Increased Expression of 5-HT_6 Receptors in the Nucleus Accumbens Blocks the Rewarding But Not Psychomotor Activating Properties of Cocaine

Susan M. Ferguson, Ellen S. Mitchell, and John F. Neumaier

Background: Repeated exposure to cocaine produces enduring forms of drug experience-dependent behavioral plasticity, including conditioned place preference (CPP) and psychomotor sensitization, a progressive and persistent increase in cocaine's psychomotor activating effects. Although serotonin-6 receptors (5-HT_6Rs) are abundantly expressed in the brain regions thought to underlie these phenomena, such as the nucleus accumbens (NAc), surprisingly little is known about the role of 5-HT_6Rs in the rewarding and psychomotor activating effects of cocaine.

Methods: Viral-mediated gene transfer was used to selectively increase 5-HT_6R expression in the NAc of rats. The effects of 5-HT_6R overexpression and the selective 5-HT_6R antagonist Ro4368554 on CPP and psychomotor sensitization were examined.

Results: Increased expression of 5-HT_6Rs in the NAc blocks a CPP to cocaine but has no effect on either the acute locomotor response to cocaine or on the development of cocaine-induced locomotor sensitization. Furthermore, antagonism of 5-HT_6Rs facilitates the acquisition of a CPP to cocaine but has no effect on cocaine-induced stereotypy.

Conclusions: These results demonstrate that 5-HT_6Rs in the NAc can selectively modulate drug reward, possibly through facilitation of reward learning.

Key Words: Conditioned place preference, drug addiction, psychomotor stimulant, rat, serotonin, viral-mediated gene transfer

Drug addiction is a serious medical problem that poses a major social and economical burden on society. Although much is known regarding the neurobiology of addiction, there is a paucity of safe and/or effective pharmacotherapies (1). For example, although it has been established that both dopamine and glutamate neurotransmission are critical for the development and maintenance of cocaine addiction, agents that target these circuits produce adverse side effects in patients, thus limiting their therapeutic value. Increasing evidence indicates that serotonin neurotransmission also plays an important role in addiction (2,3). Although serotonin reuptake inhibitors are relatively safe, they have widespread effects on the serotonergic system and have not proved useful for treating addiction. This is not surprising, given that over 14 serotonin receptor subtypes have been identified, many of which have opposing effects. However, serotonergic drugs that target the receptor subtypes that are expressed in brain regions that mediate drug craving might prove to be highly efficacious in the treatment of addiction.

One promising candidate is the serotonin-6 receptor (5-HT_6R), given its dense expression in the mesocorticolimbic brain regions (e.g., nucleus accumbens [NAc], caudate-putamen, and frontal cortex) thought to be critical for the development of addiction (4–6). Until recently there have been few pharmacological agents selective for 5-HT_6Rs; consequently, little is known regarding the role of 5-HT_6Rs in the behavioral effects of drugs of abuse. In fact, only one study has examined the role of 5-HT_6Rs in the rewarding and psychomotor activating effects of psychostimulants, and it was found that pharmacological blockade of 5-HT_6Rs enhances amphetamine-induced locomotor activity and self-administration but surprisingly had no effect on the ability of cocaine to produce these same behaviors (7). The present experiment sought to characterize the role of 5-HT_6Rs in two enduring forms of drug experience-dependent behavioral plasticity—conditioned place preference (CPP) and psychomotor sensitization (8)—by using viral-mediated gene transfer to selectively enhance expression of 5-HT_6Rs in the NAc. This strategy is advantageous because it allows for increases of 5-HT_6R levels in a discrete brain area to be activated by endogenously released serotonin (9). As a complement to this approach, pharmacological methods were used to examine the consequences of 5-HT_6R blockade on cocaine-induced CPP and stereotypy.

Methods and Materials

Subjects

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts) weighing 225–249 g upon arrival were housed two/cage and given a 1-week acclimation period. The animal rooms were temperature- and humidity-controlled, and the animals were maintained on a 12-hour light/dark cycle, with food and water available ad libitum. Experimental procedures were approved by the University of Washington Institutional Animal Care and Use Committee and were conducted in accordance with National Institutes of Health guidelines.

Drugs

Cocaine–hydrogen chloride (Sigma, St. Louis, Missouri) was dissolved in sterile .9% saline. The selective 5-HT_6R antagonist Ro4368554 (3-benzenesulfonyl-7-[4-methyl-piperazin-1-yl]1H-indole; generously provided by Roche Pharmaceuticals, Palo Alto, California) was dissolved in acetate-buffered sterile water. The Ro4368554 readily crosses the blood-brain barrier and has a 100-fold selectivity for 5-HT_6Rs compared with other receptor

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Received November 17, 2006; revised February 16, 2007; accepted February 16, 2007.

0006-3223/08/$34.00
doi:10.1016/j.biopsych.2007.02.018

subtypes (10,11). The dose of Ro4368554 used in the present study (5 mg/kg) and the pre-treatment time (30 min before cocaine or saline administration) were chosen on the basis of pilot studies. Drugs were administered intraperitoneal in a volume of 1 mL/kg.

Viral Vector

A modified herpes simplex virus (HSV) amplicon was used to express the 5-HT_6R transgene and green fluorescent protein (GFP) as described previously (12). Briefly, the pHSV-HA6/GFP (HA6; Figure 1) viral vector expresses both hemagglutinin-tagged 5-HT_6R and GFP from separate transcriptional cassettes, whereas the pHSV-GFP (GFP) viral vector only expresses GFP and is used as a control. Previous studies have found that the HSV vector system does not alter drug-related behaviors compared with sham or vehicle injections (13,14). The HA-5-HT_6R sequence of the HA6 amplicon was confirmed in its entirety with polymerase chain reaction, and the HA6 amplicon was packaged with replication-deficient helper virus (12), yielding approximately 1×10^8 infective units/mL. The HA-tagged 5-HT_6R was found to stimulate the accumulation of cyclic adenosine monophosphate with a luciferase-based cyclic adenosine monophosphate assay with the same pharmacological characteristics as the wild-type receptor (9).

Surgery and Viral Gene Transfer

The surgical and viral gene transfer procedures used in this study are described in the supplementary methods online (Supplement 1).

CPP

The rewarding effects of cocaine were measured with a CPP procedure with an unbiased, two-phase design (conditioning and post-conditioning test). The effect of viral-mediated increased expression of 5-HT_6Rs in the NAc on the development of a CPP to cocaine was determined. In addition, the effect of pharmacological blockade of 5-HT_6Rs on a CPP to cocaine was evaluated.

For the viral-mediated gene expression experiment, conditioning trials began 3 days after viral infusion and took place in a three-chamber CPP apparatus (containing two large compartments [24 × 21 × 21 cm] separated by a central neutral area [12 × 21 × 21 cm]; Med Associates, St. Albans, Vermont). The compartments differed in floor texture, wall color, and lighting. During the conditioning phase, rats received two 30-min pairings with cocaine (20 mg/kg) in one chamber and two 30-min pairings with saline in the other (one pairing of each/day for 2 consecutive days). The post-conditioning test for a CPP occurred

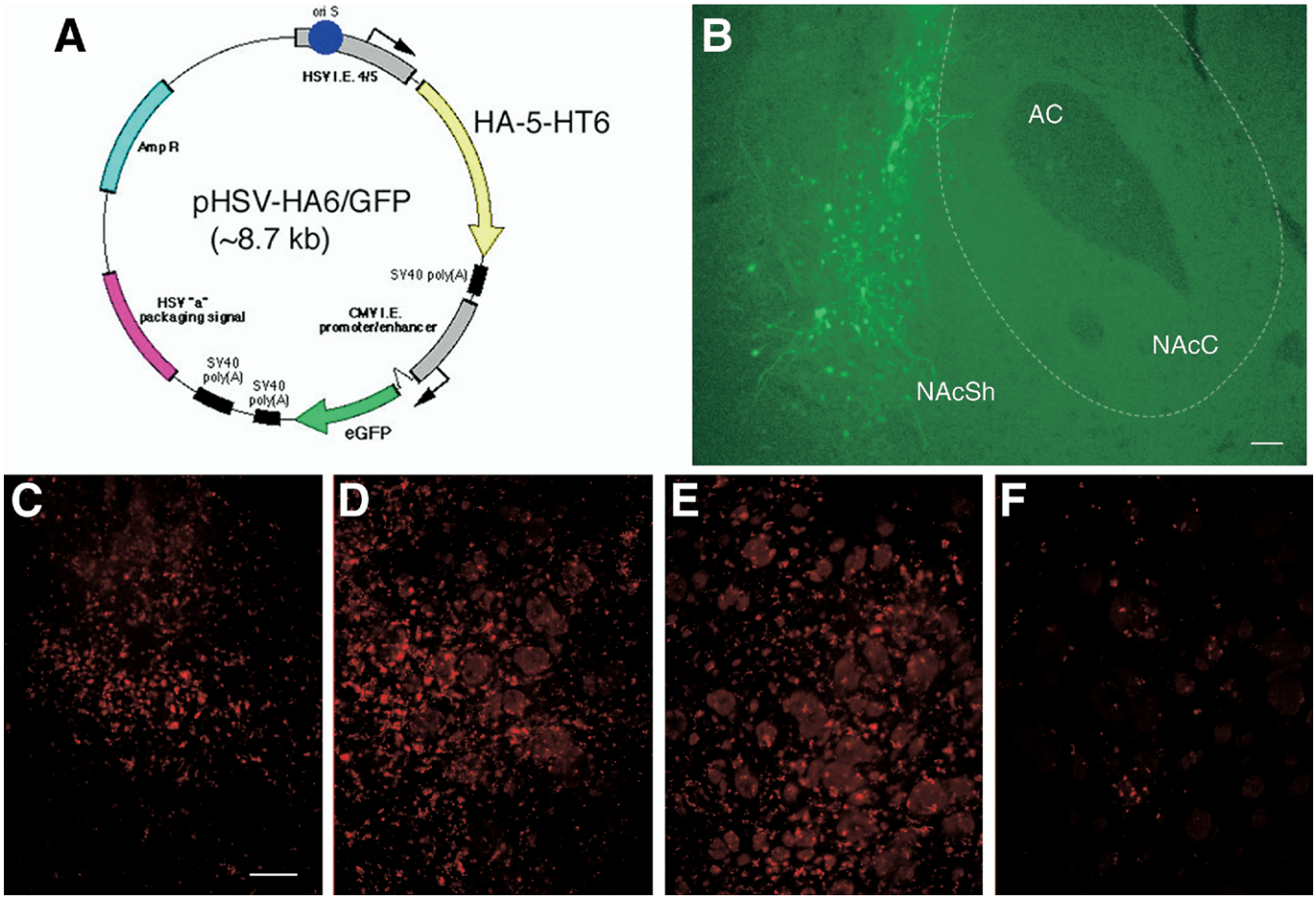

Figure 1. Time course and expression of the HA–serotonin-6 receptor (5-HT_6R) transgene. **(A)** Illustration of the p– herpes simplex virus (HSV)-HA6/green fluorescent protein (GFP) transgene amplicon. **(B)** Representative histological plate of GFP expression from a section of the NAc shell 5 days after viral infusion of the HA6 transgene. Scale bar, 100 μm. **(C–F)** The 5-HT_6R immunoreactivity at 2 days **(C)**, 4 days **(D)**, 6 days **(E)**, and 10 days **(F)** after HA6 viral infusions. Scale bar, 100 μm. NAcC, nucleus accumbens, core; NAcSh, nucleus accumbens, shell; AC, anterior commissure.

24 hours after the final conditioning trial. Rats were confined to the neutral area for a 5-min habituation period and then allowed 30-min of access to all compartments.

For the antagonist experiment, during the conditioning phase rats received an injection of the selective 5-HT_6R antagonist Ro4368554 (5 mg/kg) or vehicle 30 min before receiving cocaine (5 mg/kg) or saline pairings. To avoid potential experimental confounds with multiple antagonist injections/day, cocaine pairings took place on Day 1 and Day 3, and saline pairings took place on Day 2 and Day 4. Saline-treated rats also received Ro4368554 or vehicle pre-treatment but received four pairings of saline and no cocaine pairings. The post-conditioning test for a CPP was identical to that described in the preceding text.

Locomotor Sensitization

The effect of viral-mediated increased expression of 5-HT_6Rs in the NAc shell on the psychomotor activating effects of cocaine was measured in locomotor activity boxes (22 × 45 × 23 cm; San Diego Instruments, San Diego, California) with an adapted protocol (15,16). Three days after viral infusion rats received an escalating dose regimen of cocaine (0, 7.5, 15, and 30 mg/kg). For this acute dose response test, rats were given a 45-min habituation period to the test cage, followed by an injection of saline. Forty-five minutes later rats were given three injections of cocaine, spaced 45 min apart. Beginning 24 hours after the acute dose response test, rats received four injections of cocaine (15 mg/kg) over a 4-day treatment period (one injection every day). Rats received a 45-min habituation period before each injection, and behavior was recorded for 60 min. Forty-eight hours after the last cocaine treatment, rats were given an escalating dose challenge of cocaine (0, 7.5, 15, and 30 mg/kg) to test for locomotor sensitization. The protocol used was identical to that of the acute dose response test. Locomotor activity was used as an index of psychomotor sensitization (8) and was assessed by the number of cage crossovers, defined as two consecutive beam breaks.

Stereotypy

The stereotypy procedure used in this study is described in the supplementary methods online (Supplement 2).

Immunohistochemistry

The immunohistochemical protocol used in this study is described in the supplementary methods online (Supplement 3).

Statistical Analysis

Group differences in CPP score, locomotor activity, and stereotypy were tested with two-way analyses of variance (ANOVAs; with or without repeated measures, as warranted) followed by Bonferroni post hoc tests or planned paired *t* tests. For all comparisons, $\alpha = .05$.

Results

Time Course and Distribution of Transgene Expression

We have shown that functional 5-HT_6Rs can be transiently expressed with this vector system in dorsal striatum and that the behavioral effects of increased 5-HT_6R expression are reversed by a selective 5-HT_6R antagonist (9). Furthermore, this viral vector expresses exclusively in neurons (17). Expression of the HA6 transgene was examined at 2, 4, 6, and 10 days after viral infusion with immunohistochemistry. As seen in Figure 1 (panels C–F), 5-HT_6R immunoreactivity was substantially increased 2 days after viral infusion, remained at elevated levels through 6 days after infusion, and returned to baseline levels by 10 days after infusion. Thus, behavioral conditioning coincided with peak levels of increased 5-HT_6Rs. For the CPP experiment, injection coordinate accuracy was confirmed by visualizing GFP expression under a fluorescent microscope by an experimenter blind to the experimental conditions (Figure 1B). Animals where > 50% of the GFP signal was outside of the medial NAc shell were excluded from analysis (1 of 14). For the locomotor sensitization experiment, the challenge test occurred once viral expression of 5-HT_6Rs had returned to baseline levels. This allowed for the determination of whether increased expression of 5-HT_6Rs effected the development (rather than expression) of locomotor sensitization. It was not possible, as a result of this experimental design, to confirm GFP or HA-5-HT_6R expression histologically in this particular group of rats.

Increased Expression of 5-HT_6Rs in the NAc Blocks a CPP to Cocaine

The effect of increased expression of 5-HT_6Rs in the NAc shell on the rewarding effects of cocaine was assessed with a CPP procedure; testing was performed in a drug-free state (Figure 2). A two-way ANOVA with repeated measures on one factor (Chamber) resulted in a significant effect of Chamber [$F = 7.67$, $p = .003$] and a significant Chamber × Virus interaction [$F = 6.16$, $p = .008$]. Bonferroni post hoc tests showed that the GFP control group spent significantly more time in the chamber that had previously been paired with cocaine, compared with either the saline-paired chamber or the neutral chamber ($p < .01$). In contrast, there were no significant differences in the amount of time the HA6/GFP group spent in each of the chambers ($p > .05$). Thus, the GFP control group developed a strong CPP to cocaine, an effect that was prevented by increased expression of 5-HT_6Rs in the NAc.

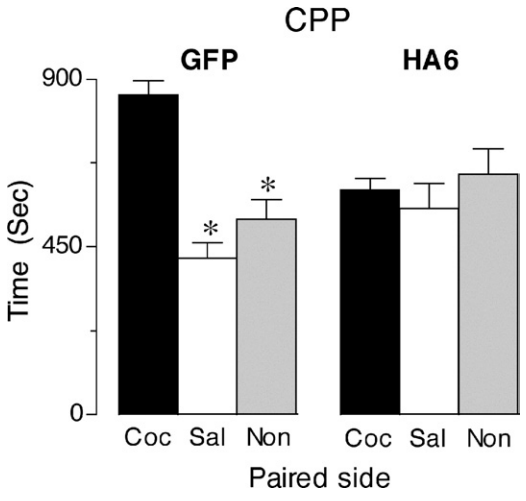

Figure 2. Increased expression of 5-HT_6Rs in the NAc blocks the development of a conditioned place preference. Data represent the mean (± SEM) amount of time spent in the compartment previously paired with cocaine (black bars), the compartment previously paired with saline (white bars), or a neutral compartment (grey bars). The left panel (GFP) represents animals that received GFP-only control viral infusions into the NAc shell, and the right panel (HA6) represents animals that received viral infusions of 5-HT_6Rs; $n = 6–7$/group. *Differs from the cocaine-paired compartment ($p < .01$, Two-way repeated measures analysis of variance [ANOVA], Bonferroni test). Abbreviations as in Figure 1.

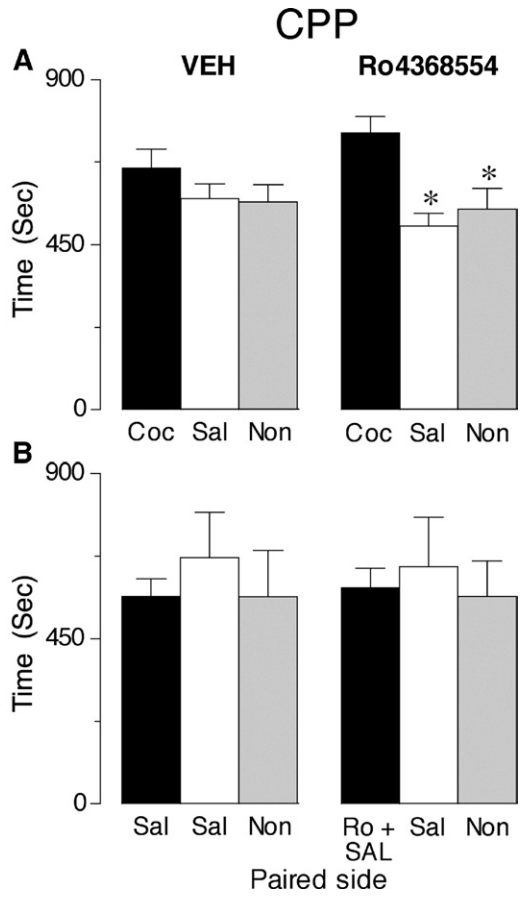

Figure 3. Pharmacological blockade of 5-HT$_6$Rs facilitates the development of a conditioned place preference to cocaine. **(A)** Data represent the mean (± SEM) amount of time spent in the compartment previously paired with cocaine (black bars), the compartment previously paired with saline (white bars), or a neutral compartment (grey bars). The left panel (VEH) represents animals that received saline pretreatment before each conditioning session, and the right panel (Ro4368554) represents animals that received pretreatment with the selective 5-HT$_6$R antagonist Ro4368554 before each conditioning session. **(B)** Data represent the mean (± SEM) amount of time spent in compartments previously paired with saline (black and white bars) or a neutral gray compartment (grey bars). The left panel (VEH) represents animals that received vehicle pretreatment before each of the conditioning sessions. The right panel (Ro4368554) represents animals that received Ro4368554 pretreatment before one-half of the conditioning sessions (black bar) and saline pretreatment before the other one-half (white bar) ($p > .05$, two-way repeated measures ANOVA); $n = 4$/group. *Differs from the cocaine-paired compartment ($p < .05$, two-way repeated measures analysis of variance [ANOVA], Bonferroni test); $n = 12$/group. Abbreviations as in Figure 1.

Pharmacological Blockade of 5-HT$_6$Rs Facilitates the Development of a CPP to Cocaine

The effect of pharmacological blockade of 5-HT$_6$Rs with the selective 5-HT$_6$R antagonist Ro4368554 on the rewarding effects of a low dose of cocaine (5 mg/kg) was assessed with a CPP procedure (Figure 3A). This dose of cocaine is below the threshold for developing a place preference in control animals using this procedure (14), so it is useful for detecting treatments that facilitate place preference conditioning. A two-way ANOVA with repeated measures on one factor (Chamber) revealed a significant effect of Chamber [$F = 5.25$, $p = .009$]. Bonferroni post hoc tests showed that the amount of time spent in each of the chambers did not differ for the saline pretreatment control group, whereas the group that received the 5-HT$_6$R antagonist pretreatment spent significantly more time in the chamber that had previously been paired with cocaine, compared with either the saline-paired chamber or the neutral chamber ($p < .05$). Thus, Ro4368554 facilitates the development of a CPP to a low dose of cocaine. We also tested whether pretreatment with Ro4368554 altered place preference conditioning to saline. There were no differences between groups that received pretreatment with Ro4368554 or saline, indicating that the antagonist alone did not produce a CPP (Figure 3B; main effect of Pretreatment [$F = 2.45$, $p = .17$], main effect of Chamber [$F = .32$, $p = .73$], and interaction between Pretreatment and Chamber factors [$F = .02$, $p = .98$] not significant).

Increased Expression of 5-HT$_6$Rs in the NAc Has No Effect on Locomotor Sensitization to Cocaine

Acute Response. Figure 4 shows the effect of viral-mediated overexpression of 5-HT$_6$Rs in the NAc on the acute locomotor response to a multiple dose regimen of cocaine (0, 7.5, 15, and 30 mg/kg). A two-way ANOVA with repeated measures on one factor (Dose) resulted in a significant main effect of Dose [Figure 4A; $F = 14.19$, $p < .0001$]. Bonferroni post hoc tests showed that both the GFP control group and the HA6/GFP group made significantly more crossovers after the highest dose of cocaine tested (30 mg/kg) compared with saline or the two other doses of cocaine tested (7.5 and 15 mg/kg) ($p < .05$). However, there were no significant differences between the GFP control group and the HA6/GFP group in the number of crossovers after an injection of saline or cocaine (Figure 4A; main effect of Virus [$F = .84$, $p = .38$] and interaction between Dose and Virus factors [$F = .48$, $p = .70$] not significant). Figure 4B illustrates the time course of the effect of cocaine on locomotor activity in GFP control and HA6/GFP groups during the acute dose response test. At the 30-mg/kg dose, a two-way ANOVA with repeated measures on one factor (Time) resulted in a significant main effect of Time [Figure 4B; $F = 2.59$, $p = .002$]. Although there were no statistically significant differences in the number of crossovers at any time point between the GFP control group and the HA6/GFP group ($p > .05$), there was a tendency for the HA6/GFP group to have fewer crossovers after onset of 30 mg/kg cocaine.

Challenge Test. Twenty-four hours after the acute dose response test, animals received four injections of cocaine over a 4-day treatment period (one injection every day). Forty-eight hours later, a multiple dose challenge test with escalating doses of cocaine was administered to test for the development of locomotor sensitization. Notably, this challenge test occurred once HA-5-HT$_6$R transgene expression had dissipated (Figure 1B), thereby allowing for a determination of whether increased 5-HT$_6$R expression altered the development rather than expression of locomotor sensitization. Figure 5 shows the effect of increasing 5-HT$_6$Rs in the NAc shell during the treatment phase on the locomotor response to a multiple dose challenge test of cocaine (0, 7.5, 15, and 30 mg/kg) administered 48 hours after the last (fourth) treatment injection. Two-way ANOVAs with repeated measures on both factors resulted in a significant main effect of Dose [Figure 4A, $F = 15.44$, $p < .0001$] and a significant interaction between Dose and Test Session factors [$F = 7.72$, $p = .002$] for the GFP control group and significant main effects of Dose [$F = 4.87$, $p = .01$] and Test Session [$F = 6.57$, $p = .05$] for the HA6/GFP group. Planned paired comparisons showed that at the two lower doses of cocaine tested (7.5 and 15 mg/kg) both the GFP control group and the HA6/GFP group made significantly more crossovers after the challenge test compared with the acute test ($p \leq .05$), indicating that both groups developed

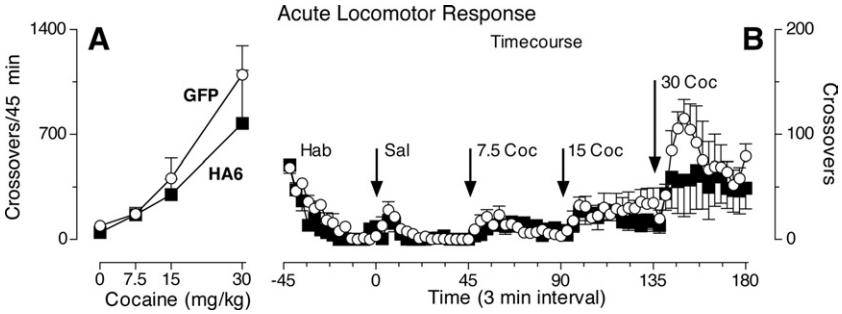

Figure 4. Increased expression of 5-HT$_6$Rs in the NAc does not alter the acute locomotor response to cocaine. **(A)** The mean (± SEM) number of crossovers made during the multiple-dose acute response test (saline treatment, followed by 7.5, 15, and 30 mg/kg cocaine, injections spaced 45 min apart). White circles represent animals that received GFP-only control viral infusions into the nucleus accumbens shell, and black squares represent animals that received viral infusions of 5-HT$_6$Rs. **(B)** The mean (± SEM) number of crossovers over time (3-min intervals) during the multiple-dose acute response test ($p > .05$, two-way repeated measures ANOVA); $n = 6$/group. Abbreviations as in Figure 1.

locomotor sensitization to cocaine. However, a two-way ANOVA with repeated measures on one factor (Dose) revealed that there were no differences in the degree of sensitization between groups (main effect of Virus [$F = 1.18$, $p = .54$] and interaction between Dose and Virus factors [$F = 4.78$, $p = .25$] not significant). Nonetheless, it should be noted that there was a decrease in the locomotor response to 30 mg/kg cocaine in the GFP group after repeated cocaine administration compared with acute treatment, although this effect did not reach statistical significance ($p = .07$). This decrease in locomotion could be a result of increased stereotypy, which would reflect a greater degree of sensitization in the GFP group. Figure 5B illustrates the time course of the effect of cocaine on locomotor activity in GFP control and HA6/GFP groups during the acute dose response test versus the challenge test.

Pharmacological Blockade of 5-HT$_6$Rs Has No Effect on Cocaine-Induced Stereotypy

Exposure to high and/or repeated doses of cocaine can produce stereotyped behaviors, an effect that can prohibit locomotion and mask sensitization (18,1918,19). Thus, it is possible that the apparent lack of a difference in the degree of locomotor sensitization between GFP and HA6/GFP groups could be attributed to differences in stereotypy. As a first step toward addressing this issue, the effect of pharmacological blockade of 5-HT$_6$Rs (with the selective 5-HT$_6$R antagonist Ro4368554) on cocaine-induced stereotypy was assessed (Figure 6A). Two-way ANOVAs conducted at each time point revealed a significant effect of Drug [$F = 9.53$–127.2, $p < .01$] for all time points except the first two (0 and 5 min after drug injection). Bonferroni post hoc tests showed that there were no differences between the groups receiving cocaine ($p > .05$), indicating that pharmacological blockade of 5-HT$_6$Rs had no effect on cocaine-induced stereotypy. In addition, a two-way ANOVA conducted on the total number of crossovers revealed that there was no effect of this dose of Ro4368554 on saline- or cocaine-induced locomotor activity (Figure 6B; main effect of Pretreatment [$F = .09$, $p = .77$] and interaction between Drug and Pretreatment factors [$F = .50$, $p = .48$] not significant). The locomotor response of the cocaine groups was also analyzed by two-way ANOVA with repeated measures on one factor (Time), which resulted in a significant main effect of Time [Figure 6B; $F = 5.98$, $p < .0001$]. Although there were no statistically significant differences in the number of crossovers at any time point between the saline pretreatment group and the Ro4368554 pretreatment group ($p > .05$), there was a tendency for the Ro4368554 pretreatment group to have fewer crossovers after onset of cocaine.

Discussion

Although 5-HT$_6$Rs are highly enriched in the brain regions thought to underlie addiction, such as the striatum (i.e., NAc and caudate-putamen) (4,5), almost nothing is known regarding their role in the development and maintenance of drug addiction. In the present study, we used viral-mediated gene transfer to examine the contribution of 5-HT$_6$Rs in the NAc shell in two long-lasting forms of drug-mediated behavioral plasticity associated with addiction, CPP and psychomotor sensitization. We found that increased expression of 5-HT$_6$Rs in the NAc blocks a CPP to cocaine but has no effect on either the acute locomotor response to cocaine (0–15 mg/kg) or on the development of cocaine-induced locomotor sensitization. Furthermore, pharmacological blockade of 5-HT$_6$Rs facilitates the acquisition of a CPP to cocaine but has no effect on cocaine-induced stereotypy. These results demonstrate that 5-HT$_6$Rs modulate the rewarding but not psychomotor activating effects of cocaine.

The findings that viral-mediated expression and pharmacological blockade of 5-HT$_6$Rs had no effect on locomotor sensitization and stereotypy, respectively, are consistent with an earlier report that antagonism of 5-HT$_6$Rs does not alter the acute locomotor response to cocaine (7). Nonetheless, this lack of effect was unexpected, given that the same 5-HT$_6$R manipulations yielded robust effects on CPP, and the NAc is known to be a critical mediator in both the rewarding and the psychomotor activating effects of cocaine (20–22). The NAc is divided into distinct subregions—the shell and the core. The shell region has been strongly implicated in drug reward (23–25), whereas the core region (and to a lesser extent the shell region, albeit

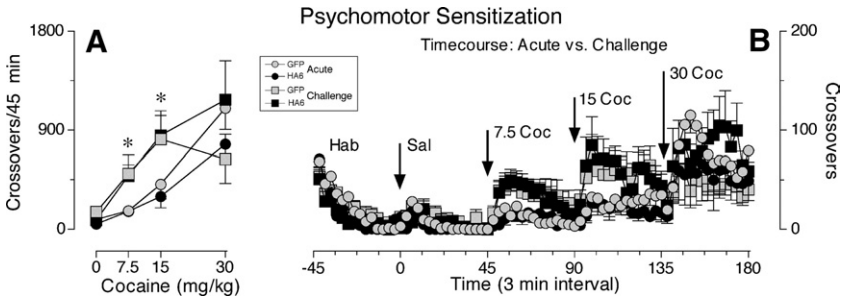

Figure 5. Increased expression of 5-HT$_6$Rs in the NAc has no effect on the development of cocaine-induced locomotor sensitization. **(A)** The mean (± SEM) number of crossovers made during the acute dose response test (circles) and the multiple-dose challenge test (squares) (saline treatment, followed by 7.5, 15, and 30 mg/kg cocaine, injections spaced 45 min apart). Grey symbols represent animals that received GFP-only control viral infusions into the nucleus accumbens shell, and black symbols represent animals that received viral infusions of 5-HT$_6$Rs. **(B)** The mean (± SEM) number of crossovers over time (3-min intervals) during the acute dose response test (circles) compared with the multiple-dose challenge test (squares) ($p > .05$, two-way repeated measures ANOVA); $n = 6$/group.

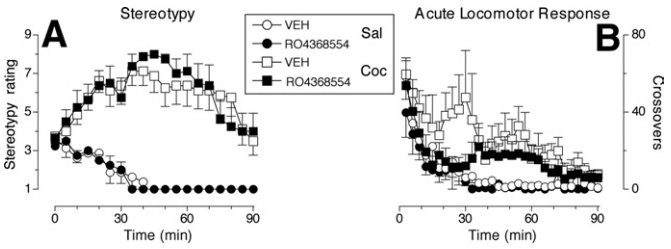

Figure 6. Pharmacological blockade of 5-HT$_6$Rs does not alter cocaine-induced stereotypy. **(A)** The mean (± SEM) stereotypy rating (with a 9-point rating scale) over time (one rating every 5 min) for the 90-min period after an injection of saline (circles) or cocaine (squares) in animals that received vehicle pretreatment (white symbols) or pretreatment with the selective 5-HT$_6$R antagonist Ro4368554 (black symbols). **(B)** The mean (± SEM) number of crossovers made during the 90-min test session ($p > .05$, two-way repeated measures ANOVA); $n = 4$/saline group and 8/cocaine group.

somewhat controversially) has been linked with the psychomotor activating effects of drugs (15,25–27). In the present study the viral injections were targeted to the NAc shell region, which could account for why increased expression of 5-HT$_6$Rs had no effect on the development of psychomotor sensitization. Future studies will be necessary to explore the role of 5-HT$_6$Rs in discreet subregions of the NAc.

Conditioned place preference is an associative learning task in that it requires animals to make a link between the drug and the environmental context paired with drug administration, whereas psychomotor sensitization is thought to primarily involve non-associative learning (28). We found that increased expression of 5-HT$_6$Rs reduced place preference conditioning whereas pharmacological blockade of 5-HT$_6$Rs enhanced place preference conditioning to cocaine. Thus, a plausible interpretation of the present results is that they reveal that the role of 5-HT$_6$Rs in addiction-related behaviors is to inhibit associative learning processes. In support of this idea, 5-HT$_6$Rs are known to be critical modulators of other forms of associative learning, such as passive avoidance and autoshaping (29,30). More recently we have found that viral-mediated expression of 5-HT$_6$Rs in the dorsomedial striatum blocks the acquisition of a reward-based instrumental learning task without altering motivation to consume the sucrose reward, an effect that is reversed by a 5-HT$_6$R antagonist9.

It is possible that, in addition to altering associative learning processes, 5-HT$_6$Rs modulate the development of a CPP to cocaine by changing the hedonic value of cocaine. Although dissociation between learning and reward was not directly tested in the present experiments, it has been reported that antagonism of 5-HT$_6$Rs has no effect on lever responding maintained by cocaine in animals that have already obtained stable levels of self-administration (7). In addition, increased expression of 5-HT$_6$Rs in dorsomedial striatum has no effect on performance of a food reward task or on sugar pellet consumption (9). Finally, we found that antagonism of 5-HT$_6$Rs did not alter CPP when paired with saline, suggesting that a 5-HT$_6$R antagonist itself is not rewarding. Although future experiments will be necessary to clarify this issue, it seems unlikely that 5-HT$_6$Rs directly alter the pleasurable effects of rewards. In conclusion, our results together with recent findings with a reward-based operant task (9) indicate that 5-HT$_6$Rs in brain reward circuits might play a critical role in delaying or inhibiting the acquisition of behaviors that depend upon associative learning.

The mechanism by which 5-HT$_6$Rs modulate drug reward is not yet known, although our results with viral-mediated gene expression suggest that the regulation is occurring within the striatum. In this region, 5-HT$_6$Rs are primarily localized to the dendrites of γ-aminobutyric acid (GABA)ergic medium spiny neurons and are found on both striatonigral neurons (i.e., those that contain the neuropeptides dynorphin and substance P) and striatopallidal neurons (i.e., those neurons that contain the neuropeptide enkephalin) (31,32). Notably, both dopamine and glutamate receptors are also found on the dendritic spines of these neurons (33–35), suggesting that serotonin, acting at 5-HT$_6$Rs, could interact with dopamine and glutamate to modulate neuronal firing and alter dendritic structure and/or function in the striatum. In addition, there is some evidence to suggest that 5-HT$_6$Rs can regulate both dopamine and serotonin neurotransmission after administration of psychostimulant drugs. For example, pharmacological blockade of 5-HT$_6$Rs enhances amphetamine-induced increases in synaptic dopamine concentrations in the striatum and frontal cortex as well as synaptic serotonin concentrations in the striatum (7,36). In these studies, 5-HT$_6$R antagonism did not alter basal levels of dopamine or serotonin (7,36), suggesting that 5-HT$_6$Rs do not play a role in the tonic regulation of dopamine and serotonin neurotransmission but rather modulate the responsiveness of post-synaptic neurons to the elevated synaptic concentrations of serotonin that occur after psychostimulant administration.

Both serotonin and dopamine are robust modulators of dopamine and cyclic adenosine 3',5'-monophosphate-regulated phosphoprotein, 32 kDa (DARPP-32), an abundantly expressed phosphoprotein in the striatum that is thought to play a critical role in the integration of synaptic inputs and has been implicated as an important intracellular mediator of drug reward (37,38). It is possible, therefore, that 5-HT$_6$Rs mediate the rewarding effects of psychostimulant drugs via DARPP-32 regulation. Although this hypothesis has not yet been tested, the effects of serotonin on DARPP-32 phosphorylation in the striatum have been attributed to 5-HT$_6$Rs, because they are blocked by a 5-HT$_6$R antagonist and mimicked by a 5-HT$_6$R agonist in striatal slices (39,40). It is worth noting, however, that even though activation of 5-HT$_6$Rs and dopamine D$_1$Rs produces similar patterns of striatal DARPP-32 phosphorylation, activation of these receptors produce opposing effects on place preference conditioning (39–43). Although the overlapping but distinct pattern of 5-HT$_6$R and dopamine D$_1$R expression in striatal neurons could account for the differences in behavior, these disparate effects certainly suggest that DARPP-32 signaling and its role in drug reward might be more complicated than previously thought.

Our findings clearly demonstrate that 5-HT$_6$Rs in the NAc shell selectively modulate the rewarding aspects of cocaine, likely through alterations in associative learning processes. Given their dense distribution on post-synaptic striatal neurons and described role in learning, it is likely that 5-HT$_6$Rs might normally serve to dampen neurochemical signaling in the striatum to inhibit the learning of new associations and habits. One hurdle that currently impedes the successful treatment of addiction is the high propensity of addicts to relapse. Although extinction and reinstatement models relevant to relapse depend on associative learning processes, studies have not yet explored the role of 5-HT$_6$Rs in these models. These are important avenues to pursue, because drugs that could modulate learning by targeting 5-HT$_6$Rs might prove to be particularly efficacious in the treatment of addiction.

This research was supported by a National Institute on Drug Abuse (NIDA) grant to JFN (DA16432); SMF was supported by a

NIDA individual National Research Service Award (DA210090); and ESM was supported by a National Alliance for Research on Schizophrenia and Depression independent investigator award (T32AG000057).

The authors have no financial disclosures to report.

Supplementary material cited in this article is available online.

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