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Protracted withdrawal from cocaine self-administration flips the switch on 5-HT_{1B} receptor modulation of cocaine-abuse related behaviors

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Abstract

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 (GFP) and 5-HT_{1B}R or GFP 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-gene transfer during maintenance shifted the dose–response curve for cocaine self-administration upward and to the left and increased break points and cocaine intake on a progressive ratio (PR) schedule, consistent with enhanced reinforcing effects of cocaine. In contrast, following 21 days of forced abstinence 5-HT_{1B}R-gene transfer attenuated break points and cocaine intake on a PR 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

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and that the therapeutic efficacy of these treatments may vary depending on the stage of the addiction cycle.

Keywords

reward; addiction; reinforcement; relapse; craving; reinstatement

Introduction

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 (FR) and progressive (PR) ratio 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 agonists also elevate intracranial self-stimulation (ICSS) thresholds and prevent cocaine-induced decreases in ICSS 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 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 break points 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 the Supplement.

Subjects

Male Sprague–Dawley rats weighing 268–308 g at the time of surgery were individually housed in a climate-controlled colony room with a 12-h reversed light/dark cycle (lights off at 6 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, VA) 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 7–10 days recovery prior to starting self-administration training.

Drugs/viral vectors

Cocaine hydrochloride (RTI International, Research Triangle Park, NC) was dissolved in sterile saline. The herpes simplex virus (HSV) based vector system utilized in the present study (see Figure 1B) has been reviewed previously (25, 29, 30). Briefly, 5-HT_{1B}R-green fluorescent protein (GFP) expresses both hemagglutinin-tagged 5-HT_{1B}R 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-HT_{1B}R-GFP vector produces a 3-fold increase in 5-HT_{1B}R mRNA in neuronal (30), but not glial (15) cells, and hemagglutinin epitope tagging does not alter the function of 5-HT_{1B}Rs (30).

Self-administration training

Rats were trained to self-administer cocaine (0.75 mg/kg/0.1 ml, i.v.) progressing from a FR1 to a FR5 schedule of reinforcement 6 days/week during 2-h sessions. Schedule completions on the active lever resulted in the simultaneous activation of a cue light, house light, and tone generator followed 1 s later by a 6-s cocaine infusion; following each infusion the house light remained activated signaling a 20-s timeout period. Inactive lever presses were recorded but produced no consequences. Once self-administration infusion rates stabilized, defined as less than 10% variability/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 min access to varying cocaine doses presented in ascending order (0.0, 0.032, 0.1, 0.32 and 1.0 mg/kg/0.1 ml, i.v.) on a FR5 schedule with a 10-min 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-h sessions. For the reinstatement and PR experiments during protracted withdrawal, once infusion rates stabilized on the training dose of cocaine (0.75 mg/kg/0.1 ml, i.v.; 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 3 consecutive sessions. Rats were assigned to groups counterbalanced for previous total cocaine intake and received bilateral microinfusions (2.0 μ l/side over 10 min) containing approximately 200,000 infective units of either 5-HT_{1B}R-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 post-infection; a subset of rats were tested on a PR schedule on day 5. Testing occurred on day 4 and 5 post infection as this time course corresponds with peak viral-mediated 5-HT_{1B}R expression for the vector utilized in this study (15, 25). Break points were defined as the highest ratio attained once rats failed to receive a cocaine infusion during a 1-h period on a schedule that progressed exponentially from an FR1 according to the formula $5 * e^{(0.2n)} - 5$, 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 selfadministration ended, which consisted of daily 1-h (6 days/wk) exposures to the selfadministration 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-HT_{1B}R-GFP (n=15) or GFP-only (n=14) as detailed above. To allow for maximal 5-HT_{1B}R expression prior to testing, rats continued extinction training for the next 3 days and were then tested for cue- and cocaine-primed reinstatement on day 4 post-infection. For cueelicited reinstatement, rats received response-contingent cue presentations on a FR 1 schedule during a 1-h test session. Immediately following the cue test phase, rats received an i.p. saline injection and were returned to the operant chambers for a 1-h 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, i.p.) and were returned to the operant chambers for a 1-h 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-HT_{1B}R-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 day 4 and 5 post-infection, 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 post-infection as described previously (34). Rats were individually placed in the center of the EPM and their exploratory behavior was measured for 5 min.

Histology

After testing, rats were anesthetized and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. Brains were then harvested, post-fixed and cryoprotected. A series of coronal sections (40 μ m) were taken throughout the NAc and VTA and were analyzed using dual laser confocal microscopy (Zeiss) in order to verify cannulae tip placement and GFP expression. Rats with misplaced cannulae or that lacked GFP expression in both the NAcsh and VTA were excluded from the analyses.

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 prior to VMGT. The total number of infusions at each dose of cocaine were analyzed using a mixed-factor 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 mixedfactor 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 0.05.

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 1D, F, respectively. GFP expression was visible in neurons

located within the NAcsh (Figure 1C and E) and VTA (Figure 1D and 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; see Figure 1F). GFP 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 for detailed attrition rates.

Effects of VMGT on cocaine intake during maintenance

Prior to 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 five 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 36–60 sessions, including those that occurred during the training and testing phases.

Figure 2 illustrates the effects of VMGT into the medial NAcsh on the cocaine self-administration dose-effect function. Prior to 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 [$F(4, 64)=59.49, p<0.0001$] but no viral group by cocaine dose interaction; there were no differences between 5-HT_{1B}R-GFP and GFP-only groups at any cocaine dose prior to 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 ($p<0.05$ 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 [$F(4, 60)=46.94, p<0.0001$] and a cocaine dose by viral treatment interaction [$F(4, 60)=3.90, p<0.001$]. 5-HT_{1B}R-GFP microinfusions shifted the cocaine dose-effect function upward and to the left, with 5-HT_{1B}R-GFP treated rats taking more infusions of the 0.032 and 0.1 mg/kg cocaine doses compared to both their pre-infection baselines and GFP-only controls ($p<0.05$ in each case); there were no changes in the number of reinforcers at any cocaine dose in GFP-only treated controls compared to baseline following VMGT. In 5-HT_{1B}R-GFP treated rats, the 0.032 mg/kg dose of cocaine supported self-administration; 0.1 mg/kg remained the peak cocaine dose, but intake was elevated compared to GFP-only controls ($p<0.05$ 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-HT_{1B}R-GFP microinfused rats achieved higher ratios [$t(11)=4.28, p<0.005$], self-administered more cocaine infusions [$t(11)=5.94, p<0.0001$] and emitted more total active

lever responses [$t(11)=4.33, p<0.005$] compared to GFP-only controls. Lever presses on the inactive lever were negligible and did not vary across groups (data not shown).

Effects of VMGT on cue- and cocaine-primed reinstatement of extinguished cocaine-seeking behavior

Prior to 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 five 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 three days of extinction ($p<0.05$ 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 prior to reinstatement testing (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 [$F(1, 27)=4.64, p<0.05$] and a viral treatment by test session interaction [$F(1, 27)=5.34, p<0.05$]. 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 (Newman-Keuls, $p<0.05$ in each case), indicating cue-elicited reinstatement of extinguished cocaine-seeking behavior regardless of viral condition. However, 5-HT_{1B}R-GFP microinfusions attenuated response rates compared to GFP-only microinfusions, indicating that elevated 5-HT_{1B}R 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 [$F(1, 27)=12.137, p=0.005$] and a viral treatment by test session interaction [$F(1, 27)=11.86, p=0.005$]. 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 (Newman-Keuls, $p<0.05$ in each case), indicating cocaine-primed reinstatement of extinguished cocaine-seeking behavior regardless of viral condition. However, 5-HT_{1B}R-GFP microinfusions attenuated response rates compared to GFP-only controls, indicating that elevated expression of 5-HT_{1B}Rs attenuated cocaine-primed reinstatement of extinguished cocaine-seeking behavior. Lever presses on the inactive lever were negligible and did not vary across groups (data not shown).

Effects of VMGT on cue-elicited cocaine-seeking behavior and cocaine intake during protracted withdrawal

Prior to 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 five 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-HT_{1B}R-GFP treated rats exhibited decreased responding on the active lever compared to GFP-only controls [$t(14)=3.46, p<0.005$], indicating that elevated expression of 5-HT_{1B}Rs 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-HT_{1B}R-GFP microinfused rats achieved lower ratios [$t(14)=4.52, p<0.0001$], self-administered fewer cocaine infusions [$t(14)=4.07, p<0.005$] and emitted less total active lever responses [$t(14)=4.57, p<0.0001$] compared to GFP-only controls. Lever presses on the inactive lever were negligible and did not vary across groups (data not shown).

Effects of VMGT on anxiety-like behavior in the EPM

5-HT_{1B}R-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-HT_{1B}Rs modulate cocaine-abuse related behaviors in opposing directions depending on the stage of addiction. During maintenance (i.e. active drug use), 5-HT_{1B}R-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., break points) 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-HT_{1B}R-VMGT decreased cue- and cocaine-primed reinstatement of extinguished cocaine-seeking behavior (Figures 4, 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-HT_{1B}R expression in these same neuronal populations increases cocaine-CPP (15, 25), and that 5-HT_{1B}R agonists administered during maintenance produce a leftward shift in the cocaine self-administration dose-effect function and increase responding on PR schedules of reinforcement (10, 11). In contrast, 5-HT_{1B}R agonists administered during protracted withdrawal decrease cue- and cocaine-primed reinstatement of extinguished cocaine-seeking behavior (12, 17), consistent with the inhibitory influence of increased 5-HT_{1B}R expression in the present study. The bi-directional effects of VMGT on PR responding may indicate that 5-HT_{1B}Rs 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-HT_{1B}R 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 5-HT systems. Although future research is needed to determine the precise mechanism by which 5-HT_{1B}Rs modulate PR responding before and during withdrawal, the present pattern of effects indicates that 5-HT_{1B}R 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-HT_{1B}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-HT_{1B}R agonist effects (12, 26). 5-HT_{1B}R-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 3-fold increase in 5-HT_{1B}R mRNA (30) exclusively in neurons (15) and hemagglutinin epitope tagging does not alter 5-HT_{1B}R function (30). Collectively, these findings preclude nonspecific motor, anxiety or viral-vector effects.

The medial NAcsh projects predominantly to VTA (40). Although 5-HT_{1B}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 and F), consistent with previously confirmed transgene expression within the VTA in the form of immunoblotted hemagglutinin-tagged 5-HT_{1B}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-HT_{1B}Rs were expressed in these neurons. GABA release in the VTA is inhibited via 5-HT_{1B}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-HT_{1B}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 (47–49). 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_{1B}R-VMGT within a single or small ensemble of medium spiny neurons would be expected to increase 5-HT_{1B}Rs in all of the related axon terminal

fields (40). Such changes closely model the consequences of increased 5-HT_{1B}R expression in NAc medium spiny neurons that occurs during exposure to cocaine (50, 51).

The present results suggest that 5-HT_{1B}R 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_{1B}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 poly-drug use in cocaine addicts presents a major challenge in developing treatments for drug dependence (52, 53). In particular, cocaine-dependent patients often exhibit co-morbidity for alcohol and opiate abuse (54–56). Interestingly, polymorphisms of 5-HT_{1B}R 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_{1B}R (57), effects similar to those detected following cocaine administration in rats (50, 51). Furthermore, systemic 5-HT_{1B}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_{1B}R 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_{1B}R on poly-drug 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_{1B}R 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_{1B}R agonists, is unique and strongly suggests that 5-HT_{1B}R should be pursued as a target for medication development for treating cocaine dependence and relapse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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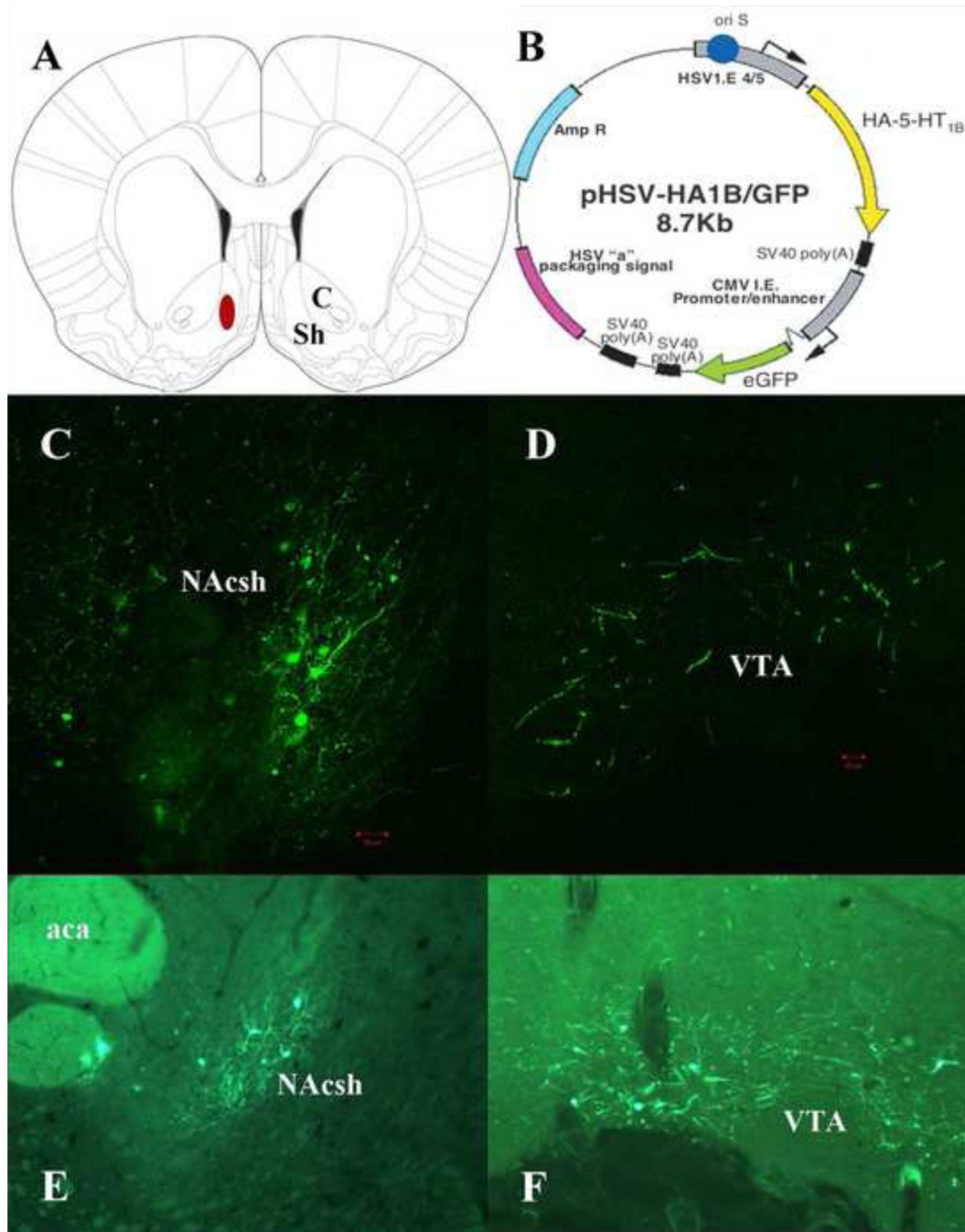


Figure 1.

Illustration of the anatomical target within the medial NAcsh for viral microinfusions (A) (reproduced with permission from (28)), schematic drawing of the HSV-hemagglutinin-tagged 5-HT_{1B}R-GFP transgene amplicon (B), and representative photomicrographs depicting viral-mediated gene transfer evident as GFP expression in sections from the NAcsh (C and E) and VTA (D and F) 5 days after viral microinfusions of the 5-HT_{1B}R-GFP transgene. Viral-mediated gene transfer into the medial NAcsh induced local GFP expression in cell bodies and transport of transgenes from the NAcsh to axon terminals of

median spiny neurons in the VTA. Scale bar, 50 μ m. Abbreviations: C (nucleus accumbens core); Sh (nucleus accumbens shell); aca (anterior commissure).

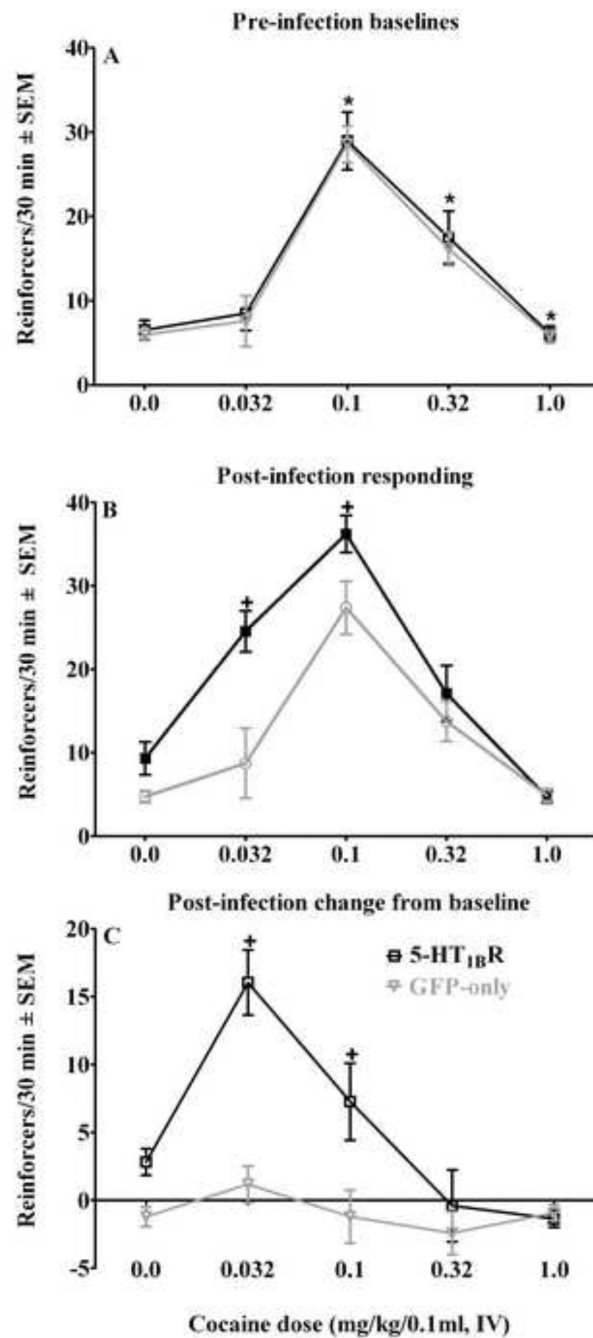


Figure 2. Effects of viral-mediated 5-HT_{1B}R-gene transfer into the medial NAcsh on the cocaine self-administration dose-effect functions expressed as the mean number of reinforcers obtained/30 min ± SEM on a FR5 schedule of reinforcement during baseline training (A), post-infection testing (B) and as a difference from baseline (C). Prior to viral-mediated gene transfer, rats in both the 5-HT_{1B}R-GFP (black squares) and GFP-only (gray triangles) viral treated groups (n=8–9/group) exhibited characteristic inverted U-shaped cocaine dose-response functions, whereas post-infection the dose-effect function was shifted upward and

to the left in 5-HT_{1B}R-GFP treated rats. Asterisk (*) represents a within-group change in cocaine intake compared to the previous cocaine dose (Newman-Keuls, $p < 0.05$); there were no differences between 5-HT_{1B}R-GFP and GFP-only groups at any cocaine dose during baseline sessions. Plus sign (+) represents an elevation compared to GFP-only controls (Newman-Keuls, $p < 0.05$ in each case).

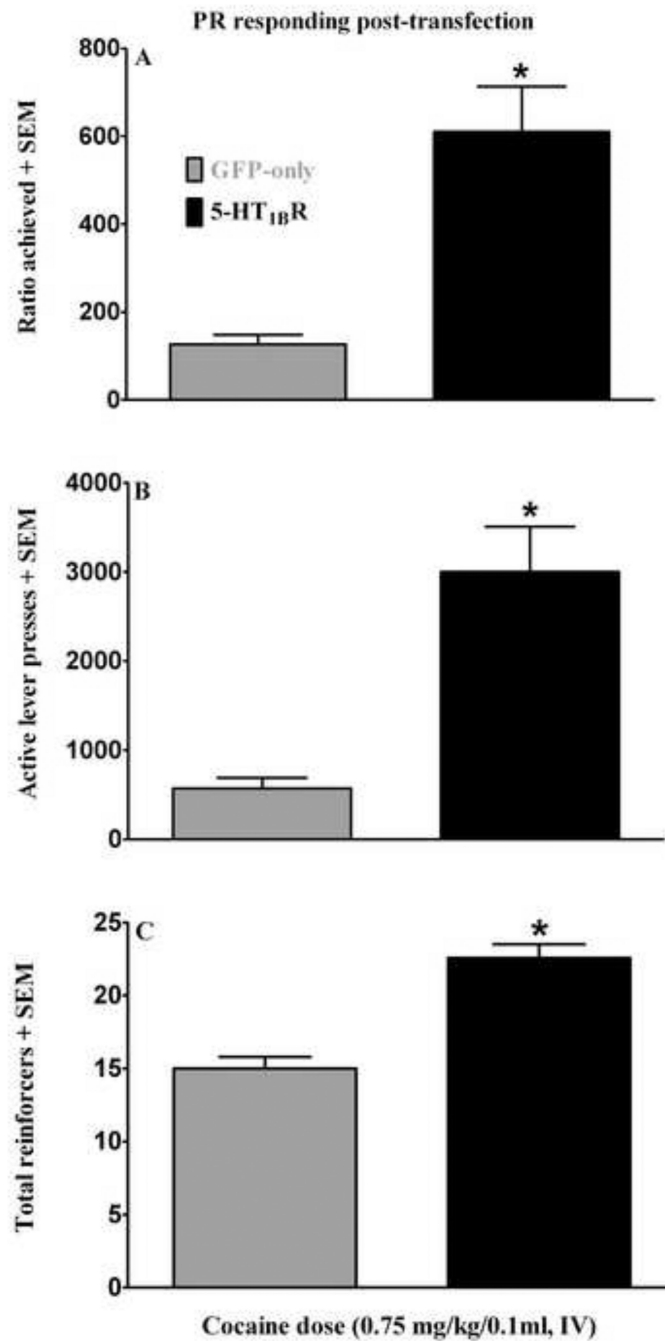


Figure 3. Effects of viral-mediated 5-HT_{1B}R-gene transfer into the medial NAcsh on responding during cocaine self-administration (0.75 mg/kg/0.1 ml, i.v.) break point testing on an exponential PR schedule of reinforcement expressed as the highest ratio achieved (A), the number of active lever responses emitted (B) and the number of cocaine reinforcers obtained (C). Five days following viral-mediated gene transfer, 5-HT_{1B}R-GFP (black bars) treated rats achieved higher final ratios, emitted more active lever responses and received more cocaine infusions compared to GFP-only (gray bars) controls; data expressed as mean +

SEM. Asterisk (*) represents an increase compared to GFP-only treated rats (t-tests, $p < 0.05$ in each case).

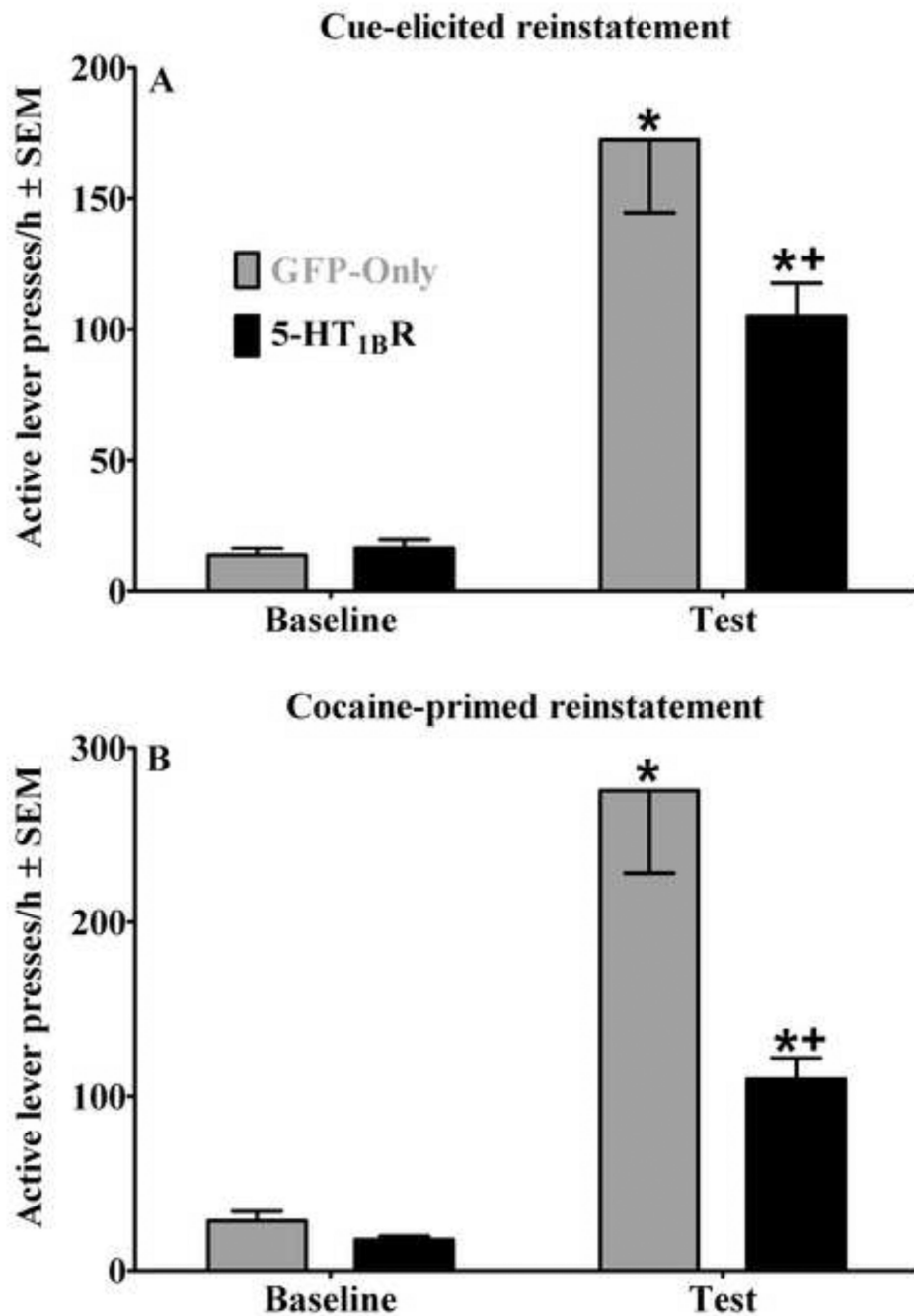


Figure 4. Effects of viral-mediated 5-HT_{1B}R-gene transfer into the medial NAcsh on cue-(A) and cocaine-primed (B) reinstatement of extinguished cocaine-seeking behavior expressed as the mean number of active lever responses during a 1-hr test session ± SEM. Baselines represent mean responses during the extinction session preceding the cue-elicited test session (A), and mean responses during the saline-primed extinction session preceding the cocaine-primed test session (B). Rats (n=14–15/group) received microinfusions of a viral vector containing either 5-HT_{1B}R-GFP (black bars) or GFP-only (gray bars) into the medial NAcsh four days

prior to testing. For cue-elicited reinstatement, cues were available response-contingently during the test session on an FR1 schedule of reinforcement. For cocaine-primed reinstatement, the cocaine prime (10 mg/kg, i.p.) was administered immediately before testing and no cues were presented during the test sessions. Asterisk (*) represents a difference from baseline and plus sign (+) represents a difference from GFP-only controls (Newman-Keuls, $p < 0.05$ in each case).

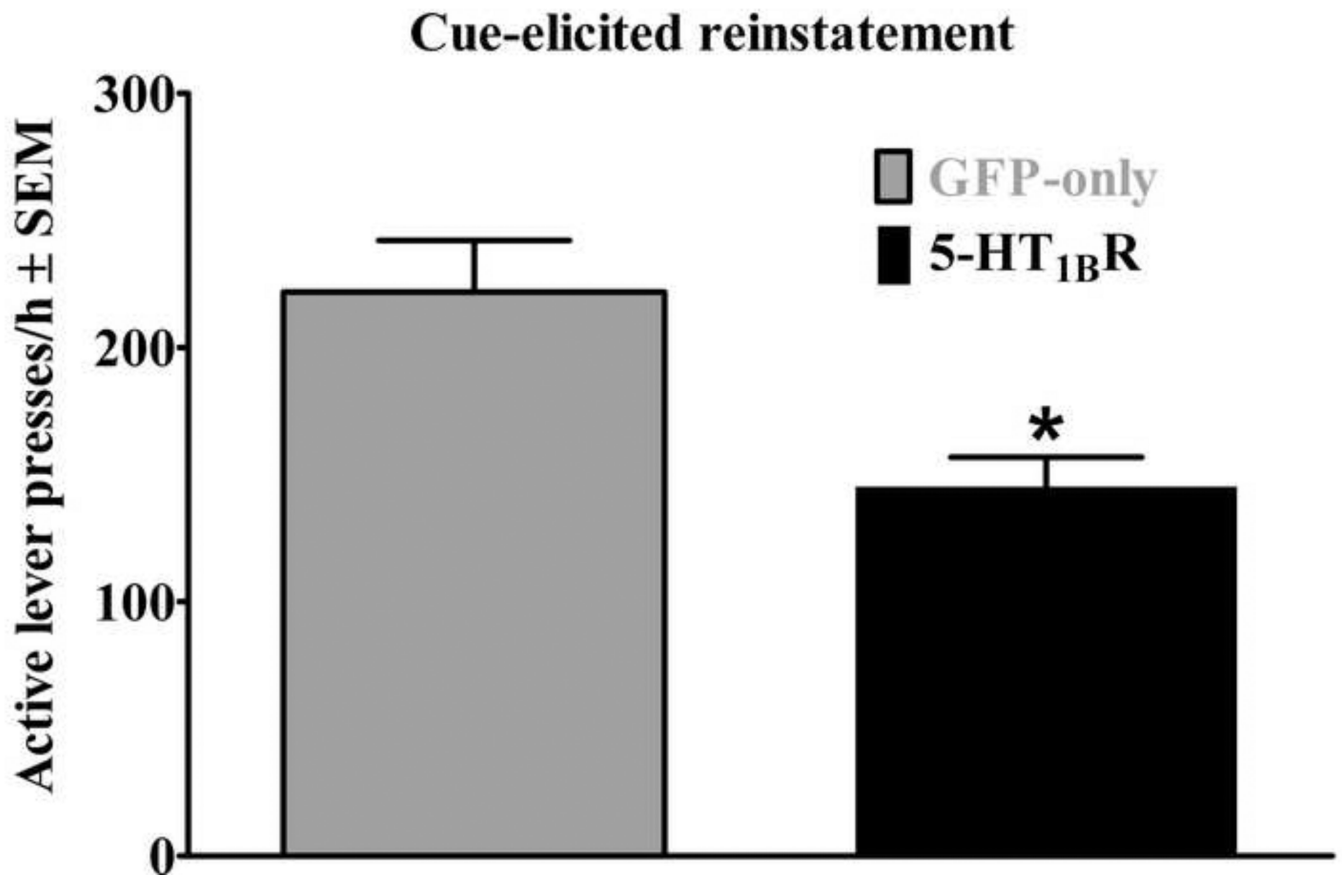


Figure 5. Effects of viral-mediated 5-HT_{1B}R-gene transfer into the medial NAcsh on cue-elicited cocaine-seeking behavior following 21 days of forced abstinence from chronic cocaine self-administration expressed as the mean number of active lever responses during a 1-hr test session + SEM. Rats (n=7–9/group) received microinfusions of a viral vector containing either 5-HT_{1B}R-GFP (black bars) or GFP-only (gray bars) into the medial NAcsh four days prior to testing. Cues were available response-contingently during the test session on an FR1 schedule of reinforcement. Asterisk (*) represents a difference compared to GFP-only controls (t-test, $p < 0.05$).

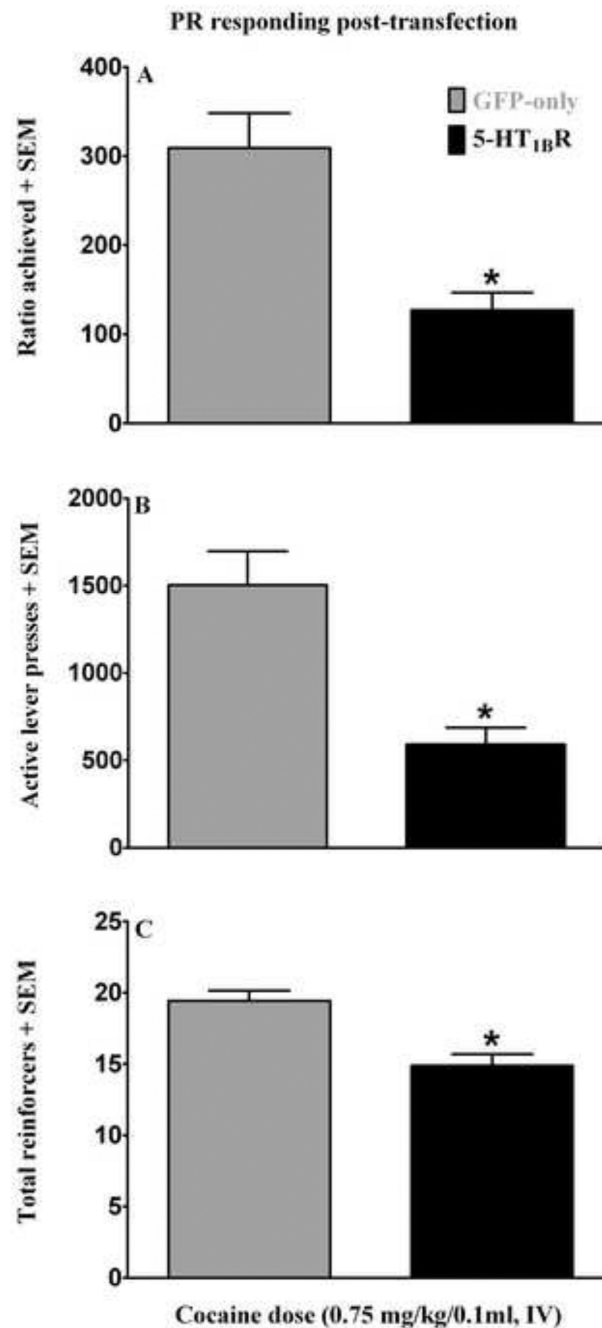


Figure 6.

Effects of viral-mediated 5-HT_{1B}R-gene transfer into the medial NAcsh following 21 days of protracted withdrawal (i.e. forced abstinence) from chronic cocaine self-administration on responding during cocaine self-administration (0.75 mg/kg/0.1 ml, i.v.) break point testing on an exponential 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-HT_{1B}R-GFP (black bars) treated rats achieved lower final ratios, emitted fewer active lever

responses and received fewer cocaine infusions compared to GFP-only (gray bars) controls; data expressed as mean + SEM. Asterisk (*) represents an increase compared to GFP-only treated rats (t-tests, $p < 0.05$ in each case).

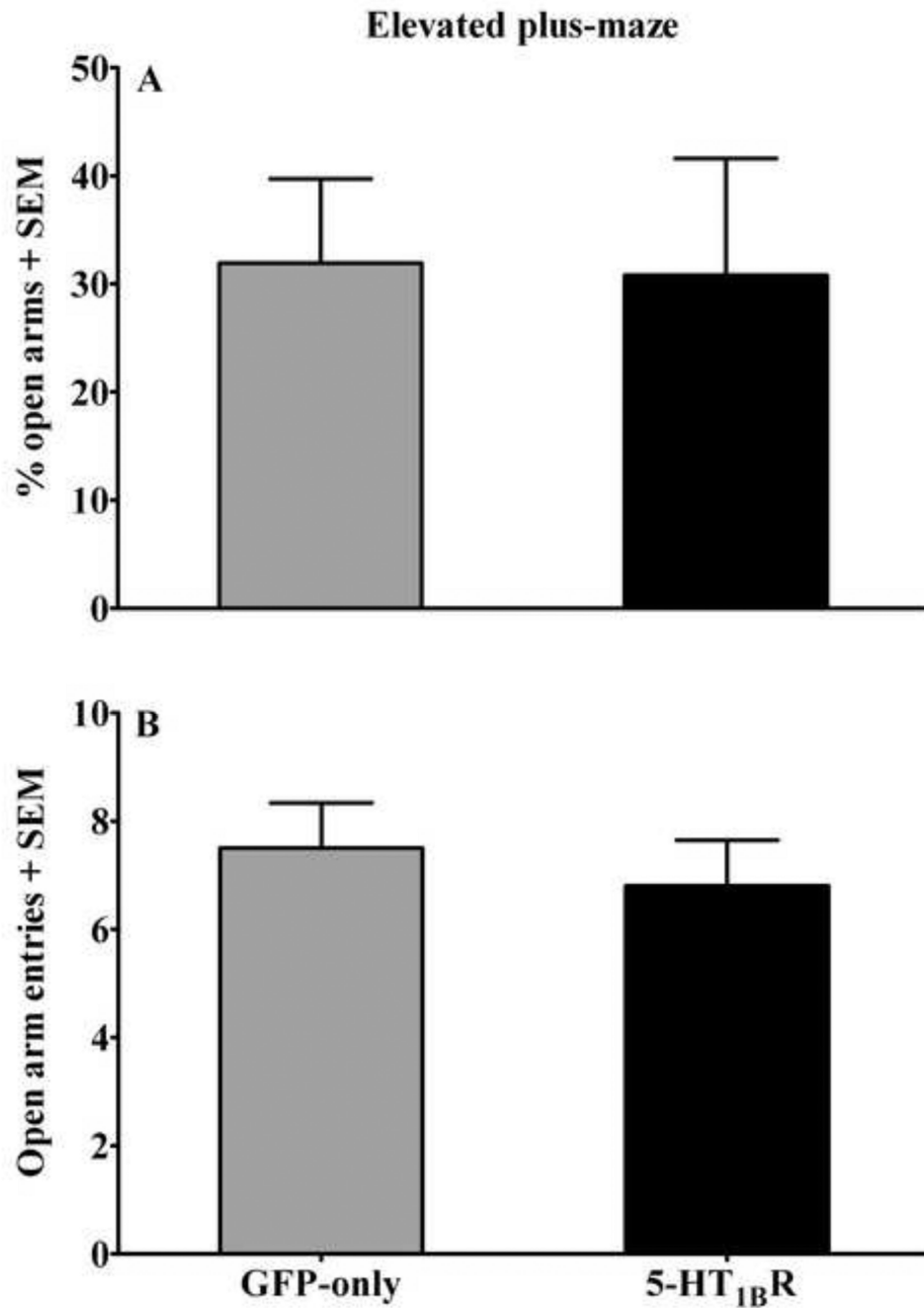


Figure 7. Effects of viral-mediated 5-HT_{1B}R-gene transfer into the medial NAcsh on anxiety-like behavior during testing in the EPM expressed as the percentage of time spent in (A) the open arms and number of entries into (B) the open arms + SEM during a 5 min test session. Rats (n=14–15/group) received microinfusions of a viral vector containing either 5-HT_{1B}R-GFP (black bars) or GFP-only (gray bars) into the medial NAcsh five days prior to testing.

Table 1Cocaine reinforcers and response rates (mean \pm SEM) during self-administration (SA) training and extinction.

Viral condition/experiment	Infusions/session last 5 training days	Total infusions	Active lever presses	
			Last SA day	First extinction day
Reinforcement:				
5-HT _{1B} -GFP (n= 9)	26.36 \pm 2.58	678.67 \pm 58.31	83.17 \pm 14.50	N/A
GFP-only (n=8)	28.05 \pm 4.22	719.25 \pm 63.63	73.31 \pm 14.91	N/A
Reinstatement:				
5-HT _{1B} -GFP (n=15)	25.56 \pm 0.95	424.43 \pm 18.63	72.10 \pm 3.98	100.33 \pm 12.29
GFP-only (n=14)	27.49 \pm 1.51	453.85 \pm 26.46	88.39 \pm 17.28	122.54 \pm 18.43
Relapse				
5-HT _{1B} -GFP (n=9)	22.40 \pm 1.05	306.63 \pm 23.31	58.19 \pm 1.61	N/A
GFP-only (n=7)	22.67 \pm 0.92	315.83 \pm 24.55	63.14 \pm 3.70	N/A

Each i.v. cocaine infusion contained 0.75 mg/kg/0.1 ml.