both dosage groups on the saline pretreatment test when a cocaine priming injection was administered relative to respective baselines (Newman–Keuls tests, $P<0.05$), demonstrating cue reinstatement. RU24969 decreased responding relative to saline pretreatment in animals receiving GR127935 vehicle (Newman–Keuls tests, $P<0.05$). However, in animals receiving 3 mg/kg GR127935, RU24969 failed to decrease responding relative to saline pretreatment. This group also exhibited increased responding relative to RU24969 co-administered with GR127935 vehicle (planned t-test, $t(24)=-2.09$, $P<0.05$). The ANOVA of response latency indicated a significant interaction between test session and dosage group [$F(1,24)=8.76$, $P<0.01$]. In animals receiving GR127935 vehicle, post-hoc comparisons demonstrated increased response latency on the RU24969 pretreatment test relative to both pretreatment tests in animals receiving 3 mg/kg GR127935 (Newman–Keuls tests, $P<0.01$). In addition, in animals receiving GR127935 vehicle, planned comparisons demonstrated increased response latency on RU24969 pretreatment test relative to saline pretreatment test $t(11)=-2.96$, $P<0.05$] (see Table 2). Thus, GR127935 also reversed the effects of RU24969 on response latency during the cocaine-primed reinstatement test.

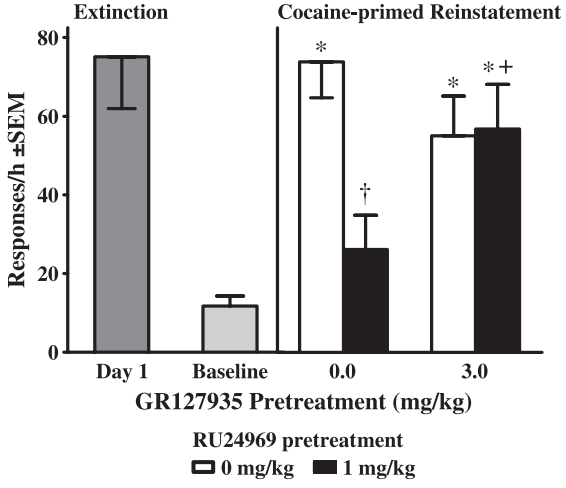

Fig. 6. GR127935 reversal (n per dose=12 and 14, respectively) of RU24969-induced attenuation of reinstatement of cocaine-seeking behavior by a cocaine priming injection (10 mg/kg, IP). To simplify presentation, extinction day 1 and baseline response rates are collapsed across all groups, however, all statistical comparisons were conducted using individual group baselines. Baselines were calculated as the mean responses during the last extinction sessions prior to each test. Animals were first pretreated with their assigned dose of GR127935 25-min prior to testing and saline 15 min prior to one test (white bars) and RU24969 (1 mg/kg) 15 min prior to the other test (black bars) with order counterbalanced. The cocaine prime was administered immediately before testing. The asterisks (*) represent a significant difference from extinction baseline (Newman-Keuls, $P<0.05$). The dagger (†) represents a significant difference from saline pretreatment session (Newman-Keuls, $P<0.05$). The plus sign (+) represents a significant difference from the respective 0 mg/kg GR127935 dosage group (planned t-tests, $P<0.05$).

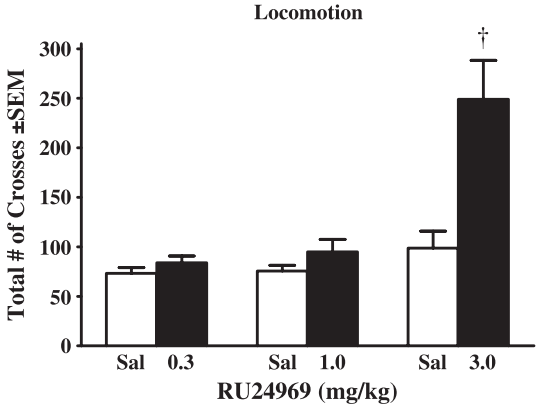

Fig. 7. Effects of RU24969 (n per dose=14) on locomotor activity. Animals were pretreated with saline 15 min prior to one test (white bars) and their assigned dose of RU24969 15 min prior to the other test (black bars) with order of tests counterbalanced. The dagger (†) indicates a significant increase from saline pretreatment (Newman-Keuls, $P<0.001$).

3.7. Effects of RU24969 on locomotor activity

The effects of RU24969 on locomotion are shown in Fig. 7. The ANOVA indicated a significant interaction between test session and dose of RU24969 [$F(1,39)=15.70$, $P<0.001$]. Post-hoc comparisons revealed that animals in the highest dose group exhibited an increase in locomotion on the RU24969 pretreatment test session relative to the saline pretreatment test session (Newman–Keuls tests, $P<0.001$). RU24969 did not alter locomotion at the two lowest doses.

4. Discussion

RU24969 dose-dependently decreased cue and cocaine-primed reinstatement of cocaine-seeking behavior and increased latency to respond. These effects were reversed by pretreatment with the 5-HT_{1B} antagonist, GR127935. However, the antagonist alone did not significantly alter cocaine-seeking behavior. These findings suggest that the effects of RU24969 on cue and cocaine-primed reinstatement are 5-HT_{1B} receptor-mediated. Surprisingly, some group differences were observed on the saline pretreatment test day. Specifically, animals in the 0.3 mg/kg RU24969 dosage group exhibited less responding on the saline pretreatment day relative to animals in the 3 mg/kg RU24969 dosage group during cue reinstatement and also exhibited less responding on the saline pretreatment day relative to animals in the 1 mg/kg RU24969 dosage group during cocaine-primed reinstatement. The lower response rate in the low RU24969 dosage group may be due to the higher response rate on the drug pretreatment day in this group relative to other dose groups, which may have

facilitated extinction of responding on the saline pretreatment day in the half of the animals that were tested with drug first. Alternatively, these effects may have resulted from individual differences in responding that are apparent from the large amount of variability across groups. Importantly, RU24969-induced decreases in reinstatement were evident from within subject comparisons at a given dose and therefore, are not an artifact of individual differences.

The effects of RU24969 were not specific to cocaine-seeking behavior since RU24969 also decreased cue reinstatement of sucrose-seeking behavior. In contrast to the effects of RU24969 on operant behavior, the highest dose of the drug increased locomotion. Previous studies have reported a more potent (1.25 and 2 mg/kg) RU24969-induced hyperactivity (Green et al., 1984; Oberlander et al., 1987). The differences in potency of RU24969 to produce hyperactivity across studies may be due to differences in drug history prior to testing and/or the procedure used to examine locomotion. In the present study, it is possible that RU24969-induced hyperactivity may have interfered with operant responding such that animals were unable to suppress this drug-induced behavior long enough to perform an operant response. However, this idea is somewhat mitigated by the findings that the 0.3 and 1 mg/kg doses of RU24969 failed to alter locomotion in the present study, but produced a robust decrease in cue reinstatement of cocaine-seeking behavior and the 1 mg/kg dose also decreased cocaine-primed reinstatement of cocaine-seeking behavior. The dissociation between the dose-dependent effects of RU24969 on cocaine-seeking behavior versus locomotion suggests that the former is not likely explained by competing hyperactivity. Furthermore, Parsons et al. (1998) have shown that these same doses of RU24969 increase operant responding maintained by cocaine reinforcement. Therefore, it seems unlikely that RU24969-induced hyperactivity can fully account for the decreases in cocaine-seeking behavior observed in the present study.

Another possible explanation for the decrease in cocaine-seeking behavior and response latency is that RU24969 may produce anxiety that interferes with operant responding. Indeed, 5-HT_{1B} receptor agonists decrease the number of entries and time spent in the open arms of an elevated plus maze, suggesting anxiogenic effects (Lin and Parsons, 2002). Although we did not quantitatively measure anxiety-related behavior, we did observe freezing behavior (immobilization) and hyper-reactivity (i.e., startle responses and vocalizations) when RU24969-treated animals were placed into and removed from the operant conditioning chamber. However, this explanation is somewhat mitigated by the findings that other anxiogenic events, such as footshock, increase rather than decrease cocaine-seeking behavior (Erb et al., 1996). Nevertheless, it is still possible that particular anxiogenic states and not others may inhibit cocaine-seeking behavior.

Another possible explanation for the effects of RU24969 on cocaine-seeking behavior is that it may produce a general decrease in motivation, perhaps via a satiation effect. Consistent with this idea, sucrose-seeking behavior was also decreased by RU24969 in the present study. Furthermore, previous literature has shown that, CP94253, another 5-HT_{1B} agonist, dose-dependently decreases the frequency and duration of feeding behavior for both food and sucrose pellets, suggesting a satiety effect, and consequently, a decrease in motivation (Lee and Simansky, 1997). Moreover, Fletcher and Korth (1999a) found that a high dose of 5-HT_{1B} selective agonists, CP93129 and 5CT, decreased responding maintained by water and water-conditioned reinforcement. Taken together, these findings suggest a general decrease in motivation for appetitive stimuli.

We had expected that RU24969 would increase, rather than decrease, cocaine-primed cocaine-seeking behavior since previous research has found that RU24969 enhances the discriminative stimulus and reinforcing effects of cocaine (Callahan and Cunningham, 1997; Parsons et al., 1998). Furthermore, $5HT_{1B}$ receptor stimulation increases DA in the nucleus accumbens by potentiating cocaine-induced decreases in GABA levels in the ventral tegmentum (O'Dell and Parsons, 2004), a pathway known to be involved in cocaine reward. Moreover, following abstinence from chronic cocaine administration, 5-HT_{1B} receptors in various brain regions are up-regulated (Przegalinski et al., 2003) which may result in sensitization to the reinstating effects of cocaine. In this regard, it should be noted that high cocaine priming doses can increase response latency resulting in less reinstatement relative to low doses (Tran-Nguyen et al., 2001), perhaps due to a satiating effect of the high cocaine priming dose. Therefore, it is possible that a RU24969-induced enhancement of the effects of the cocaine prime may result in a decrease in reinstatement during the 1-h test session due to enhanced response latency effects resulting from satiating effects of the drug (see Fig. 2 and Table 1). This explanation is consistent with the idea that RU24969 may potentiate, rather than attenuate, effects of the cocaine prime. This idea could be tested by extending the test session to 2 h in order to enhance sensitivity for detecting putative increases in responding that would be expected to occur later as occurs with higher doses of cocaine primes. Also, testing a lower dose of cocaine prime may enhance sensitivity for detecting a RU24969-induced increase in the incentive motivational effects of cocaine priming as an increase in response rates. Therefore, further research investigating effects of RU24969 at various priming doses of cocaine is needed.

Finally, it is possible that the different findings across models used to study abuse-related behaviors may be due to different mechanisms involved in the type of effects measured in these models. Consistent with this idea, dopamine D1 agonists partially substitute for the discriminative stimulus effects of cocaine (Witkin et al., 1991), yet decrease cocaine-primed cocaine-seeking behavior (Alleweireldt et al., 2002; Khroyan et al., 2000), suggesting different mechanisms are involved in discrim-

inative stimulus effects versus cocaine-primed reinstatement (Spealman et al., 1999). Previous research has also shown dissociable effects of manipulations on reinforcing effects versus cocaine-seeking behavior. For instance, selective dopamine D3 antagonists decrease reinstatement of cocaine-seeking behavior by cocaine-associated cues and cocaine priming (Di Ciano et al., 2003; Vorel et al., 2002) but have inconsistent effects on cocaine self-administration (Campiani et al., 2003; Di Ciano et al., 2003; Gál and Gyertyán, 2003). These findings suggest different mechanisms may be involved in cocaine-primed reinstatement of cocaine-seeking behavior versus cocaine self-administration.

Future studies are needed to examine the role of 5-HT_{1B} receptors in the circuitry involved in cocaine-primed and cue reinstatement of cocaine-seeking behavior. It is possible that different circuits are involved in the motivational effects of the agonist versus other effects, such as increased cocaine reinforcement and behavioral disruption. In addition, it is necessary to investigate whether the effects observed in the present study are due to a pre- or post-synaptic effect. 5-HT_{1B} receptors can function presynaptically as terminal autoreceptors, decreasing serotonin neurotransmission (Barnes and Sharp, 1999) and perhaps thereby decreasing motivation for cocaine. Understanding the circuitry involved in the effects of 5-HT_{1B} agonists may aid in developing treatments for cocaine dependence.

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