Elevated Expression of 5-HT$_{1B}$ Receptors in Nucleus Accumbens Efferents Sensitizes Animals to Cocaine

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Although the effects of psychostimulants on brain dopamine systems are well recognized, the direct actions of cocaine on serotonin systems also appear to be important to its addictive properties. For example, serotonin actions at 5-HT$_{1B}$ receptors in the ventral tegmental area (VTA) modulate cocaine-induced dopamine release in the nucleus accumbens (NAcc) and alter the rewarding and stimulant properties of cocaine. However, the mechanisms of these effects have been unclear, because several neuron types in VTA express 5-HT$_{1B}$ receptors. One possibility is that 5-HT$_{1B}$ receptors on the terminals of GABAergic projections from NAcc to VTA inhibit local GABA release, thereby disinhibiting VTA neurons. We tested this hypothesis directly by using viral-mediated gene transfer to overexpress 5-HT$_{1B}$ receptors in NAcc projections to VTA. A viral vector containing either epitope hemagglutinin-tagged 5-HT$_{1B}$ and green fluorescent protein (HA1B–GFP) cassettes or green fluorescent protein cassette alone (GFP-only) was injected into the NAcc shell, which sends projections to the VTA. HA1B–GFP injection induced elevated expression of 5-HT$_{1B}$ receptors in neuronal fibers in VTA and increased cocaine-induced locomotor hyperactivity without affecting baseline locomotion. Overexpression of 5-HT$_{1B}$ receptors also shifted the dose–response curve for cocaine-conditioned place preference to the left, indicating alterations in the rewarding effects of cocaine. Thus, increased expression of 5-HT$_{1B}$ receptors in NAcc efferents, probably in the terminals of medium spiny neurons projecting to the VTA, may contribute to psychomotor sensitization and offer an important target for regulating the addictive effects of cocaine.

Key words: herpes simplex virus; gene transfer; ventral tegmental area; conditioned place preference; locomotor; hyperactivity

Cocaine dependence is an important clinical and social problem that is often resistant to behavioral interventions alone (Crits-Christoph et al., 1999). It is therefore important to develop pharmacological strategies to modify the reinforcing effects of cocaine. These effects are mediated by natural reward circuitry in the brain, including the dopaminergic projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc) and the reciprocal GABAergic projection from NAcc to VTA (Wise, 1996). In response to cocaine exposure, VTA and NAcc neurons undergo a variety of adaptations that may affect the development of sensitization to the stimulant and rewarding effects of the drug, as well as tolerance, drug craving, and relapse (Fitzgerald et al., 1996; Churchill et al., 1999) (for review, see Nestler, 2001). For this reason, the membrane receptors and signal transduction mechanisms within the mesolimbic system are potential targets for pharmacological treatment of cocaine dependence.

The NAcc and VTA are both richly innervated by serotonergic fibers (Lavoie and Parent, 1990; Van Bockstaele et al., 1994; Phelix and Broderick, 1995), and cocaine increases extracellular concentrations of serotonin as well as dopamine in these areas (Di Chiara and Imperato, 1988; Hernandez and Hoebel, 1988; Bradberry and Roth, 1989; Klitenick et al., 1992; Parsons et al., 1995; Reith et al., 1997). The NAcc and VTA contain several types of serotonin receptors, including the 5-HT$_{1B}$ subtype, which act as inhibitory heteroreceptors in the axon terminals of GABAergic NAcc neurons that project to VTA (Johnson et al., 1992; Cameron and Williams, 1994; Morikawa et al., 2000). Although they are an intriguing target for investigation, 5-HT$_{1B}$ receptors are particularly difficult to study: they are expressed widely in mammalian brain and are translocated to axon terminals that may be some distance from the cell bodies of origin (Boschert et al., 1994; Ghavami et al., 1999; Riad et al., 2000). The sources of all 5-HT$_{1B}$ binding sites in VTA have not been delineated, and typical pharmacological or binding studies are unable to reveal which neuronal subtypes contain 5-HT$_{1B}$ receptors, even when binding sites are localized autoradiographically. This inability to identify or manipulate selectively individual 5-HT$_{1B}$ receptor populations may contribute to controversy regarding the role of 5-HT$_{1B}$ receptors in cocaine-related behaviors. Furthermore, the available 5-HT$_{1B}$ ligands are incompletely selective.

In some cases, 5-HT$_{1B}$ agonists given systemically or intracerebroventricularly sensitize rats to the effects of psychostimulants (Parsons et al., 1996, 1998, 1999). The putative mechanism of enhancement is that stimulation of 5-HT$_{1B}$ receptors on the terminals of GABAergic neurons in the VTA decreases local GABA release, thereby disinhibiting VTA dopamine neurons (Parsons et al., 1999). Conversely, 5-HT$_{1B}$ agonists have also been found to decrease the rewarding effects of amphetamine

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(Fletcher and Korth, 1999a,b), and 5-HT_{1B} knock-out mice have shown a confusing pattern of alterations in cocaine response (Rocha et al., 1997; Belzung et al., 2000; Castanon et al., 2000; Shippenberg et al., 2000). However, the cellular basis of the 5-HT_{1B}-mediated effects in these studies is not known.

The present studies were designed to clarify the role of VTA 5-HT_{1B} receptors using viral-mediated gene transfer (VMGT), a method capable of targeting a specific receptor population. One possibility is that elevated expression of 5-HT_{1B} receptors in NAcc neurons projecting to VTA increases the stimulant and rewarding actions of cocaine. To test this hypothesis, we used VMGT to increase expression of 5-HT_{1B} receptors in terminals of NAcc neurons that project to the VTA, among other places. We then evaluated the ability of several doses of cocaine to stimulate locomotor activity and establish conditioned place preferences.

MATERIALS AND METHODS

Animals. Male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were used. The rats were housed in a climate-controlled vivarium with a 12 hr light/dark cycle (lights on at 6:00 A.M.). Food and water were available *ad libitum*, except during viral-mediated gene transfer and behavioral testing. All animal procedures were approved by the Institutional Animal Care and Use Committees at our institutions and were conducted in accordance with National Institutes of Health guidelines.

Viral vectors. A herpes simplex virus (HSV) vector system using replication-deficient helper virus for packaging was used in this study; this system has been used in a number of studies and has been reviewed previously (Neve, 1999; Carlezon et al., 2000). Two plasmid amplicons were packaged into viral vectors as described previously (Clark et al., 2002). Briefly, HA1B–GFP expresses both hemagglutinin-tagged 5-HT_{1B} receptor and green fluorescent protein from separate transcriptional cassettes, whereas GFP-only contains only the gene for green fluorescent protein. The GFP-only vector serves as a control for all nonspecific aspects of the viral gene transfer procedure, and this HSV vector system does not alter drug reward or behavior compared with sham or vehicle injections (Carlezon et al., 2000). The functional expression of the HA1B–GFP and GFP-only vectors after injection into the rat dorsal raphe nucleus has also been described previously (Clark et al., 2002).

Experiment 1 design: cocaine-induced hyperactivity. Rats were handled for 1–2 min once per day on 2 consecutive days (days 3 and 4) before starting the study. On the morning of day 1, each animal was weighed and received an intraperitoneal injection of 0.9% saline solution (1 ml/kg), followed by 1 hr of automated activity monitoring. The animals then received bilateral microinjections of either HA1B–GFP ($n = 31$) or GFP-only ($n = 30$) vector into the medial NAcc shell. Anesthesia was induced and maintained using isoflurane gas delivered by mask. Briefly, each rat was placed in a stereotaxic instrument, the skull was exposed, and the bregma was located. Bilateral bore holes were drilled. Injections were aimed at the NAcc shell [relative to bregma (in mm): anteroposterior, +1.7; lateral, ±2.3; and dorsoventral, 6.8 below the dura] (Paxinos and Watson, 1986). For each injection, a 27 gauge needle was angled 10° from midline and advanced slowly to the injection coordinates. Viral particles (2.0 μl at 10^8 infective units per milliliter) were injected over a period of 10 min; the injections were controlled by a microprocessor-controlled pump (World Precision Instruments, Sarasota, FL). The needle was left in place for 5 min after the injection and withdrawn slowly over a period of 5 min. The skin was closed with surgical glue (Vetbond; 3M, St. Paul, MN). Rats were observed until spontaneous movement resumed, at which point animals were returned to the home cage. In control rats ($n = 53$) that received sham surgery, the injection needle was lowered 1.0 mm below the dura, but no injections were made. On days 2 and 3, the rats were again weighed, given a saline injection, and placed in the activity monitor cages. On day 4, the animals were injected with cocaine hydrochloride solution intraperitoneally rather than saline before activity monitoring, at a dose of 5 mg/kg (5 mg/ml; $n = 22$), 10 mg/kg (10 mg/ml; $n = 22$), or 20 mg/kg (20 mg/ml; $n = 17$). All behavioral assessments were made between 7:00 A.M. and 12:00 P.M. The accuracy of injection coordinates was confirmed by visualizing the location of GFP expression in tissue sections (40 μm) by fluorescent microscopy (described in Immunohistochemistry). Animals with injection sites outside the shell of the NAcc (6 of 61) were excluded before analysis of behavioral data.

Activity monitoring. Activity was assessed with the Cage Rack Photobeam Activity System (San Diego Instruments, San Diego, CA). Immediately after the intraperitoneal injection of cocaine or saline, each rat was placed into an individual activity monitor and left there for 1 hr. The monitor consisted of a clean cage set inside a horizontal metal frame holding seven infrared photobeam motion detectors. Movement data were recorded using San Diego Instruments software on an IBM-compatible computer. After the session, the rats were returned to their home cage. Total activity (total beam breaks) and ambulation (two sequential beam breaks) were quantified automatically by the software and were analyzed later using Microsoft (Seattle, WA) Excel and SPSS (Chicago, IL) programs. Statistical significance was assessed by averaging each of the six 10 min segments; these collapsed time points were analyzed using mixed effect repeated-measures ANOVA, using viral treatment as the between-subjects variable and time as the within-subjects variable. At doses at which there was an overall main effect of viral treatment, individual segments were tested for significance using *post hoc* Student's *t* tests.

Experiment 2 design: place conditioning. Place conditioning occurred in a three-compartment apparatus (Med Associates, St. Albans, VT), as described previously (Carlezon et al., 1998). During screening (day 0), rats (300–325 gm) were placed in the small (12 × 21 × 21 cm) central compartment of the three-chamber place conditioning apparatus and were allowed to explore the entire apparatus for 30 min. Compartments differed in color, floor texture, and lighting. Data collected during this screening session were considered to reflect baseline (i.e., before conditioning) preferences for each of the compartments. Rats that did not show strong *a priori* preferences (≥18 min) for a compartment were anesthetized (65 mg/kg, i.p., sodium pentobarbital) and given atropine (0.25 mg, s.c.) to minimize bronchial secretions. Each rat received bilateral microinjections (2.0 μl/side) of HA1B–GFP ($n = 34$) or GFP-only viral particles ($n = 33$) aimed at the NAcc shell as described for experiment 1. The medial NAcc shell was targeted specifically because we showed previously that this region is critical for the rewarding effects of cocaine (Carlezon et al., 1995) and other stimulants (Carlezon and Wise, 1996) and because it has the strongest reciprocal connections with VTA (Groenewegen et al., 1999). Control rats ($n = 53$) received sham surgery in which the injection needle was lowered 1.0 mm below the dura, but no injections were made.

After 2 d of recovery, conditioning trials (two per day) were given on 2 consecutive days (days 3 and 4). On the first conditioning trial of each day, the rats received saline (1 ml/kg, i.p.) and were confined to one of the large (24 × 18 × 33 cm) side compartments of the apparatus. Three hours later, they received cocaine (5, 10, 20, or 40 mg/kg, i.p.; Sigma, St. Louis, MO) and were confined to the other side compartment. On the final day (day 5), they were again allowed to explore the entire apparatus freely for 30 min. Data collected during this test session were considered to reflect conditioning-induced (i.e., after conditioning) preferences for each of the compartments.

Immediately after the final test sessions, rats were anesthetized with pentobarbital (130 mg/kg, i.p.) and perfused with 0.9% saline, followed by 4% paraformaldehyde. The brains were kept overnight in 20% glycerol before slicing (40 μm). Injection placements were verified by histological analyses (Carlezon et al., 1998). Data from rats with placements outside the NAcc shell (5 of 73) were excluded from analyses. Conditioning-induced changes in preference for the drug- or saline-associated compartments were analyzed using a three-way (vector treatment × cocaine dose × time, before vs after conditioning) ANOVA with repeated measures, followed by *post hoc* comparisons with Fisher's *t* tests (two-tailed).

Immunohistochemistry. A number of rats ($n = 20$) from the locomotor activity studies were selected randomly for immunohistochemistry studies. These rats were injected with 1000 U of heparin intraperitoneally, anesthetized deeply with pentobarbital, and perfused intracardially with Tyrode's solution (in mM: 126 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 0.4 NaH_2PO_4, 10 glucose, and 10 HEPES), followed by 4% paraformaldehyde. The brains were removed, postfixed for 2 hr in 4% paraformaldehyde, and stored in PBS at 4°C 1–2 d before being processed further. Free-floating sections (40 μm) were prepared on a Leica (Nussloch, Germany) VT1000S vibratome and rinsed in PBS (200 mM), pH 7.4. The sections were permeabilized in PBS–0.5% Triton X-100 for 30 min and

then blocked with 0.3% gelatin (bovine) in PBS–0.025% Triton X-100 for 1 hr at room temperature or overnight at 4°C. They were then incubated with mouse monoclonal anti-hemagglutinin antibody (HA.11; Babco, Richmond, CA) diluted 1:1000 in 0.3% gelatin in PBS–0.025% Triton X-100 and incubated overnight at room temperature with gentle agitation. Sections were then rinsed three times for 10 min each with PBS–0.025% Triton X-100 and incubated with secondary antibody (goat anti-mouse Alexa-568 conjugate, 5 μg/ml; Molecular Probes, Eugene, OR) in 0.3% gelatin PBS–0.025% Triton X-100 for 1 hr at room temperature. The sections were rinsed three times for 10 min each with PBS–0.025% Triton X-100. Bisbenzimide at 1:50,000 in PBS was applied for 5 min, after which the sections were rinsed briefly with PBS and mounted on glass slides with Vectashield mounting medium (Vector Laboratories, Burlingame, CA). The sections were analyzed using a Bio-Rad (Hercules, CA) Radiance 2000 confocal system and associated Nikon (Tokyo, Japan) fluorescence microscope using an argon–krypton laser and red laser diode with appropriate Performance filters (Bio-Rad) for detection of GFP and Alexa-568 fluorescence.

RESULTS

The microinjections of viral vectors were aimed at the NAcc shell (Fig. 1*A*). GFP and immunoreactivity for the hemagglutinin epitope tag were both visible in animals that had received HA1B–GFP (Fig. 1*B*), whereas GFP alone was detected in animals that received GFP-only vector (Fig. 1*C*). Many transgene-expressing neurons were detected at each injection site, and the morphology of infected cells was consistent with neuron-specific gene transfer, as described previously with this HSV packaging system (Fig. 1*D*) (Carlezon et al., 2000). Virtually all of the cell bodies expressing transgene were located within an area ~800 μm in diameter, although we occasionally observed cell bodies in regions that project to NAcc, indicating that a few non-NAcc cells were infected retrogradely (usually zero to two cells per VTA section). Because NAcc neurons project to VTA and 5-HT$_{1B}$ receptors are translocated to axon terminals, we examined this region for evidence of epitope-tagged 5-HT$_{1B}$ receptor expression. As can be seen in Figure 1*E*, many fine neurites demonstrated both GFP fluorescence and HA1B immunoreactivity, indicating that, as expected, the transgene receptors are translocated to axon terminals in VTA, and the NAcc projections still demonstrate normal morphology despite viral gene transfer. NAcc also projects to ventral pallidum, in which GFP and HA1B staining fibers were also detected, although these were more sparse than in VTA. These results indicate that both epitope tagging and GFP coexpression are highly sensitive methods with which to evaluate the extent of transgene expression.

In the first experiment, we tested whether HA1B–GFP altered total activity and ambulation (a subcomponent of total activity) induced by cocaine. HA1B–GFP or GFP-only gene transfer into NAcc was performed after the activity monitoring for the first day. On the fourth test day, cocaine was injected (5, 10, or 20 mg/kg, i.p.), and total and ambulatory activities were monitored. Locomotion after saline injections on day 3 did not differ between GFP-only control animals and animals overexpressing 5-HT$_{1B}$ receptors (Fig. 2*A*). The total activity profile over time after cocaine injection on day 4 is shown in Figure 2*B–D*. When the full test period was analyzed, a significant difference was apparent at the 10 mg/kg dose, with 5-HT$_{1B}$-overexpressing animals showing much greater total activity ($F_{(1,18)} = 5.88$; $p < 0.05$) (Fig. 2*C*). Ambulatory activity was also greater in HA1B–GFP-treated animals after 10 mg/kg cocaine ($F_{(1,18)} = 7.46$; $p < 0.05$). There were no overall significant differences between viral treatment groups at 5 or 20 mg/kg cocaine over the full hour. Although there appeared to be a slower decay in the rate of activity late in the period of the HA1B–GFP animals treated with 5 mg/kg cocaine, this effect was not statistically significant. Activity after 20 mg/kg (intraperitoneally) cocaine appeared to reflect a maximal "ceiling" effect, with no additional effect in 5-HT$_{1B}$ overexpressing animals at any point during the test period. Total beam

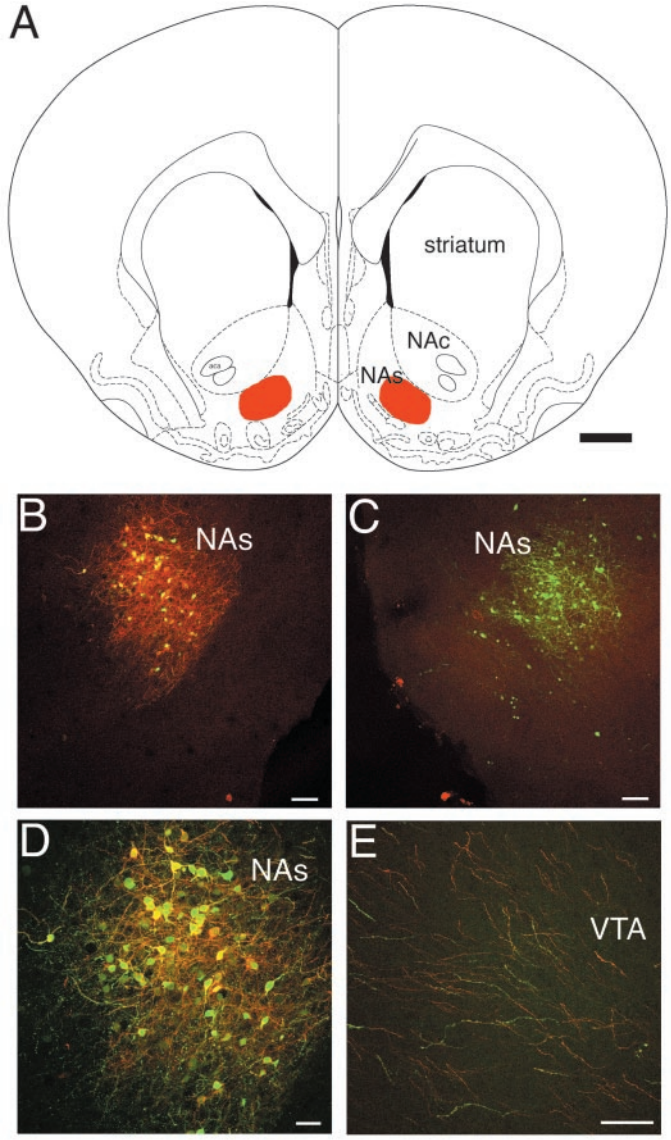

Figure 1. Viral-mediated gene transfer into NAcc induces expression and transport of transgenes from NAcc cell bodies to axon terminals in VTA. *A*, Anatomic targets for gene transfer are shown in *red shading*. Scale bar, 1 mm. GFP expression allowed rapid confirmation of expression and accuracy of injection in both experimental and control viral treatment groups. *B*, Brain section that received injections of HA1B–GFP viral vector demonstrates both hemagglutinin-tagged 5-HT$_{1B}$ immunoreactivity (*red*) and GFP direct fluorescence (*green*); 10× magnification. Scale bar, 100 μm. *C*, Brain section that received GFP-only control vector injections. Note that there is no cell labeling with the HA antibody (*red*), whereas GFP expression (*green*) is intense; 10× magnification. Scale bar, 100 μm. *D*, HA-5-HT$_{1B}$ (*red*) and GFP (*green*) are coexpressed in many cells; *yellow* indicates colocalized signal, especially in the cell membrane and proximal fibers; 20× confocal stack compressed in *z*-axis. Scale bar, 100 μm. *E*, Both HA–5-HT$_{1B}$- and GFP-positive fibers were detected in the VTA, indicating that transgenes were translocated to axon projections from cell bodies of NAcc neurons. Colocalization of both transgenes is apparent in many of the fibers, whereas GFP alone is detected in GFP-only treated animals; 40× confocal stack compressed in *z*-axis. Scale bar, 100 μm. *NAc*, Nucleus accumbens core; *NAs*, nucleus accumbens shell.

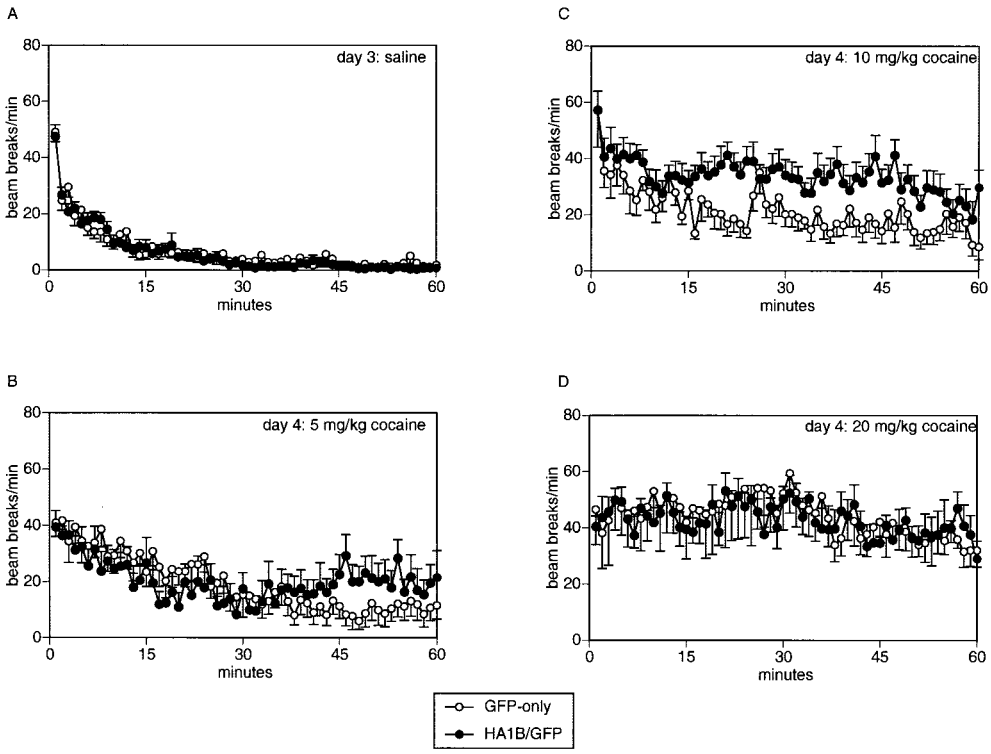

Figure 2. Time course of locomotor activity after injection with saline or cocaine. Overall activity (total beam breaks) and ambulation (consecutive beam break events) were quantified by automatic collection using infrared activity monitoring (see Materials and Methods). *A*, Total activity after the day 3 saline injection shows that animals rapidly became inactive and that 5-HT$_{1B}$ overexpression in NAcc projection neurons did not alter baseline behavior ($n = 26$, HA1B–GFP; $n = 27$, GFP-only-treated animals). *B–D*, Total activity after injection of 5 (*B*), 10 (*C*), or 20 (*D*) mg/kg cocaine (intraperitoneally) on day 4. A dose-dependent effect of cocaine on total locomotion is apparent, and 5-HT$_{1B}$ overexpression increased locomotion at the 5 and 10 mg/kg doses cocaine. The differences between HA1B–GFP- and GFP-only-treated animals were not apparent until at least 15 min after injection, presumably because of delay in cocaine availability after injection. Whether there was a late effect of 5-HT$_{1B}$ overexpression (after 60 min) on 20 mg/kg cocaine was not determined, although there appears to be a ceiling effect of maximal cocaine-induced hyperactivity for both treatment groups at the time points measured. Data represent the mean ± SEM at each time point of 8–11 animals per treatment condition.

breaks and ambulation (successive beam breaks in one direction) were further analyzed in 10 min collapsed segments during the test period (Fig. 3). After 10 mg/kg (intraperitoneally) cocaine, the HA1B–GFP-treated animals showed significantly greater total activity and ambulation than the GFP-only-treated animals during most of the test period (Fig. 3*C*,*D*).

In place conditioning studies, the time that rats spent in cocaine-associated environments depended on viral vector treatment, the dose of cocaine, and time (before vs after) (three-way interaction, $F_{(6,102)} = 2.55$; $p < 0.05$). Sham-operated rats given 10 or 20 mg/kg (intraperitoneally) cocaine spent more time ($p < 0.01$; Fisher's t tests) in cocaine-associated compartments after conditioning than they did before conditioning (Fig. 4); such data indicate conditioned place preferences and demonstrate the sensitivity of our procedures to the rewarding effects of the drug. A lower dose of cocaine (5 mg/kg) failed to alter significantly the time spent in drug-associated environments in sham-operated rats. Likewise, 40 mg/kg cocaine failed to systematically alter the time spent in drug-associated environments in sham-operated rats, raising the possibility that high doses of cocaine cause aversive, anxiogenic (Kosten et al., 1994), or memory-disrupting effects. This dose–effect relationship was virtually identical in rats given GFP-only vector into the NAcc: rats spent more time in drug-associated environments after 10 mg/kg ($p < 0.05$) or 20 mg/kg ($p < 0.01$), whereas 5.0 and 40 mg/kg did not affect place conditioning. In contrast, rats given microinjections of HA1B–GFP into the NAcc spent more time in cocaine-associated environments after 5.0 mg/kg ($p < 0.05$), indicating increased sensitivity to the rewarding effects of low doses of the drug. These rats showed normal increases in time spent in drug-associated environments after 10 mg/kg ($p < 0.01$). However, cocaine did not affect place conditioning at 20 or 40 mg/kg in rats given HA1B–GFP into the NAcc, suggesting increased sensitivity to the aversive, anxiogenic, or memory-disrupting effects of higher doses of the drug.

DISCUSSION

Elevated expression of 5-HT$_{1B}$ heteroreceptors in NAcc neurons that project to VTA causes sensitized behavioral responses to cocaine. Rats that received NAcc shell injections of a viral vector encoding 5-HT$_{1B}$ receptors tagged with GFP overexpressed the receptor in the VTA, suggesting viral uptake into the medium spiny projection neurons of the NAcc shell and translocation of overexpressed receptors from the somata of these neurons to their terminals in the VTA. Rats given this treatment were more sensitive to the locomotor stimulating effects of cocaine. Considering that the locomotor stimulating and rewarding effects of psychomotor stimulants appear to depend on common neural substrates (Wise and Bozarth, 1987), these findings raised the possibility that elevated expression of 5-HT$_{1B}$ receptors in the mesolimbic system would also affect the rewarding effects of cocaine. Indeed, lower doses of cocaine established conditioned place preferences in rats given this treatment, suggesting that these rats were more sensitive to the rewarding as well as the locomotor stimulating effects of the drug. Interestingly, rats treated with HA1B–GFP also appeared to be more sensitive to the aversive, anxiogenic (Kosten et al., 1994; Carlezon et al., 1998; Kelz et al., 1999; Pliakas et al., 2001; Andersen et al., 2002), or memory-disrupting effects of high doses of cocaine. Together, these findings suggest that mesolimbic 5-HT$_{1B}$ receptors play an important role in the regulation of the motivational effects of cocaine, presumably through their influence on the activity of VTA dopaminergic neurons.

Smaller amounts of GFP-expressing and HA1B-staining fibers were also detected in the ventral pallidum. Although the rewarding effects of cocaine are most often associated with the function

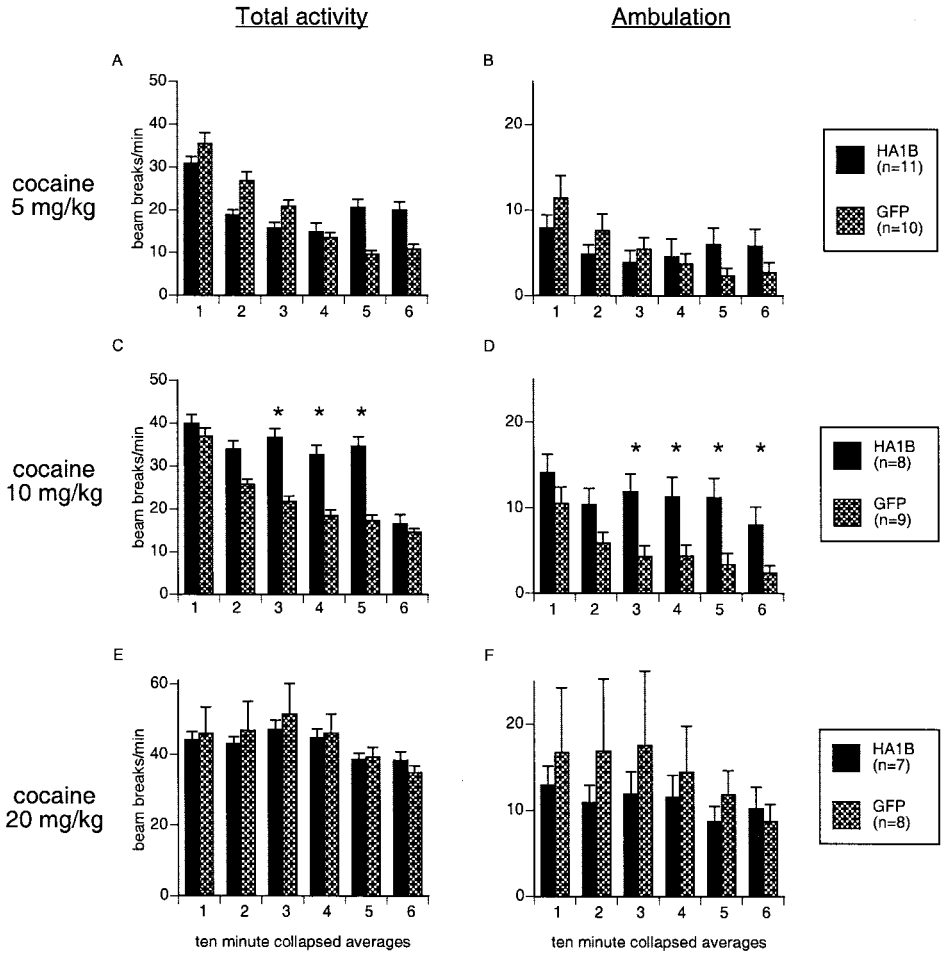

Figure 3. Elevated expression of mesolimbic 5-HT_{1B} receptors enhances cocaine-induced hyperactivity. Collapsed averages for 10 min segments (*1–6*) were calculated for total activity and ambulation. There was an overall treatment effect for both total activity and ambulation at 10 mg/kg cocaine (intraperitoneally) ($F_{(1,18)} = 5.88$ and 7.46 for total activity and ambulation, respectively; $p < 0.05$). Because there was an overall treatment effect of the HA1B–GFP treatment in the 10 mg/kg cocaine (intraperitoneally)-treated animals, *post hoc* Student's *t* tests were performed on each time segment to identify when increased 5-HT_{1B} expression altered cocaine-induced locomotor activity; $^*p < 0.05$.

of VTA dopamine cells and their terminal fields within the NAcc (Roberts et al., 1977; Phillips et al., 1983; Zito et al., 1985; Carlezon et al., 1995) and prefrontal cortex (Goeders and Smith, 1983), there is emerging evidence that the effects of cocaine in or indirectly on regions such as the ventral pallidum may also contribute to the reward-related effects of the drug (Gong et al., 1996; Johnson and Napier, 1996; McBride et al., 1999; Sizemore et al., 2000).

We detected high levels of GFP and HA–5-HT_{1B} expression, and both transgenic proteins were translocated to axon fibers in VTA and ventral pallidum. Recently, we confirmed that the HA epitope tagging does not alter the function of the 5-HT_{1B} receptor and that the same transgene receptor alters stress-induced anxiety-like behavior in the open field and elevated plus maze models (Clark et al., 2002). In that study, 5-HT_{1B} mRNA was increased threefold in the dorsal raphe nucleus after 5-HT_{1B} overexpression with the same viral vector as used in this study. The GFP-only vector controls for the presence of viral particles and the injection procedure, and, in the place conditioning studies, it produced results that were nearly identical to those in sham-injected animals. Therefore, our demonstration that elevated expression of 5-HT_{1B} in the mesolimbic system sensitizes animals to cocaine is not likely a result of nonspecific factors.

Viral-mediated gene transfer of epitope-tagged 5-HT_{1B} receptor, coexpressed with GFP, is an effective method for studying the role of 5-HT_{1B} receptors in selected neuron populations. Because 5-HT_{1B} receptors are expressed by many different neuron types and are translocated to axon terminals at varying distances from the cell bodies, manipulation of the mature receptor protein can be difficult to achieve without simultaneously involving a heterogeneous mix of 5-HT_{1B} heteroreceptors and autoreceptors. This problem is illustrated by the discrepancies between mouse studies of 5-HT_{1B} null mutations and rat studies involving local infusion of partially selective 5-HT_{1B} drugs. Although pharmacological studies in rats have suggested that 5-HT_{1B} agonists sensitize animals to cocaine, mouse "knock-out" experiments have reached the opposite conclusion in most cases (Belzung et al., 2000; Castanon et al., 2000). However, even the rat pharmacological data present an inconsistent picture. Administration of 5-HT_{1B} agonist sensitized animals to the effects of cocaine in most (Parsons et al., 1998, 1999) but not all (Przegalinski et al., 2001) studies; amphetamine effects were not enhanced by similar treatments (Fletcher and Korth, 1999a,b). CGS-12066B, a 5-HT_{1B} agonist, did enhance the reinforcing properties of GBR-12909, a dopamine-selective reuptake inhibitor (Parsons et al., 1996). These data indicate that 5-HT_{1B} agonists may enhance the rewarding properties of several classes of rewarding drugs.

Although these pharmacological studies have implicated specific circuits that contain 5-HT_{1B} receptors, the anatomic basis for altered drug sensitivity in 5-HT_{1B} knock-out mice is not known, and some of these effects have not been replicated across laboratories (Crabbe et al., 1999). The knock-out mice reportedly have significant developmental alterations in the dopaminergic system that may account for at least some of their altered behavioral

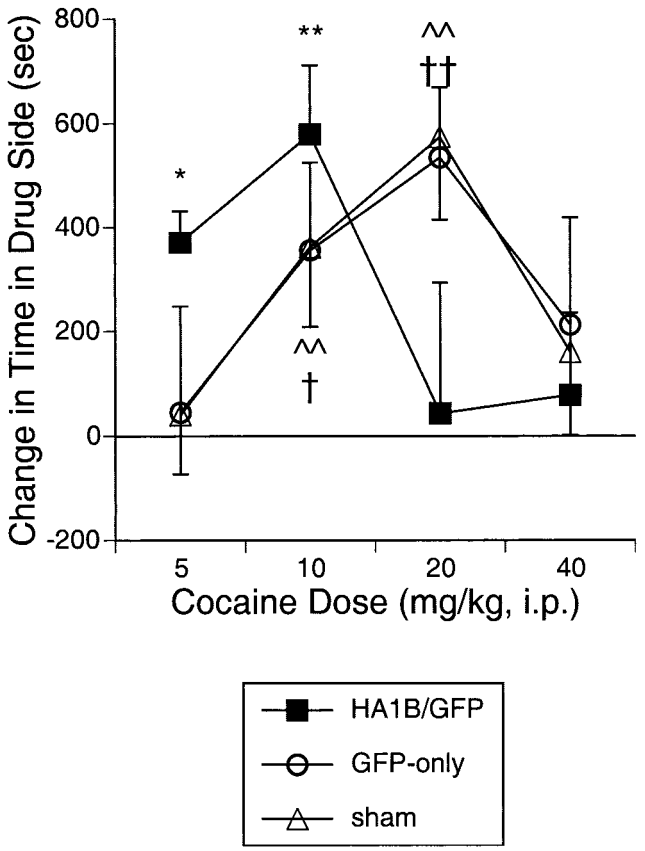

Figure 4. Effect of microinjections of viral vectors into the NAcc on cocaine place conditioning. Rats received bilateral microinjections of HA1B–GFP or GFP-only viral vector bilaterally into the NAcc or sham injections. For graphical clarity, place conditioning data (mean ± SEM; 7–14 rats per group) are expressed as the change in time spent in cocaine-associated compartments (i.e., time in seconds after conditioning minus time before conditioning). In rats given GFP-only vector or sham surgery, cocaine at 10 and 20 mg/kg established significant place preferences, whereas a higher dose (40 mg/kg) failed to have reliable effects. In contrast, treatment with HA1B–GFP shifted the cocaine dose–effect function to the left: a lower dose (5 mg/kg) established place preferences, whereas the effects of higher doses (20 and 40 mg/kg) were not reliable. Significant increases in time spent in cocaine-associated compartments are indicated as follows: $^{*}p \leq 0.05$ and $^{**}p \leq 0.01$ for rats given HA1B–GFP; $\dagger p \leq 0.05$ and $\dagger\dagger p \leq 0.01$ for rats given GFP-only vector; and $\hat{}\, p \leq 0.01$ for control rats; Fisher's *t* tests.

phenotype (Castanon et al., 2000; Shippenberg et al., 2000). Although previous studies suggested that the 5-HT$_{1B}$ agonist drugs acted on 5-HT$_{1B}$ receptors on GABA–dynorphin terminals of NAcc projections to VTA (Parsons et al., 1999), this was not demonstrated. Electrophysiological studies of 5-HT$_{1B}$ effects on cocaine in VTA slices supported but did not show conclusively this mechanism of action (Johnson et al., 1992; Cameron and Williams, 1994; Morikawa et al., 2000). Direct infusion of CP 93129 into NAcc did not enhance amphetamine discrimination (Filip et al., 2001), and the predominant electrophysiological effect of 5-HT$_{1B}$ receptor activation in NAcc itself was to inhibit glutamate release presynaptically (Muramatsu et al., 1998). Therefore, 5-HT$_{1B}$ activation in NAcc itself is not likely to explain the results of our experiments. However, by transiently increasing synthesis of 5-HT$_{1B}$ receptors in NAcc shell neurons that have efferent projections to VTA, we were able to reproduce the enhanced effects of cocaine demonstrated in other studies, strongly suggesting that the sensitization is caused by activation of 5-HT$_{1B}$ receptors in these specific neurons. Because >90% of NAcc neurons are GABAergic medium spiny projection neurons (Gerfen, 1988) and because HSV vectors are not selective for any neuronal subtype (Carlezon et al., 2000), it is likely that the majority of the HA–5-HT$_{1B}$ transgene receptors were expressed in these neurons. Furthermore, because we targeted the medial NAcc shell, the subregion that projects predominantly to VTA (Groenewegen et al., 1999), we believe that the effects we observed involve primarily 5-HT$_{1B}$ receptors in VTA. We interpret our results to suggest that cocaine-induced increase in extracellular serotonin can activate 5-HT$_{1B}$ receptors in NAcc projection fibers to VTA, thereby enhancing sensitivity to the stimulant and rewarding effects of cocaine, although the possibility of relevant actions in other regions that receive projections from NAcc (e.g., the ventral pallidum) (Johnson and Napier, 1996) cannot be excluded. Indeed, the recent observation that cocaine has rewarding effects via serotonin transporters in dopamine transporter knock-out mice indicates that serotonin participates directly in some of the actions of cocaine (Rocha et al., 1998; Sora et al., 2001). Both serotonin and dopamine levels increase significantly in NAcc during cocaine self-administration (Di Chiara and Imperato, 1988; Hernandez and Hoebel, 1988; Parsons et al., 1995; Reith et al., 1997); presumably this excess serotonin stimulates serotonin receptors in these brain regions. It is likely (although not yet demonstrated) that cocaine-induced serotonin activity participates in adaptive processes, such as those involved in sensitization, tolerance, withdrawal, and relapse. The receptor basis for these effects is poorly understood. However, *in situ* hybridization histochemistry, the most reliable method of detecting the expression of these receptors, suggests that several serotonin receptors may potentially be involved. These include 5-HT$_{1B}$ (Bruinvels et al., 1994), 5-HT$_{2A}$ (Ward and Dorsa, 1996; Mijnster et al., 1997), 5-HT$_{2C}$ (Ward and Dorsa, 1996; Eberle-Wang et al., 1997), 5-HT$_4$ (Vilaro et al., 1996), 5-HT$_6$ (Ward and Dorsa, 1996), and 5-HT$_7$ (Neumaier et al., 2001) receptors. The 5-HT$_{1D}$ receptors are represented (Bruinvels et al., 1994), and 5-HT$_{1A}$, 5-HT$_{1E}$, 5-HT$_{1F}$, 5-HT$_3$, and 5-HT$_5$ receptors are probably not present in NAcc. In summary, the present report confirms and extends accumulating evidence that 5-HT$_{1B}$ receptors seem to alter the sensitivity of brain reward circuits to cocaine. The additional use of precise molecular and anatomic strategies will be necessary to elucidate the complex roles of these serotonin receptors in cocaine addiction.

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