Protracted Withdrawal from Cocaine Self-Administration Flips the Switch on 5-HT$_{1B}$ Receptor Modulation of Cocaine Abuse-Related Behaviors

Nathan S. Pentkowski, Tim H.C. Cheung, William A. Toy, Matthew D. Adams, John F. Neumaier, and Janet L. Neisewander

**Background:** The role of serotonin-1B receptors (5-HT$_{1B}$Rs) in modulating cocaine abuse-related behaviors has been controversial due to discrepancies between pharmacological and gene knockout approaches and opposite influences on cocaine self-administration versus cocaine-seeking behavior. We hypothesized that modulation of these behaviors via 5-HT$_{1B}$Rs in the mesolimbic pathway may vary depending on the stage of the addiction cycle.

**Methods:** To test this hypothesis, we examined the effects of increasing 5-HT$_{1B}$R production by microinfusing a viral vector expressing either green fluorescent protein and 5-HT$_{1B}$R or green fluorescent protein alone into the medial nucleus accumbens shell of rats either during maintenance of cocaine self-administration (i.e., active drug use) or during protracted withdrawal.

**Results:** 5-HT$_{1B}$R receptor gene transfer during maintenance shifted the dose-response curve for cocaine self-administration upward and to the left and increased breakpoints and cocaine intake on a progressive ratio schedule, consistent with enhanced reinforcing effects of cocaine. In contrast, following 21 days of forced abstinence, 5-HT$_{1B}$R gene transfer attenuated breakpoints and cocaine intake on a progressive ratio schedule of reinforcement, as well as cue- and cocaine-primed reinstatement of cocaine-seeking behavior.

**Conclusions:** This unique pattern of effects suggests that mesolimbic 5-HT$_{1B}$Rs differentially modulate cocaine abuse-related behaviors, with a facilitative influence during periods of active drug use, in striking contrast to an inhibitory influence during protracted withdrawal. These findings suggest that targeting 5-HT$_{1B}$Rs may lead to a novel treatment for cocaine dependence and that the therapeutic efficacy of these treatments may vary depending on the stage of the addiction cycle.

**Key Words:** Addiction, craving, reinforcement, reinstatement, relapse, reward

Polymorphisms of serotonin-1B receptors (5-HT$_{1B}$Rs) have been linked to substance abuse (1–5); yet, the role of these receptors in cocaine abuse-related behaviors is unclear due to inconsistencies across studies examining their involvement in the rewarding, reinforcing, and incentive motivational effects of psychostimulants. For instance, studies examining cocaine self-administration in 5-HT$_{1B}$R knockout mice (6,7) or amphetamine self-administration in rats (8,9) suggest that 5-HT$_{1B}$Rs inhibit psychostimulant reinforcement. However, self-administration studies in rats using fixed ratio (FR) and progressive ratio (PR) schedules suggest that 5-HT$_{1B}$Rs enhance cocaine reinforcement (10–12). Furthermore, 5-HT$_{1B}$R knockout mice fail to exhibit cocaine-conditioned place preference (CPP) (13), whereas 5-HT$_{1B}$R agonists alone produce conditioned place aversion in rats, yet enhance cocaine-CPP (14), effects that depend on the timing of cocaine-context pairings (15). 5-HT$_{1B}$R receptor agonists also elevate intracranial self-stimulation thresholds and prevent cocaine-induced decreases in intracranial self-stimulation thresholds, suggesting blunted reward mechanisms (16). Furthermore, these agonists attenuate cue- and cocaine-primed reinstatement of extinguished cocaine-seeking behavior, suggesting decreased incentive motivation for cocaine (12,17). These discrepancies may be due to differences in 5-HT$_{1B}$R manipulations or the timing of their use.

5-HT$_{1B}$Rs are synthesized in medium spiny neurons throughout the striatum and are transported to axon terminals in the ventral tegmental area (VTA), ventral pallidum, and substantia nigra (18–20), where they exert inhibitory control over neuronal activity (21,22). Stimulation of VTA 5-HT$_{1B}$Rs potentiates cocaine effects via inhibiting local gamma-aminobutyric acid (GABA) release from neurons that inhibit dopaminergic neurons projecting to the nucleus accumbens shell (NAcsh) (23,24). Increased expression of 5-HT$_{1B}$Rs on terminals of GABAergic neurons projecting from the NAcsh to the VTA shifts the cocaine-CPP dose-response curve to the left (15,25), suggesting that this 5-HT$_{1B}$R population modulates cocaine reward.

The present study aimed to further elucidate the role of 5-HT$_{1B}$Rs in cocaine abuse-related behaviors utilizing viral-mediated gene transfer (VMGT) to transiently increase 5-HT$_{1B}$Rs in terminals of medial NAcsh neurons. Following VMGT, rats were tested for cocaine intake on a FR5 schedule of reinforcement across a range of cocaine doses during maintenance (i.e., active drug use) and for breakpoints and cocaine intake on a PR schedule during both maintenance and protracted withdrawal. Additional experiments examined the effects of VMGT on cue- and cocaine-primed reinstatement of cocaine-seeking behavior during protracted withdrawal. Anxiety-like behavior in the elevated plus-maze (EPM) was also measured, as anxiogenic effects of 5-HT$_{1B}$R agonists may contribute to their effects on drug-seeking behavior (12,26).

**Methods and Materials**

For detailed methodology, see Supplement 1.
Subjects

Male Sprague-Dawley rats weighing 268 g to 308 g at the time of surgery were individually housed in a climate-controlled colony room with a 12-hour reversed light/dark cycle (lights off at 6:00 AM).

Surgery

Surgical procedures were performed as described previously (27). Briefly, rats (n = 74) were anesthetized using isoflurane and catheters were implanted into their jugular veins. Next, the rats were placed into a stereotaxic alignment system (Kopf Instruments, Tujunga, California) and guide cannulae (26G; Plastics One, Roanoke, Virginia) were implanted bilaterally into the NAcsh using coordinates obtained from the Rat Brain Atlas (28): +1.60 mm anterior to bregma, ±1.1 mm from the midline, and -6.6 mm ventral from the surface of the skull; coordinates were based on previous research (15). Following surgery, rats were returned to their home cages for 7 to 10 days recovery before starting self-administration training.

Drugs/Viral Vectors

Cocaine hydrochloride (RTI International, Research Triangle Park, North Carolina) was dissolved in sterile saline. The herpes simplex virus (HSV)-based vector system utilized in the present study (Figure 1B) has been reviewed previously (25,29,30). Briefly, 5-HT1B-green fluorescent protein (GFP) expresses both hemagglutinin-tagged 5-HT1B and GFP from separate transcriptional cassettes, whereas GFP-only expresses only the GFP transcript. The GFP-only control vector does not alter drug reward or behavior compared with sham or vehicle microinfusions (25,29,31). This 5-HT1B-GFP vector produces a threefold increase in 5-HT1B messenger RNA in neuronal (30), but not glial (15), cells and hemagglutinin epitope tagging does not alter the function of 5-HT1B receptors (30).

Self-Administration Training

Rats were trained to self-administer cocaine (.75 mg/kg/1 mL, intravenous [IV]), progressing from a FR1 to a FR5 schedule of reinforcement 6 days per week during 2-hour sessions. Schedule completions on the active lever resulted in the simultaneous activation of a cue light, house light, and tone generator followed 1 second later by a 6-second cocaine infusion; following each infusion, the house light remained activated signaling a 20-second timeout period. Inactive lever presses were recorded but produced no consequences. Once self-administration infusion rates stabilized, defined as less than 10% variability per session across 3 consecutive days with no upward or downward trends (16–22 sessions), rats included in the cocaine dose-response experiments were given 30 minutes access to varying cocaine doses presented in ascending order (0, .032, .1, .32 and 1.0 mg/kg/1 mL, IV) on a FR5 schedule with

![Figure 1](https://www.sobp.org/journal)
a 10-minute timeout period between doses (32). Within-session dose-response training occurred every 2 days, alternating with access to the training dose of cocaine for 2-hour sessions. For the reinstatement and PR experiments during protracted withdrawal, once infusions rates stabilized on the training dose of cocaine (75 mg/kg/1 mL, IV; 18 sessions), rats began extinction training (17 sessions across 21 days of abstinence) or were placed into forced abstinence (21 days), respectively.

**Effects of VMGT on Cocaine Intake During Maintenance**

Testing procedures began once within-session cocaine dose-effect functions stabilized to less than 10% variability across three consecutive sessions. Rats were assigned to groups counterbalanced for previous total cocaine intake and received bilateral microinfusions (2.0 μL/side over 10 minutes) containing approximately 200,000 infective units of either 5-HT1BR-GFP (n = 10) or GFP-only (n = 9) into the medial NAcsh under light isoflurane (2%) anesthesia. To allow for maximal viral expression, rats were given access to their training dose of cocaine for the next 3 days and were then tested on the within-session cocaine dose-effect function on day 4 postinfection; a subset of rats were tested on a PR schedule on day 5. Testing occurred on days 4 and 5 postinfection, as this time course corresponds with peak viral-mediated 5-HT1BR expression for the vector utilized in this study (15,25). Breakpoints were defined as the highest ratio attained once rats failed to receive a cocaine infusion during a 1-hour period on a schedule that progressed exponentially from an FR1 according to the formula \(5^{n-1}/(2n)−5\), with \(n\) reflecting the number of reinforcers the rat received during the session (33).

**Extinction Training**

For the reinstatement experiments, extinction training began the day after self-administration ended, which consisted of daily 1-hour (6 days per week) exposures to the self-administration environment. During extinction, active and inactive lever responses were recorded but produced no consequences. Responding on the active lever in the absence of cocaine reinforcement is the operational definition of cocaine-seeking behavior. Extinction training continued until response rates on the active lever declined to less than 20% of the highest rate observed during extinction (17 sessions).

**Effects of VMGT on Cue- and Cocaine-Primed Reinstatement of Extinguished Cocaine-Seeking Behavior**

Following 14 days of extinction, rats received microinfusions of either 5-HT1BR-GFP (n = 15) or GFP-only (n = 14), as detailed above. To allow for maximal 5-HT1BR expression before testing, rats continued extinction training for the next 3 days and were then tested for cue- and cocaine-primed reinstatement on day 4 postinfection. For cue-elicited reinstatement, rats received response-contingent cue presentations on a FR1 schedule during a 1-hour test session. Immediately following the cue test phase, rats received an intraperitoneal (IP) saline injection and were returned to the operant chambers for a 1-hour extinction session. Lever responses during this test phase produced no consequences and served as the baseline for the cocaine-primed reinstatement test. Immediately after the saline-primed extinction phase, rats received a cocaine-priming injection (10 mg/kg, IP) and were returned to the operant chambers for a 1-hour test during which responses produced no consequences.

**Effects of VMGT on Cue-Elicited Cocaine-Seeking Behavior and Cocaine Intake During Protracted Withdrawal**

For these experiments, a period of forced abstinence (21 days) began the day after the last self-administration session. On the 17th day of abstinence, rats received microinfusions of either 5-HT1BR-GFP (n = 11) or GFP-only (n = 9), as detailed above. Tests for cue-elicited cocaine seeking and responding under a PR schedule of reinforcement were conducted on days 4 and 5 postinfection, respectively. Parameters for both tests were identical to those described above, except that testing commenced following 21 days of forced abstinence.

**Effects of VMGT on Anxiety-Like Behavior in the EPM**

Testing for anxiety-like behavior occurred on day 5 postinfection, as described previously (34). Rats were individually placed in the center of the EPM and their exploratory behavior was measured for 5 minutes.

**Statistical Analyses**

Infusion and response rates during self-administration and extinction training were analyzed using independent sample t tests. For the within-session cocaine dose-response experiment, baseline responding was defined as the average response rates during the three within sessions before VMGT. The total number of infusions at each dose of cocaine were analyzed using a mixed-factor analysis of variance (ANOVA) with viral condition as the between-subjects factor and cocaine dose as the repeated measure. For PR testing and cue testing following protracted withdrawal, the total number of infusions, lever responses, and the highest ratios achieved were analyzed using independent sample t tests. Lever response rates during cue- and cocaine-primed reinstatement tests were analyzed using mixed-factor ANOVAs with viral condition as the between-subjects factor and test session (extinction baseline and cue- or cocaine-primed test session) as the repeated measure. Percent open-arm duration, the number of open-arm entries, and total locomotor activity in the EPM were analyzed using independent sample t tests. Significant ANOVAs were followed by post hoc Newman-Keuls tests; \(α\) was set at .05.

**Results**

**Histology and Attrition**

The histological boundaries of injector tip placements for rats included in the analyses are shown in Figure 1A and representative photomicrographs within the NAcsh and VTA are shown in Figure 1C, E and Figure 1D, F, respectively. GFP expression was visible in neurons located within the NAcsh (Figure 1C, E) and VTA (Figure 1D, F), consistent with previous reports utilizing this HSV packaging system (15,25). Virtually all cell bodies expressing transgenic GFP were located within the NAcsh, although we occasionally observed limited GFP expression in VTA cell bodies, suggesting that a few cells were infected retrogradely (0–2 cells per VTA section; Figure 1F). Green fluorescent protein was extensively localized in beaded axon terminals within the VTA (Figure 1D, F), suggesting viral uptake into medial NAcsh medium spiny projection neurons and translocation of the receptors from the somata of these neurons to their terminals, including the VTA. See Supplement 1 for detailed attrition rates.
**Effects of VMGT on Cocaine Intake During Maintenance**

Before VMGT, rats readily acquired cocaine self-administration. There were no group differences in rates of acquisition (data not shown), the total number of cocaine infusions throughout training or across the last 5 days of training (Table 1), or the number of active lever presses on the last day of self-administration training (Table 1). Depending on individual performance, rats received 36 to 60 sessions, including those that occurred during the training and testing phases.

**Table 1.** Cocaine Reinforcers and Response Rates (Mean ± SEM) During Self-Administration Training and Extinction

<table>
<thead>
<tr>
<th>Viral Condition/Experiment</th>
<th>Infusions/Session Last 5 Training Days</th>
<th>Total Infusions</th>
<th>Active Lever Presses/Hour</th>
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<tbody>
<tr>
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<tr>
<td>Reinfocement</td>
<td>5-HT1B-GFP (n = 9)</td>
<td>62.50 ± 2.58</td>
<td>768.67 ± 58.31</td>
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<tr>
<td></td>
<td>GFP-only (n = 8)</td>
<td>28.05 ± 4.22</td>
<td>719.25 ± 63.63</td>
</tr>
<tr>
<td>Reinstatement</td>
<td>5-HT1B-GFP (n = 15)</td>
<td>25.56 ± .95</td>
<td>424.43 ± 18.63</td>
</tr>
<tr>
<td></td>
<td>GFP-only (n = 14)</td>
<td>27.49 ± 1.51</td>
<td>453.85 ± 26.46</td>
</tr>
<tr>
<td>Relapse</td>
<td>5-HT1B-GFP (n = 9)</td>
<td>22.40 ± 1.05</td>
<td>306.63 ± 23.31</td>
</tr>
<tr>
<td></td>
<td>GFP-only (n = 7)</td>
<td>22.67 ± .92</td>
<td>315.83 ± 24.55</td>
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</table>

Each intravenous cocaine infusion contained .75 mg/kg/1 mL. GFP, green fluorescent protein; 5-HT1B, serotonin-1B; NA, not applicable; SA, self-administration; SEM, standard error of the mean.

**Effects of VMGT on Cue- and Cocaine-primed Reinstatement of Extinguished Cocaine-Seeking Behavior**

Before VMGT, rats readily acquired cocaine self-administration. There were no group differences in rates of acquisition (data not shown), the total number of cocaine infusions throughout training or across the last 5 days of training (Table 1), or the number of active lever presses on the last day of self-administration training (Table 1). Active lever presses readily decreased during extinction training (data not shown), with a significant decrease across each of the first 3 days of extinction (p < .05 in each case). There were no group differences in the number of active lever presses on the first day of extinction (Table 1) or across the 17 days of extinction before reinstatement testing (data not shown).

**Figure 2** illustrates the effects of VMGT into the medial NAcsh on the cocaine self-administration dose-effect function. Before VMGT, varying the unit doses of cocaine produced a characteristic inverted U-shaped dose-effect function (Figure 2A), with the ANOVA indicating a significant quadratic trend and a main effect of cocaine dose [F(4,60) = 59.49, p < .0001] but no viral group by cocaine dose interaction; there were no differences between 5-HT1B-GFP and GFP-only groups at any cocaine dose before VMGT. Post hoc Newman-Keuls analysis revealed that on the ascending limb of the cocaine dose-response curve, increasing the unit dose of cocaine reliably increased intake at each dose relative to the previous dose of cocaine, while on the descending limb, intake decreased at each subsequent cocaine dose (p < .05 in each case); self-administration was not reliably maintained when saline was substituted for cocaine.

Following VMGT (Figure 2B), there was a main effect of viral treatment [F(4,60) = 46.94, p < .0001] and a cocaine dose by viral treatment interaction [F(4,60) = 3.90, p < .001]. 5-HT1B-GFP microinusions shifted the cocaine dose-effect function upward and to the left, with 5-HT1B-GFP treated rats taking more infusions of the .032 and .1 mg/kg cocaine doses compared with both their preinfection baselines and GFP-only control rats (p < .05 in each case); there were no changes in the number of reinforcers at any cocaine dose in GFP-only treated control rats compared with baseline following VMGT. In 5-HT1B-GFP treated rats, the .032 mg/kg dose of cocaine supported self-administration; 1 mg/kg remained the peak cocaine dose, but intake was elevated compared with GFP-only control rats (p < .05 in each case). Lever presses on the inactive lever were negligible and did not vary across groups (data not shown).

**Figure 3** illustrates the effects of VMGT into the medial NAcsh on PR responding. 5-HT1B-GFP microinjected rats achieved higher ratios [t(11) = 4.28, p < .005], self-administered more cocaine infusions [t(11) = 5.94, p < .0001], and emitted more total active lever responses [t(11) = 4.33, p < .0005] compared with GFP-only control rats. Lever presses on the inactive lever were negligible and did not vary across groups (data not shown).

**Figure 4A** illustrates the effects of VMGT into the medial NAcsh on active lever responding during cue-elicited reinstatement testing. There was a main effect of viral treatment [F(1,27) = 4.64, p < .05] and a viral treatment by test session interaction [F(1,27) = 5.34, p < .05]. Post hoc analysis indicated that both viral groups increased responding on the active lever when response-contingent cues were available, relative to baseline when responses produced no consequences (p < .05 in each case), indicating cue-elicited reinstatement of extinguished cocaine-seeking behavior regardless of viral condition. However, 5-HT1B-GFP microinusions attenuated response rates compared with GFP-only microinusions, indicating that elevated 5-HT1B expression attenuated cue-elicited reinstatement of extinguished cocaine-seeking behavior. Lever presses on the inactive lever were negligible and did not vary across groups (data not shown).

**Figure 4B** illustrates the effects of VMGT into the medial NAcsh on active lever responding during cocaine-primed reinstatement testing. There was a main effect of viral treatment [F(1,27) = 12.137, p = .005] and a viral treatment by test session interaction [F(1,27) = 11.86, p = .005]. Post hoc analysis indicated that both viral groups increased responding when cocaine-priming injections were administered, relative to baseline when saline-priming injections were administered (p < .05 in each case), indicating cocaine-primed reinstatement of extinguished cocaine-seeking behavior regardless of viral condition. However, 5-HT1B-GFP microinusions attenuated response rates compared with GFP-only control rats, indicating that elevated expression of 5-HT1BRs attenuated cocaine-primed reinstatement of extinguished cocaine-seeking be-

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Effects of VMGT on Cue-Elicited Cocaine-Seeking Behavior and Cocaine Intake During Protracted Withdrawal

Before VMGT, rats readily acquired cocaine self-administration. There were no group differences in rates of acquisition (data not shown), the total number of cocaine infusions throughout training or across the last 5 days of training (Table 1), or the number of active lever presses on the last day of self-administration training (Table 1).

Figure 5 illustrates the effects of VMGT into the medial NAcsh on active lever responding during the cue-elicited cocaine-seeking test. Following VMGT, 5-HT₁₅-R-GFP treated rats exhibited decreased responding on the active lever compared with GFP-only control rats \( t(14) = 3.46, p < .005 \), indicating that elevated expression of 5-HT₁₅Rs attenuated cue-elicited cocaine-seeking behavior. Lever presses on the inactive lever were negligible and did not vary across groups (data not shown).

Figure 6 illustrates the effects of VMGT into the medial NAcsh on PR response rates. Following VMGT, 5-HT₁₅-R-GFP microinfused rats achieved lower ratios \( t(14) = 4.52, p < .0001 \), self-administered fewer cocaine infusions \( t(14) = 4.07, p < .005 \), and emitted less...
Effects of viral-mediated serotonin-1B receptor (5-HT1BR)-gene transfer into the medial nucleus accumbens shell on cue- and cocaine-primed reinstatement of extinguished cocaine-seeking behavior expressed as the mean number of active lever responses during a 1-hour test session ± standard error of the mean (SEM). Rats were available response-contingently during the test session on a fixed ratio 1 schedule of reinforcement. For cue-elicited reinstatement, cues were available response-contingently during the test session on a fixed ratio 1 schedule of reinforcement. For cocaine-primed reinstatement, the cocaine prime (10 mg/kg, intraperitoneal) was administered immediately before testing and no cues were presented during the test sessions. *Difference from baseline; **difference from GFP-only control rats (Newman-Keuls, p < .05 in each case).

Effects of VMGT on Anxiety-Like Behavior in the EPM

5-HT1BR-GFP microinfusions into the medial NAcsh failed to alter anxiety-like behavior in the EPM. There were no group differences in the percentage of time spent in the open arms or the number of open-arm entries (Figure 7). Furthermore, following VMGT, there were no differences between viral groups in total locomotor activity (data not shown).

Discussion

The results provide convincing support for the hypothesis that 5-HT1BRs modulate cocaine abuse-related behaviors in opposing directions depending on the stage of addiction. During maintenance (i.e., active drug use), 5-HT1BR-VMGT shifted the dose-response curve for cocaine self-administration upward and to the left on a FR5 schedule of reinforcement (Figure 2) and increased cocaine intake, active lever responses, and the highest ratio achieved (i.e., breakpoints) on a PR schedule (Figure 3). This pattern of changes is similar to the effects produced by increasing the unit dose of self-administered cocaine (35,36), suggesting increased reinforcing effects of cocaine. In contrast, during protracted withdrawal, 5-HT1BR-VMGT decreased cue- and cocaine-primed reinstatement of extinguished cocaine-seeking behavior (Figures 4 and 5) and decreased cocaine intake, active lever responses, and the highest ratio achieved on a PR schedule (Figure 6). This pattern suggests a decrease in the incentive motivational effects elicited by cocaine-priming injections and exposure to cocaine-associated cues and a decrease in motivation and/or reinforcement on a PR schedule.

These findings extend upon previous research demonstrating that elevated 5-HT1BR expression in these same neuronal populations increases cocaine-CPP (15,25) and that 5-HT1BR agonists administered during maintenance produce a leftward shift in the cocaine self-administration dose-effect function and increased responding on PR schedules of reinforcement (10,11). In contrast, 5-HT1BR agonists administered during protracted withdrawal decreased cue- and cocaine-primed reinstatement of extinguished cocaine-seeking behavior (12,17), consistent with the inhibitory influence of increased 5-HT1BR expression in the present study. The bi-directional effects of VMGT on PR responding may indicate that 5-HT1BRs are differentially engaged in reinforcement processes during maintenance of cocaine self-administration versus motivation processes after a period of abstinence. Alternatively, the opposing influence on PR responding may indicate a withdrawal-induced shift in 5-HT1BR regulation over cocaine reinforcement and/or motivation. Indeed, a functional switch in multiple neurotransmitter systems has been reported following withdrawal from several drugs of abuse (37–39); however, this is the first data to suggest a withdrawal-induced switch in serotonin systems. Although future research is needed to determine the precise mechanism by which 5-HT1BRs modulate PR responding before and during withdrawal, the present pattern of effects indicates that 5-HT1BR modulation over drug seeking/taking is addiction stage-dependent with a facilitative influence during periods of active drug use, in contrast to an inhibitory influence during protracted withdrawal.
The opposing effects observed at different time points mitigate impaired performance explanations, which predict consistent behavioral changes. Furthermore, there were no differences in locomotion between 5-HT1B-R-GFP and GFP-only groups during EPM testing. The finding that anxiety-like behavior was not altered during EPM testing (Figure 7) suggests that anxiety did not interfere with cocaine-seeking behavior, as suggested previously for systemic 5-HT1B agonist effects (12,26). 5-HT1B-mediated anxiety-like effects likely involve neurons originating in the dorsal raphe (30), but the present findings suggest that the target regions involved in the behavior are outside of the mesolimbic pathway. Although all groups in the present study received virus infusions, previous studies utilizing this identical vector system detected no behavioral differences between no surgery, sham surgery, and GFP-only groups (25,31). Furthermore, this vector system produces a threefold increase in 5-HT1B-R messenger RNA (30) exclusively in neurons and hemagglutinin epitope tagging does not alter 5-HT1B-R function (30). Collectively, these findings preclude nonspecific motor, anxiety, or viral-vector effects.

The medial NAcsh projects predominantly to VTA (40). Although 5-HT1B-R expression was not quantified in the present study, transgene expression in the VTA was evident in the form of GFP located within beaded axons (Figure 1D, F), consistent with previously confirmed transgene expression within the VTA in the form of immunoblotted hemagglutinin-tagged 5-HT1B-R protein (15). The majority (>90%) of NAcsh neurons are GABAergic medium spiny projection neurons (41); thus, it is likely that the majority of transgenic 5-HT1B-Rs were expressed in these neurons. Gamma-aminobutyric acid release in the VTA is inhibited via 5-HT1B-Rs localized on axon terminals of GABAergic neurons that originate in the NAcsh (20,21). These neurons presumably synapse onto dopamine neurons directly (42) or glutamatergic neurons that provide excitatory input onto dopamine neurons (43). Therefore, the present behavioral effects likely resulted from 5-HT1B-R-induced inhibition of GABA release in the VTA and subsequent disinhibition of dopaminergic neurons (23,24,44 – 46) that influence drug abuse-related behaviors associated with cocaine and other drugs of abuse (47–

Figure 6. Effects of viral-mediated serotonin-1B receptor (5-HT1B-R)-gene transfer into the medial nucleus accumbens shell following 21 days of protracted withdrawal (i.e., forced abstinence) from chronic cocaine self-administration on responding during cocaine self-administration (.75 mg/kg/1 mL, intravenous [IV]) breakpoint testing on an exponential progressive ratio (PR) schedule of reinforcement. Data are expressed as the highest ratio achieved (A), the number of active lever responses emitted (B), and the number of cocaine reinforcers obtained (C). Five days following viral-mediated gene transfer (n = 7–9/group), 5-HT1B-R-green fluorescent (GFP) (black bars) treated rats achieved lower final ratios, emitted fewer active lever responses, and received fewer cocaine infusions compared with GFP-only (gray bars) control rats; data expressed as mean ± standard error of the mean (SEM). *Increase compared with GFP-only treated rats (t tests, p < .05 in each case).

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Figure 7. Effects of viral-mediated serotonin-1B receptor (5-HT1B-R)-gene transfer into the medial nucleus accumbens shell on anxiety-like behavior during testing in the elevated plus-maze expressed as the percentage of time spent in (A) the open arms and number of entries into (B) the open arms + standard error of the mean (SEM) during a 5-minute test session. Rats (n = 14–15/group) received microinfusions of a viral vector containing either 5-HT1B-R-green fluorescent (GFP) (black bars) or GFP-only (gray bars) into the medial nucleus accumbens shell 5 days before testing.

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However, HSV vectors are not selective for any neuronal subtype (29), and therefore, it is likely that some cells projecting to the ventral pallidum, hypothalamus, or collaterals within the NAcsh were also infected and may have contributed to the observed behavioral effects. Indeed, 5-HT<sub>1B</sub>R-VMTG within a single or small ensemble of medium spiny neurons would be expected to increase 5-HT<sub>1B</sub>Rs in all of the related axon terminal fields (40). Such changes closely model the consequences of increased 5-HT<sub>1B</sub>R expression in nucleus accumbens medium spiny neurons that occurs during exposure to cocaine (50,51).

The present results suggest that 5-HT<sub>1B</sub>Rs should be pursued as a target for medication development for treating cocaine dependence and relapse. Indeed, it may be possible to exploit the ability of 5-HT<sub>1B</sub>R agonists to inhibit cocaine intake during a relapse (i.e., reduced intake on PR schedules during protracted withdrawal), while simultaneously decreasing incentive motivational effects of stimuli that elicit craving (12,17), thereby decreasing the incidence of relapse. Importantly, pure cocaine addiction is a rare clinical disorder and polydrug use in cocaine addicts presents a major challenge in developing treatments for drug dependence (52,53).

In particular, cocaine-dependent patients often exhibit comorbidity for alcohol and opiate abuse (54–56). Interestingly, polymorphisms of 5-HT<sub>1B</sub>Rs have been linked to substance abuse not only for cocaine but also opiates and alcohol (1–5). Neuroimaging studies in humans have also revealed that alcohol dependence is associated with increased ventral striatal 5-HT<sub>1B</sub>Rs (57), effects similar to those detected following cocaine administration in rats (50,51). Furthermore, systemic 5-HT<sub>1B</sub>R agonist administration decreases ethanol (58,59), amphetamine (60), and cocaine (10–12) intake, particularly on the descending limb of the self-administration dose-effect function. Collectively, these data indicate that 5-HT<sub>1B</sub>Rs are involved in modulating drug intake across a range of abused substances, including alcohol, amphetamine, and cocaine; however, further research investigating the effects of 5-HT<sub>1B</sub>Rs on polydrug intake and the influence of these receptors on the incentive motivational effects of stimuli that elicit craving for alcohol and opiates is needed.

Conclusions

The present report offers the strongest evidence to date that during the different stages of the addiction cycle, 5-HT<sub>1B</sub>Rs produce opposite modulatory effects on cocaine abuse-related behaviors, enhancing cocaine intake during periods of active drug use in contrast to inhibiting cocaine intake and incentive motivation for cocaine elicited by exposure to cocaine-associated cues or cocaine-priming injections following protracted withdrawal. The pattern of behavioral effects observed in the present study, as well as with 5-HT<sub>1B</sub>R agonists, is unique and strongly suggests that 5-HT<sub>1B</sub>Rs should be pursued as a target for medication development for treating cocaine dependence and relapse.

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Supplementary material cited in this article is available online.


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