Published in final edited form as:

Cell Rep. 2018 June 26; 23(13): 3852–3863. doi:10.1016/j.celrep.2018.05.076.

Cocaine Inhibition of Synaptic Transmission in the Ventral Pallidum Is Pathway-Specific and Mediated by Serotonin

Aya Matsui[1] and **Veronica A. Alvarez**[1,2,3,4,*]

[1]Laboratory on Neurobiology of Compulsive Behaviors, Intramural Research Program, National Institute on Alcohol Abuse and Alcoholism (NIAAA-IRP), NIH, Bethesda, MD 20892, USA

[2]Intramural Research Program, National Institute on Drug Abuse (NIDA-IRP), Baltimore, MD 21224, USA

[3]Center on Compulsive Behaviors, Intramural Research Program, NIH, Bethesda, MD 20892, USA

[4]Lead Contact

SUMMARY

The ventral pallidum (VP) is part of the basal ganglia circuitry and a target of both direct and indirect pathway projections from the nucleus accumbens. VP is important in cocaine reinforcement, and the firing of VP neurons is modulated *in vivo* during cocaine self-administration. This modulation of firing is thought to be indirect via cocaine actions on dopamine in the accumbens. Here, we show that cocaine directly inhibits synaptic transmission evoked by selective stimulation of indirect pathway projections to VP neurons. The inhibition is independent of dopamine receptor activation, absent in 5-HT1B knockout mice, and mimicked by a serotonin transporter (SERT) blocker. SERT-expressing neurons in dorsal raphe project to the VP. Optogenetic stimulation of these projections evokes serotonin transients and effectively inhibits GABAergic transmission to VP neurons. This study shows that cocaine increases endogenous serotonin in the VP to suppress synaptic transmission selectively from indirect pathway projections to VP neurons.

Graphical Abstract

*Correspondence: alvarezva@mail.nih.gov.

AUTHOR CONTRIBUTIONS

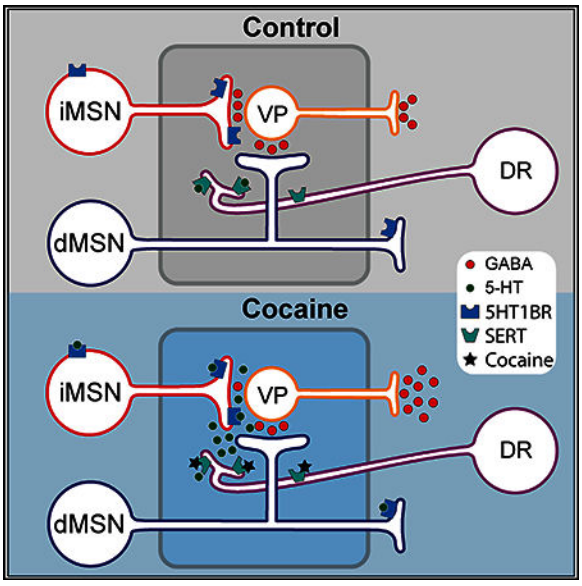
Research Design, Investigation, Analysis, Figures, and Writing, A.M. and V.A.A.; Funding, V.A.A. and A.M.; Resources: V.A.A.; Supervision: V.A.A.

DECLARATION OF INTERESTS

The authors declare no competing interests.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and one figure and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.05.076.



In Brief

Matsui and Alvarez show that acute effects of cocaine on accumbal to VP projections in brain slices are mainly mediated by serotonin, not dopamine, and involve serotonergic projections from the dorsal raphe. Cocaine inhibition selectively targets indirect pathway projections from accumbens and is expected to shift the balance of direct pathway projections toward VP.

INTRODUCTION

Cocaine is a psychostimulant drug commonly abused worldwide. It is a potent blocker of the dopamine transporter (DAT), which is thought to be the main target responsible for mediating the most rewarding and reinforcing properties of cocaine. However, cocaine blocks all three monoamine transporters— DAT, serotonin transporter (SERT) and norepinephrine transporter (NET)—with similar potency (Li et al., 1996; Richelson and Pfenning, 1984; Ritz et al., 1990; Rothman and Baumann, 2003). At high concentrations, cocaine also blocks ligand and voltage-gated ion channels (Acevedo-Rodriguez et al., 2014; Dunwiddie et al., 1988). Behavioral studies suggest a contribution of these other targets to the reinforcing properties of cocaine, but the synaptic mechanisms and site of actions of these other targets are still unclear.

In addition to dopamine (DA), cocaine increases extracellular concentrations of serotonin (5-hydroxytryptamine or 5-HT) in multiple nuclei of the basal ganglia (i.e., ventral pallidum [VP] [Sizemore et al., 2000], nucleus accumbens [NAc] [Andrews and Lucki, 2001; Bradberry et al., 1993; Li etal., 1996], and substantia nigra [Reith et al., 1997]) by inhibiting reuptake of 5-HT (Richelson and Pfenning, 1984). In rodents, blockers of SERT suppress cocaine self-administration and the motivation to seek cocaine (Carroll et al., 1990; Cunningham and Anastasio, 2014; Peltier and Schenk, 1993), suggesting that elevation of endogenous 5-HT contributes to the reinforcing properties of cocaine. DAT knockout (KO) mice acquire cocaine self-administration (Rocha et al., 1998a), while double KO mice

lacking DAT and SERT fail to acquire cocaine self-administration or cocaine-conditioned place preference (Sora et al., 2001). These findings suggest that SERT contributes to cocaine reinforcement. However, compensatory mechanisms known to develop in DAT and SERT KO mice may complicate this interpretation. Furthermore, transgenic knockin mice expressing a cocaine-insensitive DAT show impaired cocaine-induced locomotion and no conditioned place preference (Chen et al., 2006). This and other reports (Thomsen et al., 2009) seem to argue that cocaine actions on DAT are sufficient to account for cocaine behaviors and reward. Pretreatment with a DAT inhibitor, RTI-336, reduced cocaine self-administration in non-human primates. However, only a combination of DAT and SERT blockers completely suppressed cocaine self-administration in rhesus monkeys (Howell et al., 2007), suggesting that cocaine actions on DAT do not fully account for cocaine reinforcement. In fact, selective serotonin reuptake inhibitors (SSRIs), which are most commonly prescribed as antidepressants, have been used in clinical studies for pharmacological treatment of cocaine abuse (Shorter and Kosten, 2011). Some clinical studies show positive outcomes, which support a role for 5-HT in cocaine abuse and provide another avenue for treating patients suffering from both cocaine dependence and depression (Mancino et al., 2014; Moeller et al., 2007; Oliveto et al., 2012) Therefore, it is critical to determine where cocaine targets SERT and to understand the synaptic mechanisms by which 5-HT mediates the cocaine response.

A large portion of 5-HT neurons are localized to the dorsal raphe nucleus, and their axons project to multiple brain regions involved in reward, such as cortex, striatum, globus pallidus, NAc, VP, and substantia nigra. Fourteen different genes coding for 5-HT receptors have been identified and are grouped into 7 classes. A majority of 5-HT receptors are G protein-coupled receptors (GPCRs), except for 5-HT3 receptors, which are ligandgated ion channels. Pharmacological and genetic manipulations of 5-HT receptors are reported to affect cocaine reinforcement and reward. For example, administration of an agonist for 5-HT1A/B receptors potentiates cocaine-induced elevation of DA concentration in the NAc and enhances cocaine reinforcement (Parsons et al., 1998, 1999). 5-HT1B KO mice fail to develop conditioned place preference for cocaine (Belzung et al., 2000), yet show augmented locomotion and motivation to self-administer cocaine (Rocha et al., 1997, 1998b). Thus, multiple behavioral studies indicate that activation of 5-HT receptors contributes to the behavioral response of cocaine and sustains drug intake.

NAc neurons, both indirect pathway medium spiny neurons (iMSNs, also known as D2-MSNs) and direct pathway medium spiny neurons (dMSNs, also known as D1-MSNs), extend axonal projections to the VP, and activation of these projections trigger inhibitory GABA-A receptor-mediated synaptic currents (Creed et al., 2016; Kupchik et al., 2015). In addition, dMSNs also form long-range projections to the midbrain (Burke et al., 2017; Dudman and Gerfen, 2015). In the NAc, cocaine suppresses collateral GABA transmission from iMSNs to dMSNs by activating dopamine D2-receptors (D2Rs) on iMSNs (Dobbs et al., 2016). Through this DA-dependent mechanism, cocaine can disinhibit dMSNs in the NAc and change the balance between the direct and indirect pathway to contribute to the locomotor response to cocaine. In the VTA, cocaine suppresses GABA transmission to DA neurons by activating 5-HT receptors, and this cocaine effect is lost after depletion of 5-HT (Cameron and Williams, 1994). However, cocaine’s action in the VP is less understood.

The VP is important in the processing and execution of motivated behaviors and has been implicated in cocaine reinforcement. Several 5-HT receptor subtypes are present at high density in the VP (Appel et al., 1990; Chen and Lawrence, 2000; Murrough et al., 2011; Sari et al., 1999; Waeber et al., 1996), as well as immunoreactivity for SERT (Sur et al., 1996). Dorsal raphe neurons project to the VP and the firing rate of VP neurons predicts cocaine-seeking and motivated behavior (Root et al., 2010, 2013, 2015). This study examines the acute effects of cocaine from iMSN and dMSN input to the VP and the mechanism of cocaine's action in the VP.

RESULTS

Cocaine Suppresses GABA Transmission Specifically at iMSN to VP Synapses

The effect of cocaine on iMSN to VP synapses and on dMSN to VP synapses was examined using selective optogenetic stimulation of these inputs by injections of viral vector with Cre-dependent Channelrhodopsin-2 (ChR2) in iMSNs of Adora2a-Cre or in dMSNs of Drd1-Cre mice (Figures 1A and 1B). Whole-cell voltage clamp recordings were made from dorsal lateral VP neurons, which receive MSN inputs from the NAc core region. Paired pulse of light stimulation (470 nm, ISI = 50 ms) was delivered every 30 s, and light stimulations evoked inhibitory postsynaptic currents (oIPSCs) in VP neurons following activation of either iMSN or dMSN axon terminals to VP, in agreement with previous reports (Figure 1C) (Creed et al., 2016; Kupchik et al., 2015). oIPSCs were abolished by the selective antagonist gabazine (3 mM), indicating oIPSCs are mediated by activation of GABA-A receptors (2.9% ± 0.3% of baseline, $t_7 = 285.4$, $p < 0.0001$, $n = 8$). When stimulating iMSN to VP synapses, the monoamine transporter blocker cocaine (10 mM) decreased the amplitude of GABA-A oIPSCs to 66.8% ± 5.7% of baseline after 10 min application ($t_{14} = 5.9$, $p < 0.0001$, $n = 10$; Figure 1D). When stimulating dMSN to VP synapses, cocaine had no effect on oIPSC amplitude in VP neurons (92.7% ± 8.1% of baseline, $t_{14} = 1.36$, $p = 0.35$, $n = 6$; Figure 1D). The effect of cocaine on oIPSCs was significantly different between dMSN and iMSN pathways (2WRM-ANOVA, drug × pathway interaction: $F_{1,14} = 6.47$, $p = 0.023$). To determine whether a presynaptic mechanism was involved in the cocaine-induced inhibition, the paired pulse ratio (PPR) was calculated before and after cocaine application for iMSN and dMSN to VP synapses. The PPR was not affected by cocaine at dMSN to VP synapses (PPR = 0.68 ± 0.04 before and 0.71 ± 0.05 after cocaine; $t_{14} = 0.81$, $p = 0.87$), but it was increased at iMSN to VP synapses (PPR = 0.86 ± 0.1 before and 1.03 ± 0.11 after cocaine; $t_{14} = 4.87$, $p = 0.0005$; 2WRM-ANOVA, drug × pathway interaction: $F_{1,14} = 5.51$, $p = 0.034$; Figure 1E). These results indicate that cocaine exerts pathway-specific inhibition of oIPSC from iMSN in the VP and suggest that cocaine decreases the probability of GABA release specifically at iMSN to VP synapses.

Cocaine Inhibition of GABA oIPSCs Is Independent of D2R Activation

Because iMSNs express D2Rs, we examined whether cocaine-mediated suppression of iMSN to VP synaptic transmission requires D2R activation using pharmacological and genetic approaches (Dobbs et al., 2016). In the presence of D2-like receptor antagonist sulpiride (1 μM), cocaine significantly inhibited GABA-A oIPSC amplitude to 66.5% ± 0.5% of baseline ($t_{10} = 6.92$, $p < 0.0001$, $n = 11$; Figure 2A). Cocaine inhibition of oIPSC

remained intact even in the presence of a cocktail of DA receptor antagonists, which included the D1/D5 receptor antagonist SCH-23390 (3 μM), the D2/D3 receptor antagonist sulpiride (1 μM), and the D4 receptor antagonist L-745870 (100 nM; 70.2% ± 3.5% of baseline, $t_9 = 7.57$, $p < 0.0001$, n = 10; Figure 2B). Furthermore, cocaine produced a similar degree of inhibition of oIPSC amplitude in animals lacking D2Rs selectively in iMSNs (68.2% ± 2.5% of baseline, $t_{10} = 5.47$, $p = 0.0003$, n = 11; Figure 2C). There was no statistical difference in cocaine inhibition of iMSN to VP synaptic transmission between four groups (cocaine in wild-type [WT], cocaine + sulpiride in WT, cocaine + DAR antagonists in WT, and cocaine in iMSN-D2R KO mice; one-way ANOVA, $F_{3,38} = 0.12$, $p = 0.95$; Figure 2D). Thus, the cocaine inhibition of iMSN to VP synapses is not mediated by D2Rs.

Dopamine Release Was Not Detected in the VP

Our findings suggested that cocaine inhibition was independent of D2R activation in the VP; however, it is possible that DA release in the VP can play a role in this effect. Fast-scan cyclic voltammetry (FSCV) was carried out to detect evoked DA transients in the NAc and the VP from the same brain slice preparation. ChR2 was expressed in midbrain DA neurons using DATCre mice crossed with Ai32 mice, and DA terminals in the NAc and VP were stimulated optegenetically (Figure 3A). DA transients were recorded reliably in the NAc following a single light pulse (256 ±41 nM, n = 11; Figures 3B and 3C). A single electrical stimulation delivered near the carbon-fiber electrode also reliably evoked DA transients in the NAc (578 ± 103 nM, n = 11; Figures 3D and 3E). The pseudocolor voltammograms and the current-voltage (CV) plots showed peak currents at the known oxidation and reduction voltages for DA (0.6 and −0.2 V, respectively; Figures 3A, 3B and 3D). When the same carbon-fiber electrode and conditions were replicated in the VP, the same light pulse and electrical stimulation evoked undetectable DA signals in the VP (1.9 ± 0.53 nM and 1.4 ± 1.02 nM, respectively, n = 11; Figures 3B and 3D). A burst of light and electrical pulses (20 pulse, 25 Hz) were also applied in the VP; however, this strong stimulation evoked small DA transients (4.9 ± 1.35 nM, n = 11, or 7.2 ± 6.47 nM, n = 11). These experiments show that DA concentration in the VP are significantly smaller than in NAc when selectively stimulating DA axons (256 versus 1.9 and 4.9 nM; one-way ANOVA; $F_{1.002\ i1o.02} = 36.94$, $p = 0.0001$) and using electrical stimulations (578 versus 1.4 and 7.2 nM; $F_{1.004,1o.04} = 30.33$, $p = 0.0003$).

SERT Plays a Role in Cocaine Inhibition of iMSN to VP Transmission

Because cocaine can block all three monoamine transporters for DA, 5-HT, and norepinephrine, we speculated that the cocaine effect in the VP was mediated by blockade of a monoamine transporter other than DAT. To test this hypothesis, selective pharmacological blockers for each of the monoamine transporters were tested in their ability to replicate the cocaine-induced inhibition of iMSN to VP transmission. Application of the selective DAT blocker GBR12783 (1 μM) or NET blocker tamoxetine (1 μM) had no effect on the olPSC amplitude (97% ± 5.3% after GBR, $t_{26} = 1–18$, $p = 0.58$, n = 10, and 102% ± 7.8% after tamoxetine, $t_{26} = 0.23$, $p = 0.99$, n = 9). However, the selective SERT blocker citalopram (1 μM) significantly inhibited oIPSCs amplitude to 72% ± 4.7% of baseline and was significantly different from DAT and NET blocker applications ($F_{28,390} = 1.61$, $p = 0.027$;

Figures 4A and 4B). The degree of inhibition by citalopram was similar to that of cocaine ($F_{1,18}$ = 0.22, p = 0.64), and PPR was increased from 1.0 ± 0.1 to 1.2 ± 0.1 (t_7 = 3.60, p = 0.0087, n = 8), indicating a reduction of release probability (Figure 4C). GBR12783 and tamoxetine had no effect on PPR (1.05 ± 0.066 before and 1.09 ± 0.084 after GBR; t_{26} = 0.615, p = 0.905; 1.3 ± 0.18 before and 1.3 ± 0.14 after tamoxetine; t_{26} = 0.67, p = 0.88). Thus, a selective SERT blocker mimics the cocaine-induced inhibition on iMSN to VP transmission and suggests that cocaine actions on SERT are responsible for this action.

We examined the possible sources of innervation from SERT- positive neurons using retrograde tracing and immunohisto- chemistry techniques. Cholera toxin subunit B (CTB) conjugated with Alexa 488 was injected in the VP (Figure 4D). Two weeks after the injection, CTB-positive neurons were found in the dorsal raphe, a major 5-HT nucleus in the brain (Figure 4E). Subsequent immunostaining for SERT showed that 77% of CTB-positive neurons (n = 687) in the dorsal raphe were positive for SERT immunostaining, while the remaining neurons lacked detectable SERT (23%, n = 204) (Figures 4F and 4G). Thus, dorsal raphe is a source of SERT-positive innervation to the VP.

Stimulation of Dorsal Raphe Projections to VP Evokes 5-HT Transients

5-HT release in the VP was measured using FSCV with a modified voltage-ramp delivered through Nafion-coated carbon-fiber electrode (Figure 5A) (Hashemi et al., 2009; Rice and Nicholson, 1989). The first set of experiments were performed to validate the ability of the Nafion-coated carbon-fiber electrodes to detect 5-HT using the modified voltage ramp and recording conditions. Direct application of 1 μM 5-FIT produced a 34 ± 7.0 nA current (n = 7) while 1 μM DA produced no significant current (4.1 ± 2.7 nA, n = 5; Figure 5B). The CV plot obtained during 5-FIT application showed the expected current peaks at the oxidation and reduction potential for 5-FIT (0.6 V and −0.1 V; Figure 5B) (Hashemi et al., 2009). These tests confirm that this recording configuration can selectively detect 5-FIT without contribution from DA.

SERT-expressing dorsal raphe neurons are known to release 5-HT within the dorsal raphe from local axon collaterals. ChR2 was expressed in 5-HT neurons in the dorsal raphe by injections of viral vector with Cre-dependent ChR2 in SERT-Cre animals, and brain slices containing the dorsal raphe were prepared several weeks later. FSCV recordings were performed in the dorsal raphe to detect 5-HT transients by stimulating dorsal raphe neurons in acute brain slices. A train of light pulses (10 pulses at 25 Hz) evoked 5-HT transients of 44 ± 19.3 nM (n = 5) in the dorsal raphe (Figure 5G). The CV plots were similar to those obtained during exogenous 5-FIT application and showed the expected oxidation peaks for 5-FIT (Figure 5C). The selective SERT blocker citalopram (1 μM) prolonged the evoked transients and doubled the area from 87 ± 57 nM × s in artificial cerebrospinal fluid (aCSF) to 163± 74 nM × s after citalopram (t_4 = 4.187, p = 0.014) but it had no effect on the peak amplitude (42 ± 17 nM; Figures 5C and 5D). Evoked 5-FIT transients were sensitive to TTX, which is consistent with previous findings showing that activation of voltage-sensitive sodium channels is required for 5-FIT release (Figure 5C) (Carboni and Di Chiara, 1989). After TTX application, the peak transient was significantly reduced to 0.6 ±1.9 nM, revealing a small artifact of the light- induced oxidation of the carbon fiber, which peaked at

1.0 V (Figure 5C). The TTX-insensitive portion was subtracted from all traces, and the peak amplitude and area were calculated (see Experimental Procedures). These results confirm that the current method for FSCV can successfully detect 5-HT release in brain slices on selective stimulation of 5-HT neurons in dorsal raphe. The selective SERT blocker citalopram prolonged the duration of the evoked transients, strengthening the evidence of 5-HT release.

Smaller 5-HT transients were evoked by optogenetic stimulation (20–25 pulses at 25 Hz) of dorsal raphe axons in the VP (peak amplitude 18 ± 5.2 nM; Figures 5E–5G). Similar to the dorsal raphe, the CV plot in the VP showed the characteristic oxidation peak of 5-HT, and citalopram increased the area of the evoked transients from 29 ± 16 to 75 ± 26 nM × s in citalopram ($t_7 = 3.73$, $p = 0.0073$; Figure 5F) without affecting the peak amplitude (19 ± 5.0 nM in citalopram; $t_7 = 0.40$, $p = 0.7036$). TTX application significantly reduced the peak transient to 4.0 ± 2.3 nM ($F_{1,3,9,2} = 8.08$, $p = 0.00145$) and this peak largely consisted of light stimulation artifacts from the longer light pulse stimulation compared to dorsal raphe recording. Thus, stimulation of dorsal raphe axons evoked 5-HT release in the VP, and blocking SERT enhanced the concentration of extracellular 5-HT in the VP.

Serotonin Inhibits iMSN to VP Transmission in Dose-Dependent Manner

The effect of exogenously applied 5-HT was tested on the iMSN to VP synapses, the pathway selectively inhibited by cocaine. 5-HT application, in a dose-dependent manner, inhibited GABA-A olPSCs evoked by stimulation of iMSN axons (Figures 6A and 6B). The IC_{50} was calculated as 104 nM 5-HT in aCSF. In the presence of cocaine (10 μM), the dose-response curve shifted to the left, and the IC_{50} was reduced by one order of magnitude to 18.4 nM (Figure 6B). This result suggests that there is some degree of 5-HT release in the slice similar to spontaneous DA release in midbrain slices (Gantz et al., 2013), and cocaine blocks 5-HT reuptake to elevate 5-HT concentration in the VP. These results then allow us to estimate the extracellular concentration of endogenously released 5-HT in the presence of 10 μM cocaine alone where olPSCs were inhibited to 66.8% ± 5.7% of baseline (red dash line). Based on this percent inhibition, the extracellular concentration of endogenous 5-HT at the iMSN to VP synapse is estimated to reach ~60 nM.

Cocaine Effect in the VP Required 5-HT1B Receptors

The Gi protein-coupled 5-HT1B receptor is highly expressed in MSNs in the NAc (Sari, 2004). We hypothesized that 5-HT1B receptors are mediating the cocaine inhibition of GABA-A olPSCs. First, a pharmacological method was used to examine this hypothesis. The application of 5-HT1 B/D receptor agonist sumatriptan (1 μM) potently inhibited GABA-A olPSCs from iMSN to VP synapse (31.5% ± 6.6% of baseline, $t_{22} = 8.2$, $p < 0.0001$, $n = 7$; Figures 6C, 6D and S1). Similar to cocaine, the actions of sumatriptan were pathway-specific, and sumatriptan had no effects on GABA transmission from dMSN to VP synapse (98% ± 4.9% of baseline, $t_{22} = 0.06$, $p = > 0.99$, $n = 8$; Figures 6C and 6D). Statistical analysis revealed a significant interaction in the effect of cocaine between pathway-specific stimulations (2WRM-ANOVA: $F_{1,}13 = 67.95$, $p < 0.0001$; Figure 6D). Sumatriptan increased PPR only at iMSN to VP synapses from 0.81 ±0.15 to 1.18 ± 0.24 after sumatriptan ($t_{13} = 4.054$, $p = 0.0027$) and had no effect on PPR at dMSN synapses in

the VP (PPR = 0.79 ± 0.06 in aCSF and 0.76 ± 0.06 in sumatriptan; $t_{13} = 0.424$, p = 0.90; 2WRM-ANOVA, drug × pathway interaction: $F_{1,14} = 5.51$, $p < 0.05$). Interestingly, stimulation of dMSN axons in the substantia nigra reticulata (SNr) evoked olPSCs on GABAergic neurons and sumatriptan significantly inhibited the amplitude of olPSCs to 56.9% ± 13.4% of baseline at dMSN to SNr ($t_{22} = 5.14$, p = 0.0001, n = 6; Figure S1). Thus, in the VP, a selective 5-HT1 B/D receptor agonist can mimic the pathway-specific inhibition of GABA transmission, suggesting that 5-HT1B/D receptors in iMSN terminals are responsible for the effect of cocaine.

To further test this idea, we probed the effect of 5-HT and cocaine in mice lacking 5-HT1B receptors (Rocha et al., 1998b; Saudou et al., 1994). In these experiments, ChR2 could not be targeted to a specific subclass of MSNs but it was instead expressed in a non-Cre-dependent manner in the NAc. Light stimulation of axon terminals in the VP evoked olPSCs, likely mediated by stimulation of both iMSN and dMSN axons. In 5-HT1B KO mice, 5-HT (1 μM) had no significant effect on olPSC amplitude (99% ± 4.8% of baseline, $t_{12} = 0.093$, p = 0.995, n = 8), while it produced the expected inhibition of GABA-A olPSCs in WT littermate mice (56% ± 15% of baseline, = 3.28, p = 0.013, n = 6; Figure 6E). Additionally, application of cocaine (10 μM) failed to block olPSCs in 5-HT1B KO mice (90.4% ± 8.6% of baseline, $t_{16} = 0.166$, p = 0.983, n = 8), while it inhibited olPSCs in WT littermate mice (61.3% ± 3.7% of baseline, $t_{16} = 7.36$, $p < 0.0001$, n = 10; Figure 6F). Thus, 5-HT1B receptors are required for the 5-HT and the cocaine-mediated inhibition of iMSN transmission in the VP.

Stimulation of Dorsal Raphe Projections to VP Inhibits eIPSC

The effect of endogenous-released 5-HT on GABAergic synaptic transmission in the VP was examined using optogenetic stimulation of dorsal raphe projections to the VP. ChR2 was expressed in dorsal raphe neurons by injections of viral vector with Cre-dependent expression in the dorsal raphe region of SERT-Cre mice. Brain slices containing the VP were prepared several weeks later, and recordings from VP neurons were performed. A train of light pulses (20 pulses at 25 Hz), previously shown to evoke 5-HT transients, was used to evoke endogenous 5-HT release, while paired IPSCs (ISI = 50 ms) were evoked using electrical stimulation in the VP (Figure 7A). A first pair of electrical pulses (P1 and P2) was used to evoke eIPSCs and determine the baseline amplitude. A second pair of eIPSC (P3 and P4) was evoked 15 s later with (Figures 7C and 7E) or without (Figure 7B) the light train pulse stimulations. This protocol was repeated every 90 s and the P3/P1 ratio of eIPSC amplitudes were calculated for the control condition (no light stimulation; Figure 7B) and for those with light stimulation of dorsal raphe projection (Figure 7C). In the absence of light stimulation, the P3/P1 ratio of IPSC amplitude was 1.01 ± 0.034 (n = 16; Figures 7B and 7D), indicating little intrinsic variability nor rundown of IPSC amplitude without optogenetic stimulation. Following optogenetic stimulation of dorsal raphe projections to the VP, the P3/P1 ratio of eIPSC amplitude was significantly decreased to 0.82 ± 0.032 of baseline ratio (p = 0.003 n = 16; Figures 7C and 7D). The PPR between the first pair (P2/P1) and the second pair (P4/P3) was not changed in the absence of light stimulation (PPR = 1.23 ± 0.08 for P2/P1, and 1.25 ± 0.08 for P4/P3; $t_{15} = 0.55$, p = 0.59); however, the PPR for the second pair (P4/P3) was increased following light stimulation from PPR = 1.17 ± 0.07 to

1.39 ± 0.11 for P4/P3 in aCSF (t_{15} = 3.25, p = 0.005). Application of cocaine (10 mM together with 100 nM WAY 100635, a 5-HT1A receptor antagonist for minimizing autoreceptor-mediated inhibition of 5-HT release) decreased the amplitude of P1 to 82% ± 4.9% of baseline P1 in aCSF (t_{15} = 3.77, p = 0.002; Figures 7E and 7F), in agreement with the main findings described in this study. Additional optogenetic stimulation of dorsal raphe projections further decreased eIPSC amplitude (P3 in cocaine) to 70% ± 6.2% of baseline P1 in aCSF (t_{15} = 4.84, p = 0.002, n = 16; Figures 7E and 7F). The light stimulation also significantly increased the PPR, even in the presence of cocaine, from 1.29 ± 0.10 for P2/P1 cocaine to 1.66 ± 0.23for P4/P3cocaine (t_{15} = 2.48, p ≤ 0.0001).Thus, these experiments provide direct evidence that endogenously released 5-HT from dorsal raphe terminals in the VP is sufficient to inhibit GABA transmission, and cocaine can enhance this effect of 5-HT in the VP.

DISCUSSION

This study reveals direct actions of cocaine in the VP that are pathway-specific and mediated by 5-HT through cocaine blockade of SERT. Cocaine acutely inhibits GABA synaptic transmission from a select subpopulation of accumbal inputs onto VP neurons: iMSNs (D2-MSNs). Yet, synaptic transmission from bridging collaterals to VP that are formed by dMSNs (D1-MSNs) in the NAc is insensitive to cocaine.

We previously showed that cocaine exerts a similar pathway-specific inhibition of GABA transmission within the NAc where iMSNs extend axon collaterals synapses onto neighboring MSNs. This cocaine-induced inhibition of collateral transmission within the NAc is mediated by D2Rs expressed on iMSNs (Dobbs et al., 2016). However, the D2R-mediated inhibition in the VP is much weaker than in the axon collaterals in the NAc. To our surprise, we found that cocaine inhibits GABA transmission from long-range iMSN projections in the VP to a similar extent as that from collateral axons in the NAc. In the VP, the mechanism of cocaine's inhibition of GABA transmission is not mediated by canonical elevation of DA and activation of D2Rs. Instead, our experiments revealed that 5-HT and 5-HT1B receptors mediate this effect.

This study provides empirical evidence for a differential receptor efficacy at specific axon terminals from the same neuron, similar to our previous finding regarding the efficacy of D2Rs on iMSNs to suppress GABA transmission (Dobbs et al., 2016). This study shows that the agonist for 5-HT1B receptor sumatriptan had no effect on synaptic transmission from dMSN to VP neurons while it suppressed synaptic transmission from dMSN to the SNr (Figure S1), in agreement with previous reports (Cameron and Williams, 1994). These results reveal differential efficacy of 5-HT1B receptors depending on the location of axon terminals and suggest distinct trafficking and targeted expression of 5-HT1B receptors at specific synaptic terminals within a neuron. An alternative explanation is that 5-HT1B receptors are expressed only on a subset of dMSNs, which project to the midbrain, but are not expressed on dMSN for those projecting to VP. We anticipate that with the increased use of optogenetic approaches, more reports will contribute further evidence of differential efficacy at different axon terminals from a same neuron, and the mechanisms responsible for differential efficacy will be discovered.

Neither DAT- nor NET-selective blockers had any inhibitory effect on iMSN to VP GABA transmission. Moreover, 5-HT1B receptor agonist, but not D2-like receptor agonist, inhibited this synaptic transmission from the indirect pathway to VP neurons. These results provide strong evidence that cocaine actions in the VP are mainly on the serotonin system. This serotonin-mediated inhibition of iMSN inputs to VP might contribute positive reinforcing or anti-depressant actions. In behavioral observation, activation of iMSN inputs to VP implicates negative motivational state where animals stall their action sequences or lower reinforcement (Bock et al., 2013; Heinsbroek et al., 2017). 5-HT inhibition of this synaptic input could disinhibit this pathway, leading to enhanced motivation to seek cocaine.

Our experiments using FSCV for the electrochemical detection of DA failed to detect DA transients in the VP when using by both electrical and optogenetic stimulations. As a control, similar stimulation evoked large and reliable DA transients in the NAc from the same slices, ruling out most technical artifacts. While this experiment does not completely rule out the possibility for DA release in the VP, it suggests that DA release in the VP is very low (i.e., below the detection limit of this technique under the tested conditions). Furthermore, our experiment was carried out *in vitro,* while the microdialysis studies that were able to detect DA in the VP were performed *in vivo.* The findings are in agreement with the sparse DA projections observed in the VP compared to neighboring NAc (Klitenick et al., 1992; Prensa and Parent, 2001) and with the classic view that the globus pallidus and VP are indirectly influenced by DA via inputs from DA-sensitive brain regions (Yang and Mogenson, 1989). How ever, other studies have shown evidence that DA can directly modulate the firing rate of VP neurons *in vivo* (Napier and Potter, 1989; Napier et al., 1991), and anatomical studies showed the presence of DA containing axons from midbrain DA neurons (Voorn et al., 1986). A possible explanation for the discrepancy is that DA can affect the firing of VP neurons via activation of D1 receptors expressed directly on VP neurons. In fact, significant binding for D1 receptor agonist was observed in the VP, while radiolabeled ligand binding for D2R agonist was not detected in the VP (Beckstead et al., 1988).

In this study, we present evidence that 5-HT, rather than DA, is the main neuromodulator mediating the effect of cocaine in the VP. 5-HT inhibits GABA synaptic transmission from iMSNs, but not from dMSNs, in the VP. Cocaine also induced pathway-specific inhibition of synaptic inputs to the VP that can be mimicked by a selective SERT blocker. In doing so, cocaine alters the balance between direct and indirect pathway projections from NAc to the VP, and this synaptic effect might contribute the rewarding property of cocaine. These findings also suggest that pharmacological manipulation of 5-HT1B receptors could be used as therapeutic treatment for individuals that suffer from cocaine abuse and addiction.

EXPERIMENTAL PROCEDURES

Animals

All procedures were performed in accordance with the guidelines of National Institute on Alcohol Abuse, and Alcoholism and the Animal Care and Use Committees approved all experimental procedures. Male and female (>50 days old) heterozygote Adora2a-Cre mice (B6.FVB(Cg)-Tg(Adora2a-Cre)KG139Gsat/ Mmucd), Drd1-Cre mice (B6.FVB(Cg)-

Tg(Drd1-cre)EY262Gsat/Mmucd), DAT-Cre mice (B6.SJL-Slc6a3$^{tm1.1(cre)Bkmn}$/J), and SERT-Cre mice (Tg(Slc6a4-cre)ET33Gsat/Mmucd) were used to express Channelrhodopsin-2 (ChR2), in iMSNs, dMSNs, DA neurons, and dorsal raphe neurons, respectively, by targeted virus injection or cross with Ai32 mice (B6.Cg-Gt(ROSA)26Sor$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J). 5-HT1B receptor knockout mice (Ramboz et al., 1996) maintained on a BALB/c background (C.129- Htr1b^{tm1Rhn}/MpenJ) were provided by Dr. Williams (Vollum Institute). Male and female (>50 days old) 5-HT1B receptor knockout and littermate wild-type mice were used. Mice lacking D2 receptor from iMSN (iMSN-Drd2 KO) were generated by crossing Drd2$^{loxp/loxp}$ mice (B6.129S4(FVB)-Drd2$^{tm\ 1.1Mrub}$/J) with Adora2a-Cre mice and characterized previously (Lemos et al., 2016). Both male and female iMSN-Drd2 KO mice were used. All mice were group- housed in standard plastic containers on a 12-hr light and dark cycle, with food and water available ad libitum.

Stereotaxic Virus Injections

Mice (4–8 weeks) were anesthetized with isoflurane and placed in the stereotax (Kopf Instruments). Adeno-associated viral (AAV) vectors were bilaterally injected into NAc (<MI>250 nLfrom bregma: AP, +1.2; ML, ±1.0; DV, —4.6 mm), and dorsal raphe (<MI>500 nL: AP, —4.2; ML, ±0.5; DV, —3.2 mm) using a Nanoject II (Drummond Scientific). Vectors with Cre-dependent ChR2 expression (rAAV5- EF1a-DIO-hChR2(H134R)-EYFP; 4.5×10^{12}, UNC) were used to target iMSN with Adora2a-Cre mice, dMSN with Drd1-Cre mice, or dorsal raphe neurons with SERT-Cre mice. Additionally, constitutive AAV vector (rAAV5-CaMKII-hChR2(H134R)-EYFP; 8.5×10^{12}, Penn Vector Core) were used to express ChR2 in neurons of the NAcfor5-HT1BKOand littermate WT mice.ChR2expres- sion at the injection sites was confirmed before experiments. When ChR2 expressed outside of the target, animals were excluded from the study.

Slice Preparation

Mice were anesthetized with isoflurane and transcardially perfused with warm (33°C) artificial cerebrospinal fluid (aCSF) containing the following (in mM): 124 NaCl, 2.5 KCl, 1.3 MgCl2,2.5 CaCl2,1.0 NaH2PO4,26.2 $NaHCO_3$, 20 D-glucose, 0.4 ascorbate, and 3 kynurenic acid. Brains were removed and placed in a vibratome (Leica). Sagittal slices (230 μm) were prepared in warm aCSF. Slices were incubated in warm (33°C) 95%O_2/5%CO_2 oxygenated aCSF containing kynurenic acid (3 mM) for at least 30 min and moved to room temperature (22°C-24°C) until used. Slices containing VP were then transferred to the recording chamber that was constantly perfused with warm (33°C) 95%O_2/5%CO_2 oxygenated aCSF at the rate of 1.5–2 mL/min.

Electrophysiology

VP neurons were visualized with a 40× water-immersion objective on an upright fluorescent microscope (BX51WI, Olympus, USA) equipped with gradient contrast infrared optics. Whole-cell voltage clamp recordingswere madefrom dorsal lateral VP neurons using an Axopatch-200B amplifier (Molecular Devices). GABA-A IPSCs were recorded with patch pipettes (2.0–3.5 MΩ) filled with an internal solution containing the following (in mM): 57.5

KCl, 57.5 K-methylsulfate, 20 NaCl, 1.5 MgCl2, 5 HEPES, 10 BAPTA, 2 ATP, 0.2 GTP, and 10 phosphocreatine, pH 7.35, 290 mOsM. To isolate GABA-A currents, excitatory synaptic blockers, NBQX (5 mM) and 3-((R)-2-carboxypi- perazin-4-yl)-propyl-1-phosphonic acid (CPP 5 mM) were added to the aCSF. All neurons were voltage clamped at —60 mV. Series resistance was monitored throughout the experiment (range, 3–15 MU). GABA-A IPSCs were evoked byapaired light stimulation (2 stimuli at 20 Hz; every30 s).A fiber optic (200 mm/0.22 NA) connected to a blue LED (470 nm; Thorlabs) was placed near the recording, and light stimulation (0.2–2 ms) was given to evoke GABA-A IPSCs. Data were acquired using pClamp 10 software, sampled at 50 kHz, and filtered at 1 kHz. Analysis was performed with AxoGraphX (Axograph Scientific). The peak amplitude of GABA-A IPSCs were averaged over the last 3 min of drug application and normalized to the average 5 min before drug application.

Fast-Scan Cyclic Voltammetry

Fast-scan cyclic voltammetry (FSCV) was performed in the NAc and VP of acute brain slices. For DA measurements, carbon-fiber electrodes were prepared with T650fiberswith 7 μm diameterinserted into aglasspipette (~150 μm of exposed fiber). The carbon fiber electrode was held at —0.4 V versus Ag/AgCl and the DA voltage rampfrom —0.4Vto +1.2Vand backto —0.4Vat 400V/swasdelivered every 100 ms. Before the experiments started, electrodes were conditioned by delivering the voltage ramp at 60 Hz for 10 min.

For 5-HT measurements, carbon fibers electrodes were prepared as described previously (Hashemi et al., 2009). After the carbon fiber electrodes were constructed as described for DA measurement, the exposed carbon fibers were soaked into isopropyl alcohol for 30 min to clean the surface and coated with Nafion solution to improve the detection sensitivity (Rice and Nicholson, 1989). Specifically, the reference Ag/AgCl wire was twisted into a circle with 3 mm diameterand Nafion solution wasdropped intothe loop.Thecarbon fiber was inserted into the Nafion drop for 30 s with constant +1.0 V potential being applied. The carbon fiber electrode was first dried in the air and then placed in a 70°C oven for 10min.A modified voltage-ramp protocol was delivered to detect 5-HT. The carbon fiber electrode for 5-HT was held at +0.2 V versus Ag/AgCl, and the 5-HT voltage ramp from +0.2 V to +1.0 V and down to —0.1 V and back to +0.2 V at 1,000 V/s was delivered every 100 ms. Electrodes were conditioned by delivering the voltage ramp at 60 Hz for 10 min before the experiments started.

Electrical and optical stimulations were delivered to evoke DA and 5-HT transients every 2 min (Adrover et al., 2014). For electrical stimulation, a glass pipette filled with aCSF was placed near the tip of the carbon fiber (100 μm), and a single pulse (200 μA, 0.2 ms) was applied. For optical stimulation, a fiber optic as described above was placed near the carbon fiber, and a single (0.6 ms duration) or a train of light pulses was delivered to evoke release of DA (0.6 ms; 20 pulses at 25 Hz) or 5-HT (0.6 ms; 10 pulses for dorsal raphe and 20–25 pulses for VP at 25 Hz). Data were collected using a Chem-Clamp amplifier (Dagan Corporation) after low-pass filter at 3 kHz and digitized at 100 kHz using an I/O board (National Instruments) with a custom written software VIGOR using Igor Pro (Wavemetrics) running mafPC (courtesy of M.A. Xu-Friedman). All data were analyzed with VIGOR and

peak current amplitude of DA, and 5-HT transients were converted to concentrations according to the post-experimental calibration of carbon fiber using 1 μM DA or 5-HT. Peak and area of 5-HT transients were determined by measuring the maximum current and subtracting it from the current remaining after TTX application (presumed to be light-induced oxidation of the carbon fiber).

Statistical Analysis

Statistical analysis was performed with Prism (GraphPad Software) using unpaired and paired two-tailed Student's t tests, two-way ANOVA with or without repeated-measures (2WRM-ANOVA). Data are presented as mean ± SEM and statistical significance was achieved by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. Violation of sphericity in RM-ANOVA was corrected using the Greenhouse-Geisser method.

Immunofluorescence

The retrograde tracer cholera toxin B (CTB) conjugated with Alexa Fluor 488 (Invitrogen, 1:1,000) was injected into the VP (<MI>200 nL from bregma: AP, +0.5; ML, ±1.4; DV, —5.0 mm) using a Nanoject II (Drummond Scientific Company). Two weeks after the injection, mice were anesthetized with pentobarbital and transcardially perfused with PBSfollowed by 4% formaldehyde in PBS. Brains were removed and postfixed overnight in 4% formaldehyde at 4°C. Coronal or sagittal brain sections (50–75 mm thick) were obtained (Vibra- tome 1000 plus). Floating sectionswere blocked for4 hr in 5% BSAand 0.2% Triton X in PBS then incubated in serotonin transporter antibody (Immunostar) at a 1:1,000 dilution overnight at 4°C. Following 3 × 30 min rinse in PBS, sections were incubated in an Alexa Fluor 568 secondary antibody (Invitrogen goad anti-rabbit, 1:1,000) overnight. Sections were then rinsed 3 × 30 min in PBS and mounted on glass slides using Vectashield with DAPI (Ventor Laboratories). Images were obtained using confocal microscope (Zeiss LSM 880) using a 10× objective (NA:0.3) or a 40× water immersion lenses (NA:1.2). 64 fluorescent confocal images (850 × 850 mm for x-y,1024 × 1024 pixels) were combined in a composite figure in Figure 4D using Zen software (Zeiss). Alexa Fluor488-labeled CTB positive neurons were identified inthe DR from 34 confocal images (200 × 200 × 20 μm forx-y-z, 1024 × 1024 pixels) taken from 3 animals that received CTB injection in the VP. Images were analyzed using Fiji (NIH), and neuronswith CTB labeling were manually identified and outlined. The green and red fluorescent intensities were measured by the software, and background fluorescence was subtracted and compared between CTB-positive/SERT-positive neurons and CTB-positive/SERT-negative neurons.

Drugs

Cocaine HCl was obtained from the National Institute on Drug Abuse. NBQX, CPP, sulpiride, quinpirole, sumatriptan, GBR12783, tamoxetine, citalopram, 5-HT, and L-745870 were purchased from Tocris. Gabazine, kynurenic acid (sodium salt), and WAY 100635 were obtained from Abcam. All other chemicals were from Sigma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

The authors would like to thank Roland Bock for a custom-written Igor program, VIGOR, for the voltammetry for DA and 5-HT and members of the Alvarez laboratory for providing valuable feedback on this manuscript. This work was supported by the Japan Society for the Promotion of Science (toA.M.),the Intramural Research Programs of the National Institute on Alcohol Abuse and Alcoholism and the National Institute of Neurological Disorders and Stroke (ZIA-AA000421 to V.A.A.), and the DDIR Innovation Award program (to V.A.A.).

REFERENCES

Acevedo-Rodriguez A, Zhang L, Zhou F, Gong S, Gu H, De Biasi M, Zhou F-M, and Dani JA (2014). Cocaine inhibition of nicotinic acetylcholine receptors influences dopamine release. Front. Synaptic Neurosci 6, 19. [PubMed: 25237305]

Adrover MF, Shin JH, and Alvarez VA (2014). Glutamate and dopamine transmission from midbrain dopamine neurons share similar release properties but are differentially affected by cocaine. J. Neurosci 34, 3183–3192. [PubMed: 24573277]

Andrews CM, and Lucki I (2001). Effects of cocaine on extracellular dopamine and serotonin levels in the nucleus accumbens. Psychopharmacology (Berl.) 755,221–229.

Appel NM, Mitchell WM, Garlick RK, Glennon RA, Teitler M, and De Souza EB (1990). Autoradiographic characterization of (+-)-1-(2,5-dimethoxy-4-[125I] iodophenyl)-2-aminopropane ([125I]DOI) binding to 5-HT2 and 5-HT1c receptors in rat brain. J. Pharmacol. Exp. Ther 255, 843–857. [PubMed: 2243353]

Beckstead RM, Wooten GF, and Trugman JM (1988). Distribution of D1 and D2 dopamine receptors in the basal ganglia of the cat determined by quantitative autoradiography. J. Comp. Neurol 268, 131–145. [PubMed: 2964456]

Belzung C, Scearce-Levie K, Barreau S, and Hen R (2000). Absence of cocaine-induced place conditioning in serotonin 1B receptor knock-out mice. Pharmacol. Biochem. Behav 66, 221–225. [PubMed: 10837864]

Bock R, Shin JH, Kaplan AR, Dobi A, Markey E, Kramer PF, Gremel CM, Christensen CH, Adrover MF, and Alvarez VA (2013). Strengthening the accumbal indirect pathway promotes resilience to compulsive cocaine use. Nat. Neurosci 76, 632–638.

Bradberry CW, Nobiletti JB, Elsworth JD, Murphy B, Jatlow P, and Roth RH (1993). Cocaine and cocaethylene: microdialysis comparison of brain drug levels and effects on dopamine and serotonin. J. Neurochem 60, 1429–1435. [PubMed: 8455033]

Burke DA, Rotstein HG, and Alvarez VA (2017). Striatal local circuitry: a new framework for lateral inhibition. Neuron 96, 267–284. [PubMed: 29024654]

Cameron DL, and Williams JT (1994). Cocaine inhibits GABA release in the VTA through endogenous 5-HT. J. Neurosci 14, 6763–6767. [PubMed: 7965077]

Carboni E, and Di Chiara G (1989). Serotonin release estimated by transcortical dialysis in freely moving rats. Neuroscience 32, 637–645. [PubMed: 2481242]

Carroll ME, Lac ST, Asencio M, and Kragh R (1990). Fluoxetine reduces intravenous cocaine self-administration in rats. Pharmacol. Biochem. Behav 35, 237–244. [PubMed: 2315363]

Chen F, and Lawrence AJ (2000). 5-HT transporter sites and 5-HT1A and 5- HT3 receptors in Fawn-Hooded rats: a quantitative autoradiography study. Alcohol. Clin. Exp. Res 24, 1093–1102. [PubMed: 10924015]

Chen R, Tilley MR, Wei H, Zhou F, Zhou FM, Ching S, Quan N, Stephens RL, Hill ER, Nottoli T, et al. (2006). Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter. Proc. Natl. Acad. Sci. USA 703, 9333–9338.

Creed M, Ntamati NR, Chandra R, Lobo MK, and Lüscher C (2016). Convergence of reinforcing and anhedonic cocaine effects in the ventral pallidum. Neuron 92, 214–226. [PubMed: 27667004]

Cunningham KA, and Anastasio NC (2014). Serotonin at the nexus of impulsivity and cue reactivity in cocaine addiction. Neuropharmacology 76, 460–478. [PubMed: 23850573]

Dobbs LK, Kaplan AR, Lemos JC, Matsui A, Rubinstein M, and Alvarez VA (2016). Dopamine regulation of lateral inhibition between striatal neurons gates the stimulant actions of cocaine. Neuron 90, 1100–1113. [PubMed: 27181061]

Dudman JT, and Gerfen CR (2015). The basal ganglia In The Rat Nervous System, Fourth Edition, Paxinos G, ed. (Amsterdam: Elsevier), pp. 391–440.

Dunwiddie TV, Proctor WR, and Tyma J (1988). Local anaesthetic actions of cocaine: effects on excitatory and inhibitory synaptic responses in the hippocampus in vitro. Br. J. Pharmacol 95, 1117–1124. [PubMed: 2851358]

Gantz SC, Bunzow JR, and Williams JT (2013). Spontaneous inhibitory synaptic currents mediated by a G protein-coupled receptor. Neuron 78, 807–812. [PubMed: 23764286]

Hashemi P, Dankoski EC, Petrovic J, Keithley RB, and Wightman RM (2009). Voltammetric detection of5-hydroxytryptamine release in the rat brain. Anal. Chem 87,9462–9471.

Heinsbroek JA, Neuhofer DN, Griffin WC, 3rd, Siegel GS, Bobadilla A-C, Kupchik YM, and Kalivas PW (2017). Loss of plasticity in the D2- accumbens pallidal pathway promotes cocaine seeking. J. Neurosci 37, 757–767. [PubMed: 28123013]

Howell LL, Carroll FI, Votaw JR, Goodman MM, and Kimmel HL (2007). Effects of combined dopamine and serotonin transporter inhibitors on cocaine self-administration in rhesus monkeys. J. Pharmacol. Exp. Ther 320, 757–765. [PubMed: 17105829]

Klitenick MA, DeWitte P, and Kalivas PW (1992). Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. J. Neurosci 12, 2623–2632. [PubMed: 1319478]

Kupchik YM, Brown RM, Heinsbroek JA, Lobo MK, Schwartz DJ, and Kalivas PW (2015). Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. Nat. Neurosci 18, 1230–1232. [PubMed: 26214370]

Lemos JC, Friend DM, Kaplan AR, Shin JH, Rubinstein M, Kravitz AV, and Alvarez VA (2016). Enhanced GABA transmission drives bradykine-sia following loss of dopamine D2 receptor signaling. Neuron 90, 824–838. [PubMed: 27196975]

Li MY, Yan QS, Coffey LL, and Reith ME (1996). Extracellular dopamine, norepinephrine, and serotonin in the nucleus accumbens of freely moving ratsduring intracerebral dialysiswith cocaineand othermonoamineuptake blockers. J. Neurochem 66, 559–568. [PubMed: 8592125]

Mancino MJ, McGaugh J, Chopra MP, Guise JB, Cargile C, Williams DK, Thostenson J, Kosten TR, Sanders N, and Oliveto A (2014). Clinical efficacy of sertraline alone and augmented with gabapentin in recently abstinent cocaine-dependent patients with depressive symptoms. J. Clin. Psycho- pharmacol 34, 234–239.

Moeller FG, Schmitz JM, Steinberg JL, Green CM, Reist C, Lai LY, Swann AC, and Grabowski J (2007). Citalopram combined with behavioral therapy reduces cocaine use: a double-blind, placebo-controlled trial. Am. J. Drug Alcohol Abuse 33, 367–378. [PubMed: 17613964]

Murrough JW, Henry S, Hu J, Gallezot JD, Planeta-Wilson B, Neumaier JF, and Neumeister A (2011). Reduced ventral striatal/ventral pallidal serotonin1B receptor binding potential in major depressive disorder. Psychopharmacology (Berl.) 213, 547–553. [PubMed: 20480149]

Napier TC, and Potter PE (1989). Dopamine in the ratventral pallidum/substantia innominata: biochemical and electrophysiological studies. Neuropharmacology 28, 757–760. [PubMed: 2474767]

Napier TC, Simson PE, and Givens BS (1991). Dopamine electrophysiology of ventral pallidal/ substantia innominata neurons: comparison with the dorsal globus pallidus. J. Pharmacol. Exp. Ther 258, 249–262. [PubMed: 1677041]

Oliveto A, Poling J, Mancino MJ, Williams DK, Thostenson J, Pruzin- sky R, Gonsai K, Sofuoglu M, Gonzalez G, Tripathi S, and Kosten TR (2012). Sertraline delays relapse in recently abstinent cocaine-dependent patients with depressive symptoms. Addiction 107, 131–141. [PubMed: 21707811]

Parsons LH, Weiss F, and Koob GF (1998). Serotonin1B receptorstimulation enhances cocaine reinforcement. J. Neurosci 18, 10078–10089. [PubMed: 9822762]

Parsons LH, Koob GF, and Weiss F (1999). RU 24969, a 5-HT1B/1A receptor agonist, potentiates cocaine-induced increases in nucleus accumbens dopamine. Synapse 32, 132–135. [PubMed: 10231132]

Peltier R, and Schenk S (1993). Effects of serotonergic manipulations on cocaine self-administration in rats. Psychopharmacology (Berl.) 110,390–394. [PubMed: 7870907]

Prensa L, and Parent A (2001). The nigrostriatal pathway in the rat: A single-axon study of the relationship between dorsal and ventral tier nigral neurons and the striosome/matrix striatal compartments. J. Neurosci 21, 7247–7260. [PubMed: 11549735]

Ramboz S, Saudou F, Amara DA, Belzung C, Segu L, Misslin R, Buhot MC, and Hen R (1996). 5-HT1B receptor knock out-behavioral consequences. Behav. Brain Res 73, 305–312. [PubMed: 8788525]

Reith ME, Li MY, and Yan QS (1997). Extracellular dopamine, norepinephrine, and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration of cocaine and other uptake blockers. Psychopharmacology (Berl.) 134, 309–317. [PubMed: 9438681]

Rice ME, and Nicholson C (1989). Measurement of nanomolar dopamine diffusion using low-noise perfluorinated ionomer coated carbon fiber microelectrodes and high-speed cyclic voltammetry. Anal. Chem 61, 1805–1810. [PubMed: 2802146]

Richelson E, and Pfenning M (1984). Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: most antidepressants selectively block norepinephrine uptake. Eur. J. Pharmacol 104, 277–286. [PubMed: 6499924]

Ritz MC, Cone EJ, and Kuhar MJ (1990). Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure-activity study. Life Sci. 46, 635–645. [PubMed: 2308472]

Rocha BA, Ator R, Emmett-Oglesby MW, and Hen R (1997). Intravenous cocaine self-administration in mice lacking 5-HT1B receptors. Pharmacol. Biochem. Behav 57, 407–412. [PubMed: 9218264]

Rocha BA, Fumagalli F, Gainetdinov RR, Jones SR, Ator R, Giros B, Miller GW, and Caron MG (1998a). Cocaine self-administration in dopamine-transporter knockout mice. Nat. Neurosci 1, 132–137. [PubMed: 10195128]

Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ, and Hen R (1998b). Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature 393, 175–178. [PubMed: 9603521]

Root DH, Fabbricatore AT, Ma S, Barker DJ, and West MO (2010). Rapid phasic activity of ventral pallidal neurons during cocaine self-administration. Synapse 64, 704–713. [PubMed: 20340176]

Root DH, Ma S, Barker DJ, Megehee L, Striano BM, Ralston CM, Fabbricatore AT, and West MO (2013). Differential roles of ventral pallidum subregions during cocaine self-administration behaviors. J. Comp. Neurol 521, 558–588. [PubMed: 22806483]

Root DH, Melendez RI, Zaborszky L, and Napier TC (2015). The ventral pallidum: Subregion-specific functional anatomy and roles in motivated behaviors. Prog. Neurobiol 130, 29–70. [PubMed: 25857550]

Rothman RB, and Baumann MH (2003). Monoamine transporters and psychostimulant drugs. Eur. J. Pharmacol 479, 23–40. [PubMed: 14612135]

Sari Y (2004). Serotonin1B receptors: from protein to physiological function and behavior. Neurosci. Biobehav. Rev 28, 565–582. [PubMed: 15527863]

Sari Y, Miquel MC, Brisorgueil MJ, Ruiz G, Doucet E, Hamon M, and Verge D (1999). Cellular and subcellular localization of 5-hydroxytryptami- ne1B receptors in the rat central nervous system: immunocytochemical, auto-radiographic and lesion studies. Neuroscience 88, 899–915. [PubMed: 10363826]

Saudou F, Amara DA, Dierich A, LeMeur M, Ramboz S, Segu L, Buhot MC, and Hen R (1994). Enhanced aggressive behavior in mice lacking 5-HT1B receptor. Science 265, 1875–1878. [PubMed: 8091214]

Shorter D, and Kosten TR (2011). Novel pharmacotherapeutic treatments for cocaine addiction. BMC Med 9, 119. [PubMed: 22047090]

Sizemore GM, Co C, and Smith JE (2000). Ventral pallidal extracellular fluid levels of dopamine, serotonin, gamma amino butyric acid, and glutamate during cocaine self-administration in rats. Psychopharmacology (Berl.) 150, 391–398. [PubMed: 10958080]

Sora I, Hall FS, Andrews AM, Itokawa M, Li X-F, Wei HB, Wichems C, Lesch K-P, Murphy DL, and Uhl GR (2001). Molecular mechanismsof cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. Proc. Natl. Acad. Sci. USA 98,5300–5305. [PubMed: 11320258]

Sur C, Betz H, and Schloss P (1996). Immunocyto chemical detection of the serotonin transporter in rat brain. Neuroscience 73, 217–231. [PubMed: 8783244]

Thomsen M, Hall FS, Uhl GR, and Caine SB (2009). Dramatically decreased cocaine self-administration in dopamine but not serotonin transporter knock-out mice. J. Neurosci 29, 1087–1092. [PubMed: 19176817]

Voorn P, Jorritsma-Byham B, Van Dijk C, and Buijs RM (1986). The dopaminergic innervation of the ventral striatum in the rat: a light- and electron- microscopical study with antibodies against dopamine. J. Comp. Neurol 251, 84–99. [PubMed: 3760260]

Waeber C, Sebben M, Bockaert J, and Dumuis A (1996). Regional distribution and ontogeny of 5-HT4 binding sites in rat brain. Behav. Brain Res 73, 259–262. [PubMed: 8788514]

Yang CR, and Mogenson GJ (1989). Ventral pallidal neuronal responses to dopamine receptor stimulation in the nucleus accumbens. Brain Res. 489, 237–246. [PubMed: 2568154]

Highlights

- Cocaine inhibits GABA transmission from nucleus accumbens to ventral pallidum
- Inhibition is pathway-specific affecting only indirect pathway MSN projections
- Cocaine actions are independent of DA and D2 receptors in ventral pallidum
- Mechanism involves elevation of serotonin and activation of 5-HT1B receptors

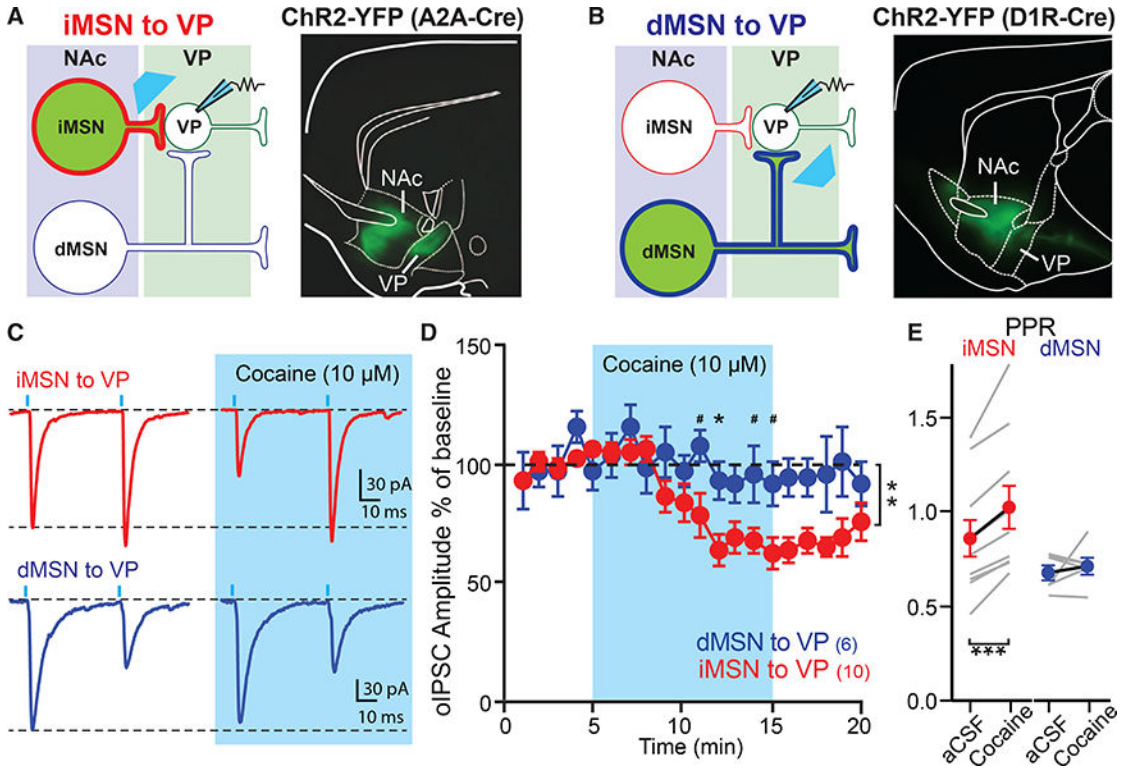


Figure 1. Cocaine Suppresses GABAergic Transmission at iMSN to VP but Not dMSN to VP Synapses

(A and B) Left: schematic diagrams of the experimental configuration where ChR2 expression was targeted to iMSN (A) or dMSN (B), and whole-cell recordings were made from VP neurons. Right: sagittal brain images of Adora2a-Cre mouse (A) and Drd1-Cre mouse (B) showing fluorescent-tagged ChR2 in cell bodies in the NAc and axonal projections to the VP.

(C) Averaged traces of GABA-A receptor-mediated olPSCs in VP neurons that were evoked by paired-pulse light stimulation (tick) of iMSN axonal projections (red, top) and dMSN axon projections (blue, bottom). Cocaine application (10 μM, blue box) reduced amplitude of oIPSC evoked by iMSN stimulation but not dMSN stimulation.

(D) Time course of cocaine effect on oIPSC amplitude when stimulating iMSN to VP (red) and dMSN to VP (blue) synapses.

(E) Paired pulse ratios of olPSCs (ISI = 50 ms) evoked by iMSN or dMSN axon stimulation were calculated before and after cocaine application. NAc, nucleus accumbens; VP, ventral pallidum. #$p < 0.07$.

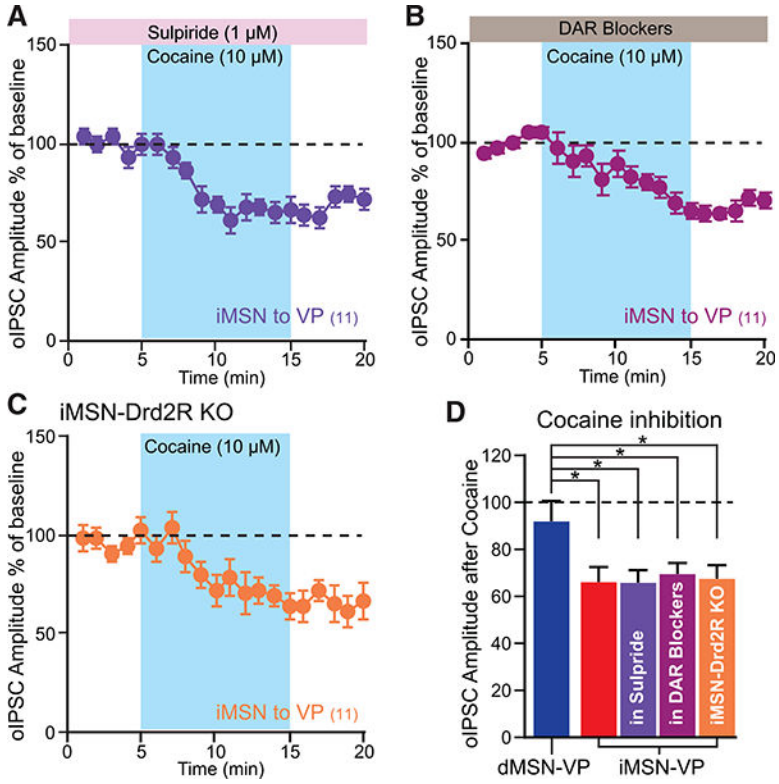


Figure 2. Cocaine Inhibition of oIPSCs Is Independent of D2 Receptor Activation

(A) In the presence of the D2/D3 receptor antagonist sulpiride (1 μM), the time course of the inhibition by cocaine of oIPSC amplitude from MSN to VP inputs was as similar as that without antagonist.

(B) Cocaine inhibition of oIPSC amplitude from MSN toVP inputs was similar in the presence of a cocktail of dopamine receptor (DAR) antagonists, including D1/D5 receptor antagonist SCH-23390 (3 μM), D2/D3 receptor antagonist sulpiride (1 μM), and D4 receptor antagonist L-745870 (100 nM).

(C) In brain slices from iMSN-Drd2 KO animals, the time course of cocaine inhibition of oIPSC amplitude from iMSN to VP inputs was similar to that in WT mice.

(D) Summary of the cocaine effect on oIPSC amplitude from dMSNs to VP inputs (blue) and from iMSN to VP inputs in aCSF (red), in sulpiride (purple), in DAR blocker cocktail (magenta), and in iMSN-Drd2 KO animals (orange). Bars represent amplitude of oIPSC after cocaine expressed as percent of baseline before cocaine.

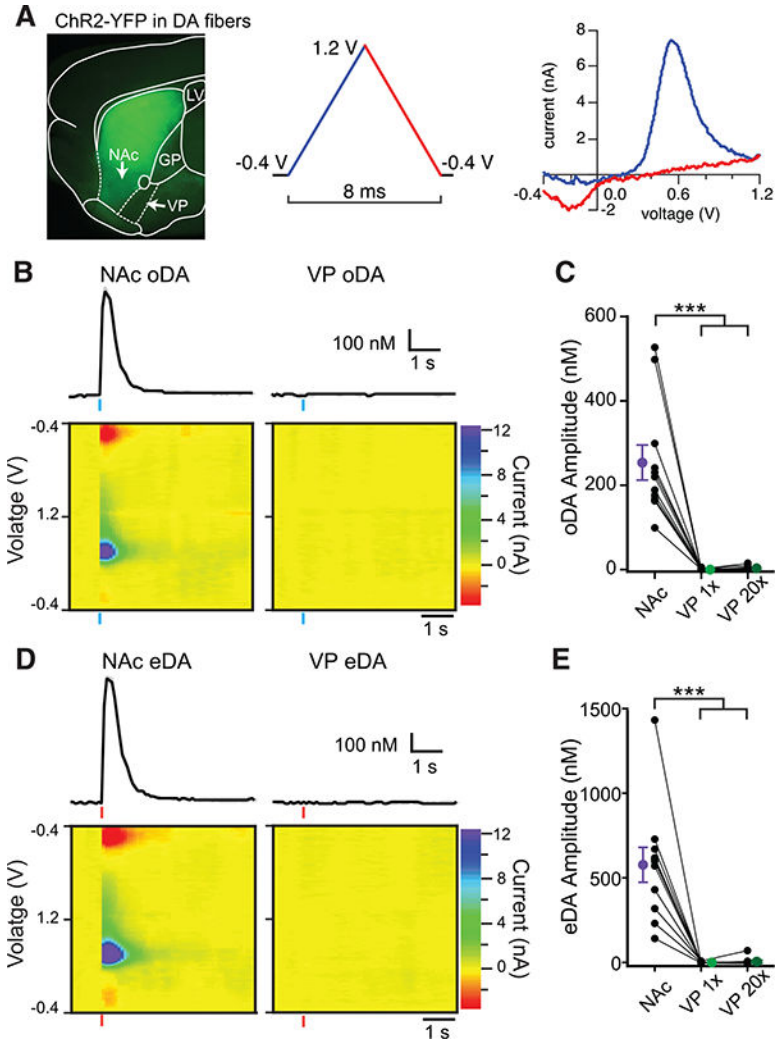


Figure 3. Evoked Dopamine Transients Can Be Measured in the NAc but Not the VP

(A) Left: sagittal brain image of forebrain showed fluorescent-tagged ChR2-expression of axon terminals from midbrain DA neurons in Dat-Cre mice crossed with Ai32. Middle: voltage ramp protocol used for detection of DA with FSCV. Right: representative CV plot of the electrochemical current (background-subtracted) evoked by optogenetic stimulation in the NAc showing the characteristic current peaks at 0.6 V and —0.2 V that match the oxidation and reduction potentials for DA.

(B) Top: averaged DA transients (oDA) triggered by a single light pulse (blue tick) when carbon fiber electrode and the stimulation were placed in the NAc (left) or the VP (right). Bottom: representative 3D voltammograms showing background-subtracted current (false colorscale) as a function oftime (xaxis, 6 s) and voltage applied (y axis).

(C) Peak amplitude of oDA transients evoked by a single pulse light stimulation in the NAc and VP and a train of light pulse (20 pulses at 25 Hz) in the VP. Black circles connected by the line represent each pair of measurements made in the NAc and the VP with the same carbon fiber electrodes, stimulation, and brain slice. Colored circles represent the mean and SEM for all measurements in the NAc and VP.

(D) Averaged DA transients (eDA, top) evoked by a single pulse (red tick) of electrical stimulation from NAc (left) and VP (right) and their representative 3D voltammograms (bottom).

(E) Peak amplitude of eDA transients evoked by electrical stimulation in the NAc and VP. Black circles and lines represent each pair of measurements made in the NAc and the VP with the same carbon fiber electrodes, stimulation, and brain slice. Colored circles represent the mean and SEM for all measurements in the NAc and VP.

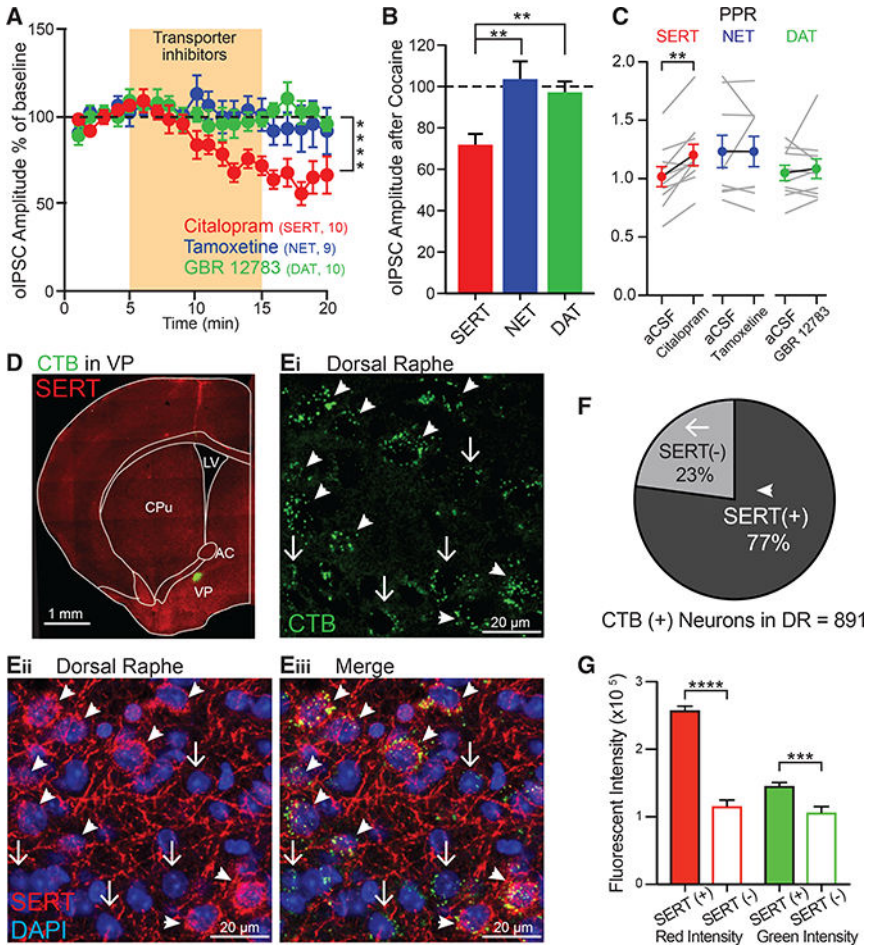


Figure 4. SERT Blockers Mimicked Cocaine Inhibition of iMSN to VP Transmission

(A) Time courses of olPSC amplitude from iMSN to VP inputs during application of the SERT blocker citalopram (1 μM, red), the NET blocker tamoxetine (1 μM, blue), or DAT blocker GBR 12783 (1 μM, green).

(B) Summary of the effect of the monoamine transporter blocker on oIPSC amplitude expressed as percent of baseline.

(C) Paired pulse ratios of olPSCs before and after application of each monoamine transporter blocker.

(D) Composite confocal image obtained at low magnification of a coronal brain section showing the site of injection of retrograde tracer cholera toxin b (CTB) conjugated with Alexa488 (green) in the VP and immunostaining for SERT (red) throughout the brain.

(E) High-power magnification confocal images of the dorsal raphe nucleus showing retrogradely transported CTB (green) in cell bodies of SERT (red)-positive cells (arrowheads). CTB-labeled neurons without SERT immunostaining neurons were also observed (arrow). DAPI staining is shown in blue. CPu, caudate putamen; LV, lateral ventricle; AC, anterior commissure.

(F) Percentage of SERT-positive and SERT-negative neurons among VP projecting CTB-positive neurons are shown.

(G) Quantification of fluorescent intensity in CTB- positive (green) and SERT-positive (red) neurons in the dorsal raphe.

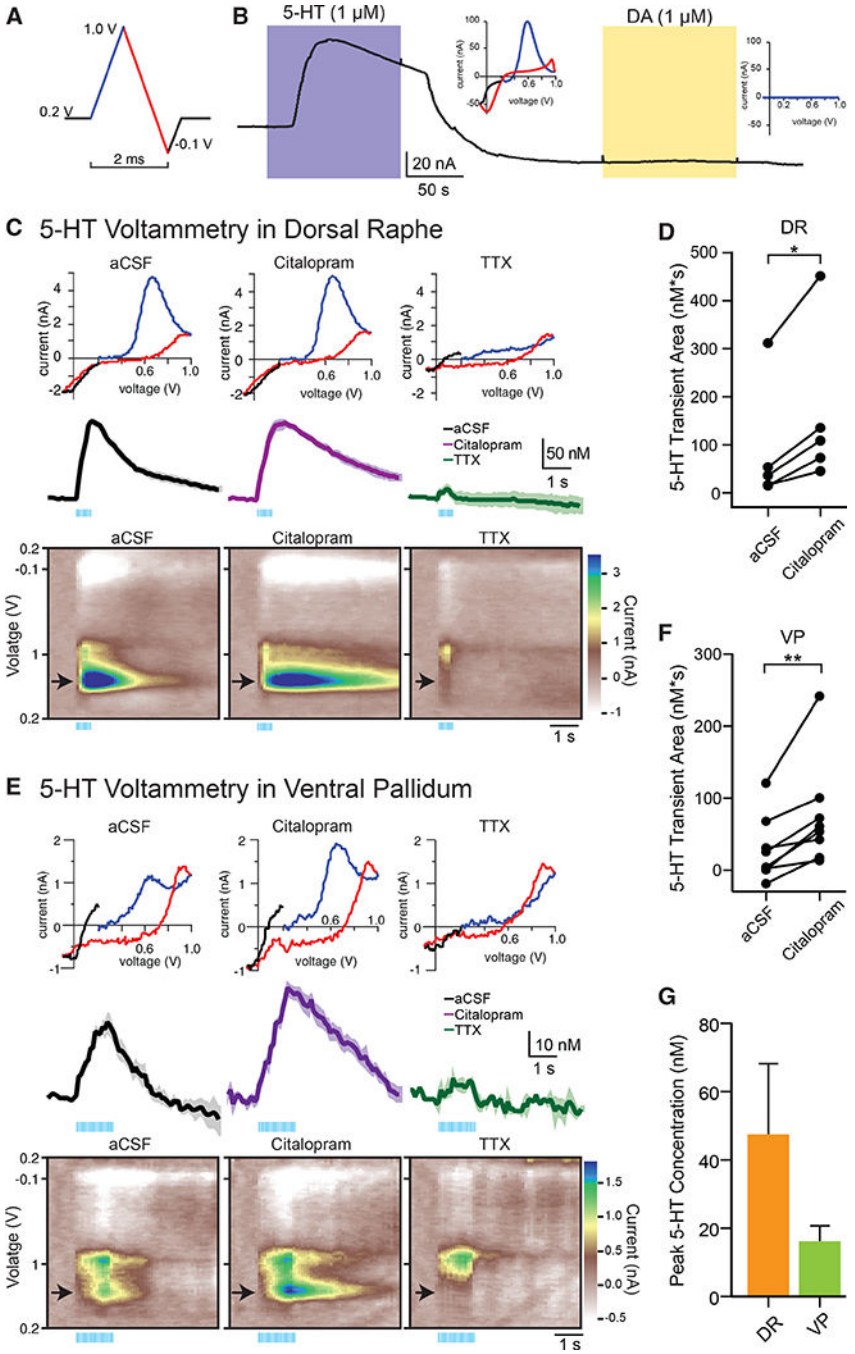


Figure 5. Evoked 5-HT Transients in the VP Are Recorded during Stimulation of Dorsal Raphe Axon Fibers

(A) Voltage ramp protocol used for detection of 5-HT with FSCV.

(B) Representative current traces measured when applying 5-HT (1 μM, purple) or DA (1 μM, yellow) during the calibration of Nafion-coated carbon-fiber electrode using the 5-HT voltage ramp. Insets: CV plots of the electrochemical current (background-subtracted) obtained during 5-HT (left) and DA (right) application.

(C) Evoked 5-HT transients were triggered by a train light pulses (10 pulses at 25 Hz) and detected in the dorsal raphe of SERT-Cre mice expressing ChR2 in 5-HT neurons during

perfusing of aCSF (left), which are prolonged by SERT blocker Citalopram (1 μM; middle) and dramatically reduced by TTX (500 nM; right). Top: representative CV plots of the electrochemical current in aCSF, citalopram, and TTX. Middle: average 5-HT transients under each condition. Bottom: 3D voltammograms of background-subtracted current (false color scale) as a function of time (x axis, 6-s) and voltage applied (y axis).

(D) Area of 5-HT transients recorded in the dorsal raphe before and after citalopram application. Black circles and lines represent the paired measurements made in the dorsal raphe before and after citalopram.

(E) Evoked 5-HT transients were triggered by a train of light pulses (25 pulses at 25 Hz) and detected in the VP of SERT-Cre mice expressing ChR2 in SERT-positive axon terminals during each condition. Representative CV plots (top), average 5-HT transients (middle), and 3D voltammograms (bottom).

(F) Area of 5-HT transients before and after citalopram application in the VP. Black circles and lines represent the paired measurements made in the VP before and after citalopram.

(G) Peak amplitude of 5-HT transients evoked in the dorsal raphe and VP.

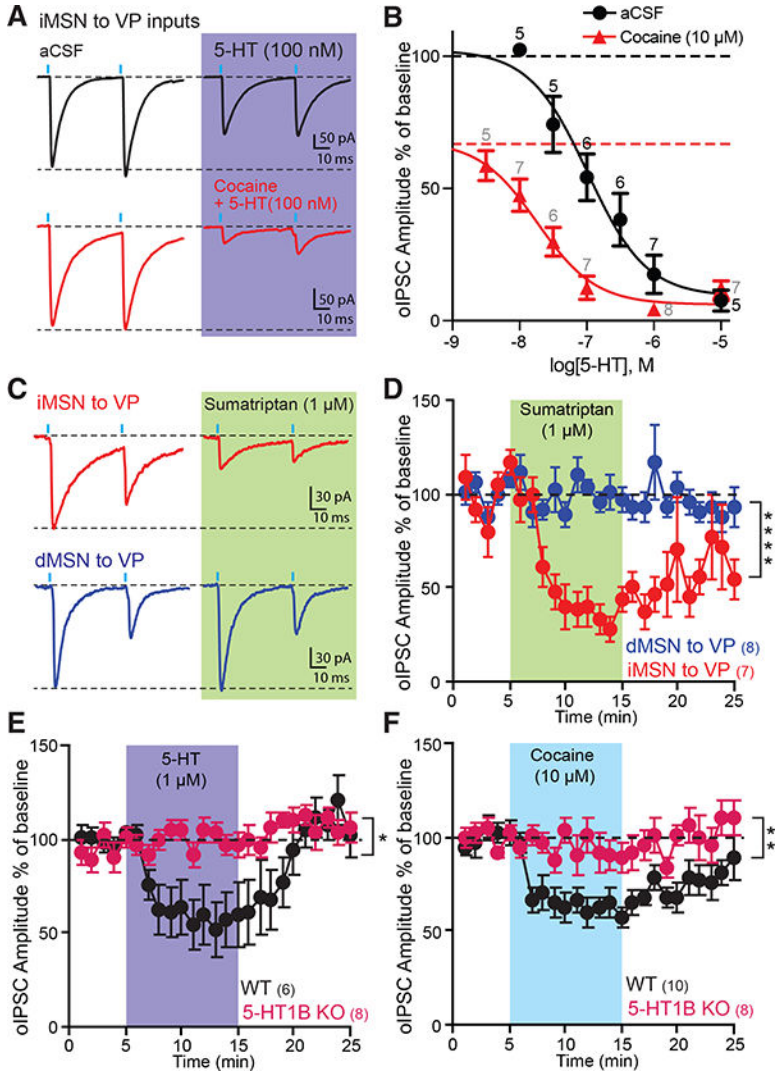


Figure 6. Cocaine Inhibition in the VP Is Mediated by Activation of 5-HT1B Receptors

(A) Average trances of olPSCs from iMSN to VP inputs before and during application of 100 nM 5-HT(purple box) in aCSF (black traces) and in presence of 10 μM cocaine (red traces).

(B) Concentration-response curves show 5-HT inhibition of olPSCs amplitude from iMSN to VP in aCSF (black) and during co-application of cocaine (10 μM, red). In the presence of cocaine, there is basal degree of olPSC inhibition (red dash line) that matches the inhibition by 10 μM cocaine alone.

(C and D) Average traces (C) and time course (D) of olPSCs amplitude change before and during application of the 5-HT1B/D agonist sumatriptan (1 μM) that were evoked by light stimulation of iMSN inputs (top red) and dMSN inputs (bottom blue) to the VP. See also Figure S1.

(E and F) Time course of olPSC amplitude during application of 5-HT (E) and cocaine (F) in brain slices taken from 5-HT1B KO animals (purple) and WT littermate (black). For these experiments, ChR2 was expressed in the NAc using a viral vector with non-cre-dependent expression and olPSCs were evoked by light stimulation in the VP.

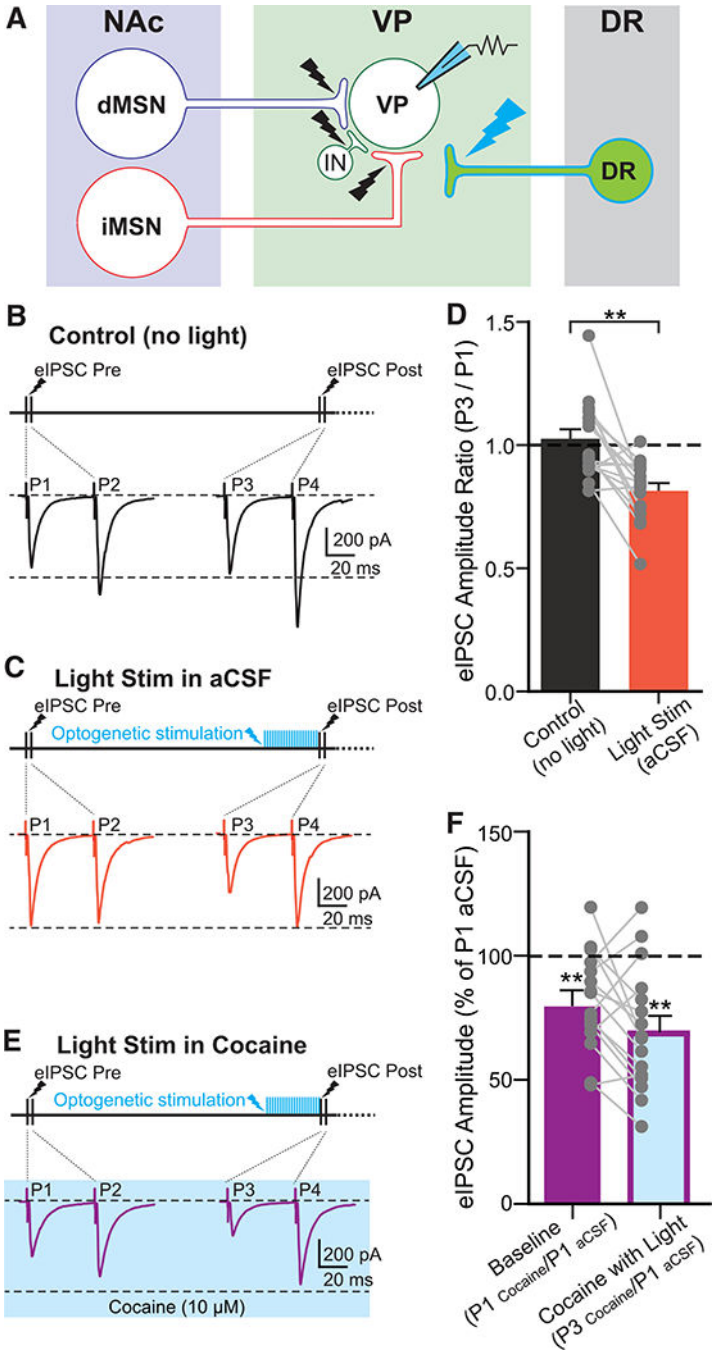


Figure 7. Optogenetic Stimulation of Dorsal Raphe Projections to the VP Inhibits Evoked GABA IPSC

(A) A schematic diagram of the experimental configuration. GABA-A IPSCs were evoked with electrical stimulation (black flash) while optogenetic stimulation of ChR2-expressing dorsal raphe terminals (blue flash) was delivered right before electrical stimulation in the VP.

(B and C) A paired pulse of electrical stimulation (P1 and P2) was delivered without dorsal raphe stimulation and, 15 s later, another paired pulse stimulation (P3 and P4) was delivered right after a train of optogenetic stimulation (20 pulses at 25 Hz, C) or sham control (without

light stimulation, B). Averaged traces of eIPSC evoked pre- (P1 and P2) and post- (P3 and P4) optogenetic stimulation of dorsal raphe projections are shown.

(D) Averaged and individual values of eIPSC amplitude ratio for P3/P1 are shown. The ratio of eIPSC amplitude was significantly reduced after optogenetic stimulation of dorsal raphe projections to the VP.

(E) Averaged traces of eIPSC recorded during application of cocaine (10 μM) and the 5-HT1A receptor antagonist, WAY 100635 (100 nM). Note that P1 was inhibited by application of cocaine, and optogenetic stimulation had an additional effect on P3 amplitude.

(F) Averaged and individual P1 values in cocaine and cocaine + optogenetic stimulation normalized to P1 in aCSF. The eIPSC P1 amplitudes were significantly reduced compared to P1 in aCSF.