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Selective Activation of Cholinergic Interneurons Enhances Accumbal Phasic Dopamine Release: Setting the Tone for Reward Processing

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SUMMARY

Dopamine plays a critical role in motor control, addiction, and reward-seeking behaviors, and its release dynamics have traditionally been linked to changes in midbrain dopamine neuron activity. Here, we report that selective endogenous cholinergic activation achieved via in vitro optogenetic stimulation of nucleus accumbens, a terminal field of dopaminergic neurons, elicits real-time dopamine release. This mechanism occurs via direct actions on dopamine terminals, does not require changes in neuron firing within the midbrain, and is dependent on glutamatergic receptor activity. More importantly, we demonstrate that in vivo selective activation of cholinergic interneurons is sufficient to elicit dopamine release in the nucleus accumbens. Therefore, the control of accumbal extracellular dopamine levels by endogenous cholinergic activity results from a complex convergence of neurotransmitter/neuromodulator systems that may ultimately synergize to drive motivated behavior.

INTRODUCTION

The mesolimbic dopamine system, comprised mainly of the rostral dopaminergic projection from the ventral tegmental area to the nucleus accumbens, (Wise, 2004; Sulzer, 2011), is crucial for decision making, motivated behaviors, and addiction. Patterns and levels of dopamine in nucleus accumbens are traditionally determined by the combination of firing of ventral tegmental area neurons and the dynamics of dopamine release from axon terminals.

Although cholinergic interneurons are 2%–5% of all striatal neurons, they establish an extensive arrangement of axons and form a diffuse neurotransmission system (Descarries et al., 1997; Descarries and Mechawar, 2000). Therefore, cholinergic activity in the striatum has long been hypothesized to play a role in the modulation of dopamine release (Giorguieff et al., 1976; Zhou et al., 2001; Zhang and Sulzer, 2004; Threlfell et al., 2010). Genetic deletion or pharmacological manipulation studies of nicotinic cholinergic (nAChRs) and muscarinic (mAChRs) receptors have shown that they modulate electrically-evoked dopamine release in the striatum (Exley et al., 2008, 2011, 2012; Zhou et al., 2001; Zhang et al., 2009; Threlfell et al., 2010). Moreover, nAChR-targeted drugs differentially alter dopamine release in a frequency-dependent manner. This finding has led to the notion that a high-pass filter, dependent on antagonism of nAChRs or nicotine, facilitates burst release of dopamine (Exley and Cragg, 2008), an activity pattern observed mainly following the presentation of reward and reward-predicting cues (Mirenowicz and Schultz, 1996; Nakahara et al., 2004; Roesch et al., 2007; Schultz, 2007; Apicella et al., 1991).

However, the precise role that endogenous cholinergic activity exerts on dopamine release in the nucleus accumbens (NAc) has not been explored. Endogenous release of acetylcholine (ACh) obtained by optogenetic control of cholinergic interneurons allows for the elucidation of cholinergic receptor action when activation occurs by the natural ligand. To examine the effect of endogenously released ACh on terminal dopamine release, we used a combination of optogenetic techniques for selective stimulation of cholinergic interneurons, combined with electrophysiology, real-time detection of dopamine levels and pharmacology. Activation of cholinergic interneurons is sufficient to evoke dopamine release in the NAc, independently of contingent activation of the ventral tegmental area, in vitro and in vivo. We further reveal that endogenous cholinergic control of dopamine release is not only mediated by nAChRs and modulated by mAChRs, but also results from actions at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Hence, our results show that endogenous cholinergic activity exerts a powerful influence on accumbal dopamine release and that this phenomenon recruits

a previously unrecognized convergence of neurotransmitter systems.

RESULTS

Endogenous Cholinergic Activity Elicits Terminal Dopamine Release

Studies of nAChR dynamics (desensitization or antagonism) have long suggested that endogenous cholinergic tone may establish the baseline for the probability of dopamine release in the striatum (Giorguieff et al., 1976; Zhou et al., 2001; Zhang and Sulzer, 2004; Threlfell et al., 2010). This phenomenon has a presynaptic locus of action, as studied by application of exogenous acetylcholine or ligands of the nAChR in striatal synaptosomes (Rapier et al., 1990; Wonnacott et al., 2000; Chéramy et al., 1996) or slices (Giorguieff et al., 1977; Wonnacott et al., 2000). However, it is not known whether selective activation of cholinergic interneurons and subsequent release of endogenous ACh can directly control dopamine release. To test this hypothesis, we utilized optogenetic techniques to selectively activate cholinergic interneurons in the NAc. Briefly, we injected an adeno-associated virus encoding channelrhodopsin2 (ChR2) and enhanced yellow fluorescent protein (EYFP) into the NAc of mice expressing Cre-recombinase downstream of the choline-acetyltransferase (ChAT) promoter (ChAT-Cre mice) (see Extended Experimental Procedures). Four weeks after viral injection, studies were performed in coronal slices of the NAc.

First, we tested the functionality of cholinergic interneurons in our in vitro preparation by performing whole-cell recordings. Under the current-clamp configuration, YFP-positive neurons (putative cholinergic interneurons) displayed the characteristic spontaneous, tonic firing at a rate of ~10 Hz [the average of the membrane potential value cycles between action potentials was −28 mV (Figure 1A)]. Under voltage clamp, delivery of a long (100 ms) or brief (4 ms) optical pulse elicited an inward current that lasted for the length of the pulse (n = 6, Figure 1A). Furthermore, delivery of a 4 ms blue light pulse under current clamp conditions induced a single action potential on YFP-positive cells (Figure 1A). Mean latency between the start of the light pulse and the start of the action potential was 5.0 ± 0.36 ms (n = 5, confirming that optical excitation of ChR2 reliably drives generation of action potentials on accumbal cholinergic interneurons). Additionally, histological analyses confirmed expression of ChR2-eYFP (Figure 1B; see also Figure S1).

To verify if selective stimulation of cholinergic interneurons is sufficient to evoke accumbal dopamine release, optical stimulation was delivered by a 125 μm optical fiber placed on the surface of the slice, while real-time changes in dopamine release were measured with fast-scan cyclic voltammetry using a glass-encased carbon fiber placed 100 μm away from the optical fiber (Figure 1C). Single pulse optical stimulation (4 ms duration square, 10 mW) in coronal slices of nucleus accumbens from ChR2-expressing ChAT-Cre mice elicited an immediate and robust increase in dopamine levels, which was comparable to single pulse electrical stimulation (400 μA; 4 ms duration square; 23 paired measurements made in 12 animals) (Figures 1D–1G). This result confirms that endogenous ACh actions on dopamine terminals are sufficient to elicit dopamine release in the NAc.

Frequency Dependence of Cholinergic Control of Dopamine Release

Because cholinergic receptor agonists modulate the release of dopamine, we hypothesized that changes in cholinergic interneuron firing rate should correspondingly enhance dopamine release. To test this, we measured dopamine following the application of different patterns of cholinergic interneuron optical stimulation. First, we monitored dopamine levels in response to paired pulse optical stimulations at different intervals. When two pulses of blue light (10 mW, 4 msec duration) were delivered at an interval of 2 s, the second pulse triggered a second peak of dopamine, which was 8.3 ± 1.6% (n = 4) of the first peak (Figure 2A). Subsequent tests showed lessened reduction in the second peak of 16 ± 2.1% at 5 s intervals (n = 4), 31.5 ± 3.1% at 10 s (n = 4), 66.0 ± 3.0% at 30 s (n = 4), and 84.75 ± 1.1% at 60 s interval (n = 4) (Figures 2A and 2B). This suggests that when the conditions for endogenous ACh release are met, a frequency-dependent limiting factor for ACh/dopamine release exists, most probably resulting from activation of dopamine or/and ACh auto-receptors. Next, we monitored dopamine levels following different patterns of optical stimulation to determine the functional range of cholinergic interneuron activity resulting in enhanced dopamine release. When compared to single pulse stimulation (n = 17), analysis of T_{80} values (see Extended Experimental Procedures) of dopamine concentration versus time curves showed a significant T_{80} increase following stimulation at 5 Hz ($p < 0.0001$, n = 15) and at 10 Hz ($p < 0.0001$, n = 16) but not at 30 Hz (n = 10) (Figure 2C, top). Dopamine peak values were also significantly different when triggered by 5 Hz ($p < 0.05$) and 10 Hz ($p < 0.001$), when compared to single pulse stimulation (Figure 2C, bottom). This suggests that cholinergic interneuron firing rates between ~5 Hz and10 Hz (frequencies well within the normal range of cholinergic interneuron firing) preferentially control release mechanisms. Application of tetrodotoxin completely abolished dopamine release triggered by optical activation of cholinergic interneurons with a single pulse or 10 Hz trains (n = 4; data not shown), confirming that the control of cholinergic interneuron activity over dopamine release is action potential-dependent.

Application of nicotine acts as a high-pass filter, facilitating burst release of dopamine (Exley and Cragg, 2008). Specifically, electrical stimulation frequencies $\leq$ 10 Hz elicit lower dopamine levels compared to control, while stimulation frequencies $\geq$ 25 Hz elicit higher dopamine levels (Rice and Cragg, 2004). We tested if this filtering phenomenon occurs following sustained cholinergic interneuron activation. To do this, we applied brief electrical stimulation at different frequencies, while long optical stimulation trains to cholinergic interneurons (50 pulses of 4 ms duration delivered at 5 Hz, 10 mW) were applied. Under these conditions, all of the tested protocols of electrical stimulation (1 pulse, 6 pulses at 30 Hz, and 6 pulses of 100 Hz) evoked lower peak levels of dopamine release, compared to peak levels obtained in absence of optical stimulation (17.6% at 1 pulse, $p < 0.0001$; 51.3% at 30 Hz, $p < 0.0001$, 74.1% at 100 Hz, $p > 0.05$; n = 3–6) (Figure 2D). Therefore, endogenous

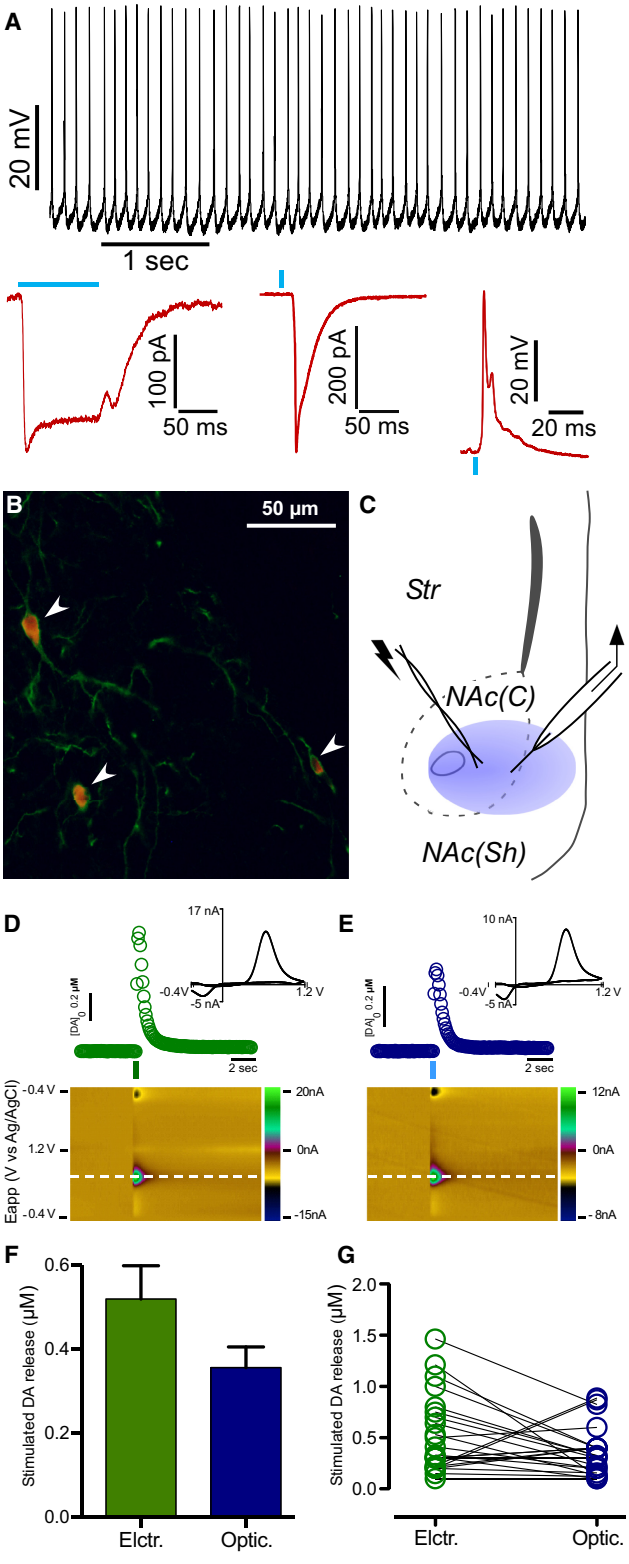

Figure 1. Selective Optical Stimulation of ChR2-Expressing Cholinergic Interneurons Elicits Accumbal Dopamine Release

(A) Top: Trace from a whole-cell recording of a YFP positive neuron [putative cholinergic interneuron]. YFP-positive neurons displayed spontaneous, tonic firing at a rate of ~10 Hz. Bottom: Under whole-cell voltage-clamp recording from a YFP-positive neuron, blue light exposure (100 ms, left or 4 ms, middle) induced a ChR2-mediated inward current. Under whole-cell current-clamp recordings of YFP-positive neurons, delivery of a blue light pulse (4 ms, right) induced firing of a single action potential.

(B) Image of eYFP-positive (green) cell bodies (arrowheads) counterstained for ChAT (red) and processes from the nucleus accumbens (NAc) of a ChAT-Cre mouse transfected with a ChR2-eYFP viral vector.

(C) Scheme of the recording arrangement from coronal NAc striatal slices. Dopamine levels were measured with voltammetry through a carbon fiber microelectrode (right) while performing electrical stimulation (left) and/or optical stimulation (blue circle) delivered by an optical fiber in apposition with the tissue.

(D) Concentration trace (top) and color plot (bottom) for dopamine release triggered by electrical stimulation of the NAc. Top: Representative trace shows concentration of dopamine (nM) over time in response to electrical stimulation (indicated by green line). Inset shows characteristic dopamine voltammogram. Bottom: Corresponding color plot depicts the voltammetric data with time on the x axis, applied scan potential (E_{app}) on the y axis and background-subtracted faradaic current shown on the z axis in pseudocolor. Dopamine can be identified by an oxidation peak (green) at +0.6V and a smaller reduction peak (yellow) at −0.2V.

(E) Concentration trace (top) and color plot (bottom) for dopamine release triggered by optical stimulation of cholinergic interneurons. Top: As in (D), representative trace shows concentration of dopamine (nM) over time in response to optical stimulation (indicated by blue line) Inset shows characteristic dopamine voltammogram. Bottom: Corresponding color plot of voltammetric data.

(F) Bar graph represents peak values of accumbal dopamine release obtained by electrical and optical stimulation.

(G) Dispersion plot indicating peak values for all the experiments performed under electrical (green circles) and optical stimulation (blue circles).

cholinergic activity under the present experimental conditions does not produce the high-pass filtering elicited by bath application of nicotine. To confirm whether cholinergic interneurons follow high-frequency patterns of optical stimulation, we performed intracellular electrophysiological recordings. Under whole-cell current clamp, optical stimulation of cholinergic interneurons at 10 Hz elicited a corresponding sequence of action potentials at 10 Hz (Figure 2E). However, optical stimulation at 30 Hz elicited a sequence of action potentials in which only the first action potential reached full amplitude (Figure 2F), suggesting that cholinergic interneurons functionally follow optical stimulation at a rate of ~10 Hz. However, cholinergic interneuron responsiveness is limited at higher stimulation frequencies (although this may also result from intrinsic ChR2 channel dynamics).

β2-Containing Nicotinic Receptors Mediate DA Release Evoked by Endogenous Cholinergic Activity

nAChRs are involved in the presynaptic control of dopamine release in the striatum (Hersch et al., 1994; Marshall et al., 2002; Rapier et al., 1990; Wonnacott et al., 2000). To confirm that cholinergic interneuron-driven enhancement of dopamine release is mediated by activation of nAChRs, we monitored dopamine levels while performing selective optical activation of cholinergic interneurons in the absence and presence of nAChR antagonists. Mecamylamine (n = 6) decreased cholinergic interneuron stimulation-induced dopamine release with a half maximal inhibitory concentration (IC_{50}) of 0.61 µM (Figure 3A). The highest dose used (20 µM) decreased dopamine levels by 85.8 ± 3% compared to pre-drug conditions.

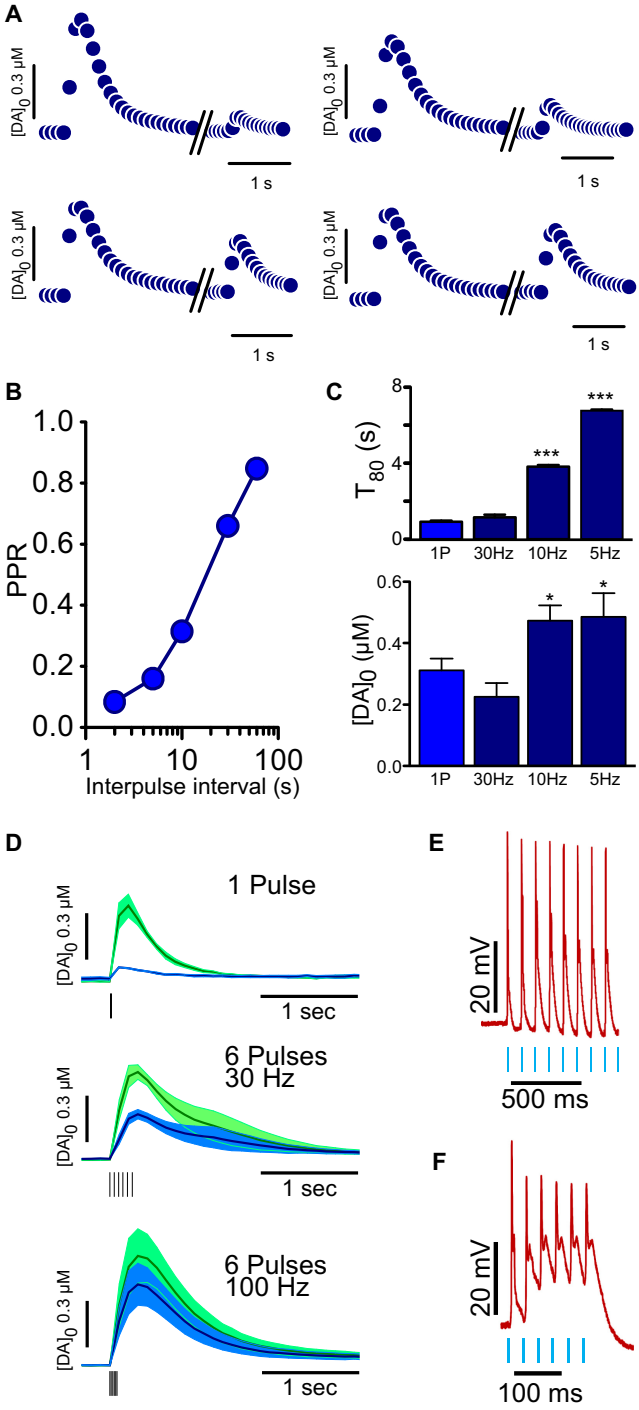

Figure 2. Frequency-Dependent Response of Dopamine Release Evoked by Stimulation of Cholinergic Interneurons

(A) Average traces of dopamine levels evoked by paired pulses of optical stimulation of cholinergic interneurons delivered at 5, 10, 30, and 60 s intervals.

(B) Summary plot of dopamine peak amplitudes in response to paired pulse ratio stimulation of cholinergic interneurons.

(C) Top: Summary bar graph showing the average T_{80} decay values for dopamine release evoked by 5, 10, 30 Hz and single pulse stimulation. Bottom: Summary graph of peak values of dopamine release evoked by 5, 10, 30 Hz and single pulse stimulation.

(D) Average traces of dopamine levels triggered by different patterns of electrical stimulation, while sustained optical stimulation of cholinergic interneurons was being performed.

(E) Representative traces from whole-cell recordings under current clamp of cholinergic interneurons showing responsiveness to 10 Hz optical stimulation.

(F) As in (E), representative trace depicting cholinergic interneuron responsiveness to 30 Hz optical stimulation.

Error shadows or bars represent SEM.

In mammals, combinations of nAChR subunits from subfamilies II (α7) and III (α2–α6, β2–β4) result in the formation of functional heteropentamers and homopentamers (Le Novère et al., 2002). In striatal dopamine terminals, heteropentamers display two α/β pairs in the form of α4/β2 and/ or α6/β2 and/or α4/β4 (Champtiaux et al., 2003). Nicotinic control of striatal dopamine release depends on β2 subunit-containing nAChRs (Zhou et al., 2001; Exley et al., 2012). To verify the role of β2-containing receptors in the augmentation of accumbal dopamine release by selective activation of cholinergic interneurons, we examined the effect of the β2-containing nAChR antagonist dihydro-β-erythroidine (DHβE). Application of DHβE (1 μM) resulted in a potent reduction of dopamine peak levels elicited by optical stimulation (89.05% relative to pretreatment values; $p < 0.0001$; $n = 4$) (Figure 3B).

Glutamate Acting at AMPA Receptors Mediates Dopamine Release Evoked by Selective Activation of Cholinergic Interneurons

Glutamate receptors also control dopamine release presynaptically (Desce et al., 1992; Krebs et al., 1991; Chéramy et al., 1986a, 1996, 1998) and cholinergic interneurons have recently been shown to release glutamate (Higley et al., 2011; Guzman et al., 2011). Moreover, several effects evoked by cholinergic interneuron activity are thought to be mediated by glutamate and not by ACh (Guzman et al., 2011). Because of our findings that endogenous cholinergic activity drives dopamine release in a nAChR-dependent fashion, we next examined if this occurred, at least in part, through activation of AMPA receptors. First, we confirmed that stimulation of cholinergic interneurons led to glutamate release. Under whole-cell voltage-clamp, single pulse optical stimulation of cholinergic interneurons evoked a single excitatory postsynaptic potential in medium spiny neurons (Figure 3C). Furthermore, optical train stimulation failed to produce subsequent excitatory postsynaptic potentials following the initial pulse in the trains (5 pulses at 2 or 30 Hz; see Figure S2). Bath application of the AMPA receptor antagonist 2, 3-dihydroxy-6-nitro-7-sulfamoylbenzo (F) quinoxaline (NBQX) (5 μM) attenuated the excitatory postsynaptic potential amplitude to 18.6% ± 3.4% of the pretreatment value ($p = 0.0002$, $n = 4$). This confirms that selective optical stimulation of cholinergic interneurons drives AMPA receptor activation on medium spiny neurons (Higley et al., 2011). Next, we monitored dopamine release changes triggered by selective cholinergic interneuron optical stimulation following NBQX. When added to the bath solution, NBQX (5 μM) significantly decreased the peak amplitude of dopamine release by 40.3% ($p < 0.01$, $n = 7$) (Figure 3D), confirming that AMPA receptors contribute

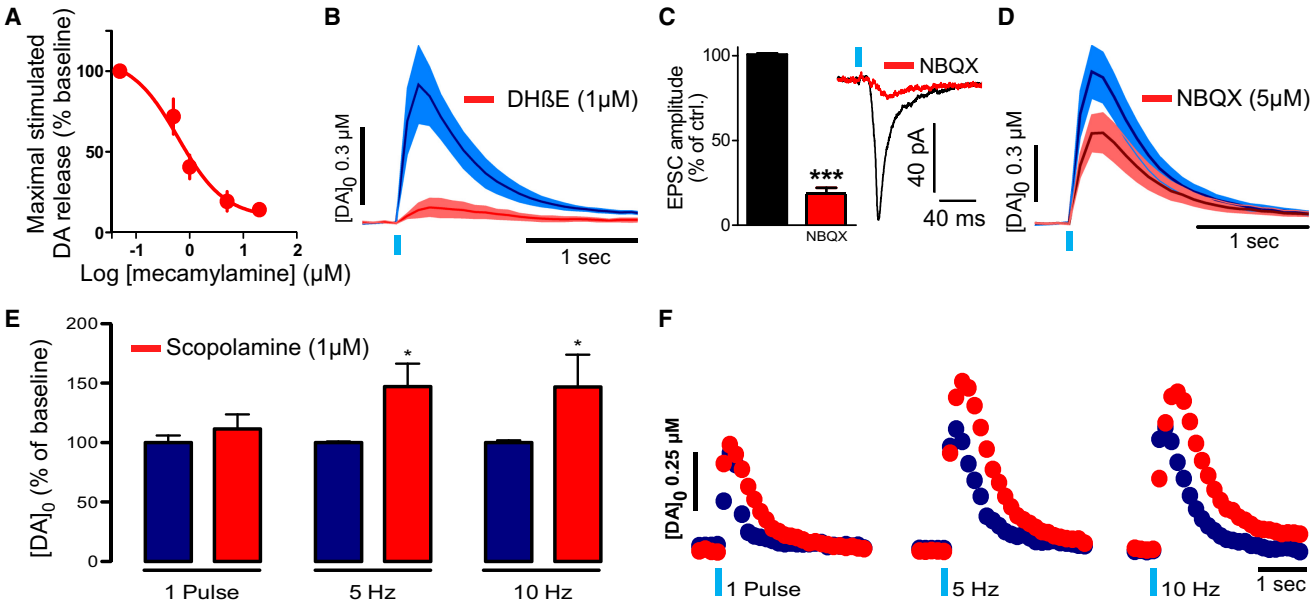

Figure 3. Modulation of Dopamine Levels Evoked by Endogenous Cholinergic Activity

(A) Concentration-response plot showing the effect of increasing concentrations of the nAChR antagonist mecamylamine on dopamine peak levels evoked by single pulses of optical stimulation. Inhibitory concentration (IC) = 0.61 μM.

(B) Effect of the β2 subunit nAChR antagonist DHβE (1 μM) on dopamine levels evoked by single pulse (left) and train (right) optical stimulation of cholinergic interneurons (CINs).

(C) Trace of an excitatory postsynaptic potential (EPSC) from a medium spiny neuron under voltage clamp, elicited by single pulse optical stimulation of CINs. Effect of NBQX (5 μM) on the medium spiny neuron EPSC.

(D) Effect of the application of the AMPA receptor antagonist NBQX (5 μM) on dopamine levels evoked by single pulse optical stimulation of CINs.

(E) Summary bar graph showing the effect of the mAChR antagonist scopolamine (1 μM) on dopamine peak levels evoked by single pulse, 5 Hz and 10 Hz optical stimulation of cholinergic interneurons, compared to pretreatment.

(F) Representative traces of dopamine concentration transients triggered by single pulse, 5 Hz and 10 Hz optical stimulation of cholinergic interneurons in the presence of scopolamine, compared to pretreatment.

Error shadows or bars represent SEM.

to the control of dopamine release evoked by cholinergic interneuron activation in the NAc.

mAChR Receptor Activation Modulates Cholinergic-Driven Release of Dopamine

mAChRs are also present in the striatum (Hersch et al., 1994) and the mAChR agonist oxotremorine decreases dopamine release evoked by single pulse electrical stimulation, but increases release evoked by high frequencies (Threlfell et al., 2010). Furthermore, genetic deletion of the muscarinic M4 subunit prevents this modulation of dopamine release in the NAc (Threlfell et al., 2010). However, in contrast to nAChRs, there is no evidence of M4 expression on dopamine terminals, suggesting that mAChR activation preferentially inhibits ACh release in the NAc (Threlfell et al., 2010; Threlfell and Cragg, 2011).

To identify the role of mAChRs on dopamine release evoked by endogenous cholinergic activity, we next tested the effect of the mAChR antagonist scopolamine on dopamine levels triggered by selective stimulation of cholinergic interneurons. When scopolamine (1 μM) was applied to the bath, dopamine peak levels evoked by a single pulse of selective cholinergic interneuron stimulation only modestly increased to 112% of pretreatment values; $p = 0.38$. When cholinergic interneurons were optically stimulated by a train of 30 pulses at 10 or 5 Hz (10 mW, 4 msec duration per pulse), peak dopamine levels significantly increased to 146%–148% ($p < 0.05$) of pretreatment values ($n = 6–12$) (Figures 3E and 3F), suggesting that blocking mAChRs relieves the inhibition of ACh release and that this effect is preferentially seen when trains of action potentials drive mobilization of the ACh releasable pool.

In Vivo Selective Stimulation of Accumbal Cholinergic Interneurons Induces Dopamine Release

The present results suggest that termino-terminal endogenous cholinergic activity controls dopamine release and that this involves activation of glutamate receptors in vitro. However, it is not known if promoting dopamine release by selective activation of cholinergic interneurons occurs in vivo. To test this possibility, fast-scan cyclic voltammetry recordings combined with optical stimulation from adjacent sites (200 μm separation) were performed by implantation of an optical fiber/carbon fiber arrangement (optrode, see Extended Experimental Procedures; Figure 4A) into the NAc of urethane-anesthetized mice. Because under these recording conditions the recording electrode cannot be optimally placed in the area of highest fluorescence under visual control, the conditions necessary to obtain cholinergic

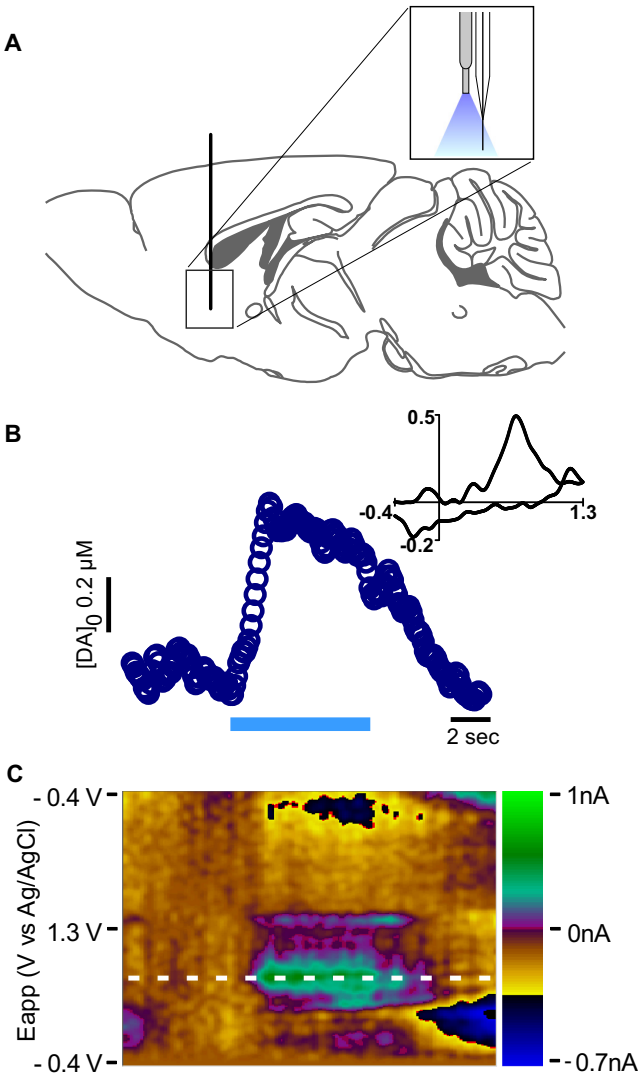

Figure 4. In Vivo Selective Stimulation of Cholinergic Interneurons Evokes Accumbal Dopamine Release

(A) Scheme depicting implantation of the optrode (optical fiber/carbon fiber arrangement) used to optically stimulate and record from a contiguous area in the NAc in vivo.

(B) Concentration trace for dopamine release triggered by a 7-s-long (20 Hz, 150 pulses, 10 mW) optical stimulation of the NAc. Representative trace shows concentration of dopamine (nM) over time in response to optical stimulation (indicated by the blue line). Inset shows characteristic dopamine voltammogram.

(C) Corresponding color plot depicts the voltammetric data with time on the x axis, applied scan potential (E_{app}) on the y axis and background-subtracted faradaic current shown on the z axis in pseudocolor.

interneuron-evoked release were different from those used in the slice. Optical stimulation was reliably achieved by delivery of a train of blue light (473 nm wavelength, 10 mW, 4 msec duration/pulse, 150 pulses, 20 Hz) through the optical fiber of the implanted optrode. Selective activation of accumbal cholinergic interneurons triggered a significant increase in dopamine concentration (18.2 ± 2.1 nM), with respect to baseline values (n = 3) (Figures 4B and 4C), providing unambiguous in vivo evidence that cholinergic interneuron activity locally enhances dopamine release in the NAc.

DISCUSSION

Cholinergic receptor activation potently controls striatal levels of dopamine, a neuromodulator crucial for the expression of coordinated motor activity and Pavlovian cue-reward associations (reviewed by Wise, 2004; Sulzer, 2011). In this report, we characterize the effects of selective cholinergic interneuron activation on accumbal dopamine levels. We find that, while having a relatively sparse distribution, cholinergic interneurons profoundly modulate dopaminergic output in NAc.

We show that selective optogenetic stimulation of cholinergic interneurons evokes dopamine release in a β2-containing nAChR-dependent manner. While electrophysiological studies have hypothesized that dopamine can be released in a manner that is not contingent upon ongoing activity in dopaminergic fibers (Ding et al., 2010), our data reveal previously unseen dynamics of this release process directly. Furthermore, we identify the convergence of different neurotransmitter systems participating in this phenomenon. Increased dopamine concentration during blockade of mAChRs suggests a critical role of these receptors in controlling ACh release. Consistent with recent reports demonstrating glutamate release from cholinergic interneuron terminals, interfering with AMPA receptor signaling weakens optically evoked dopamine release. More importantly, we determine that dopamine release can also be evoked by blue light activation of cholinergic interneurons in vivo.

Study of frequency-dependent relations between cholinergic interneuron stimulation and dopamine levels showed a clear paired-pulse depression, suggesting strong mechanisms of presynaptic control of release at either or both cholinergic interneuron and dopamine neuron terminals. Although this has been described separately at dopamine and ACh synapses, more detailed studies are necessary to demonstrate how interactions between these two sites of release interact to determine final dopamine levels. Moreover, we report that sustained optical stimulation of cholinergic interneurons does not mimic the nicotine-dependent high-pass filtering of electrically evoked dopamine release (Exley and Cragg, 2008). Together, these results point to a crucial role of mAChR activation in limiting the effects of persistent endogenous ACh activity on nAChRs. This feedback mechanism is absent under the effect of nicotine, which promotes desensitization of nAChRs, thought to be the main mechanism underlying nicotine-evoked high-pass filtering of dopamine release (Rice and Cragg, 2004; Exley and Cragg, 2008). In support of this notion, we confirmed that β2-containing nAChRs mediate ACh-evoked release of dopamine, and that mAChRs play a predominant role in limiting endogenous ACh release, because ACh-evoked dopamine release is enhanced (albeit modestly) following blockade of mAChRs.

Glutamate modulates dopamine release by acting on dopaminergic terminals (Chéramy et al., 1986b; Krebs et al., 1991; Chéramy et al., 1996, 1998), and because cholinergic interneurons mediate glutamatergic transmission (Guzman et al., 2011;

Higley et al., 2011), we hypothesized that a fraction of the dopamine released by selective stimulation of cholinergic interneurons involves activation of glutamate receptors. Supporting this view, we found that cholinergic interneuron-evoked dopamine release relies—at least partially—on activation of AMPA receptors. This establishes even broader implications, given that glutamate released from cholinergic interneurons mediates not only excitation of medium spiny neurons, as previously described (Higley et al., 2011), but also shapes accumbal dopamine release.

The present experiments uncover a multiplicity of regulatory mechanisms that converge to control dopamine release elicited by the selective activation of cholinergic interneurons. In behaving animals, cholinergic interneurons encode reward-related events (Morris et al., 2004). While dopamine neurons increase or decrease their basal firing rate in response to the presentation or omission of reward, cholinergic interneurons respond with a brief pause independently of the outcome (Aosaki et al., 1994; Morris et al., 2004). This has been interpreted as the establishment of the appropriate temporal window for contingencies to be encoded, while dopaminergic responses are theorized to carry a learning signal about future outcomes (Morris et al., 2004). Here, we determined that in vivo dopamine release is, in fact, triggered by endogenous release of ACh. This allows new considerations to be taken into account for the way that cholinergic interneuron activity may set the stage for dopamine neuron activity to produce its postsynaptic effects. Reward-related activity of cholinergic interneurons consists of several phases (initial rise, pause, and second rise) (Morris et al., 2004; Aosaki et al., 1994; Shimo and Hikosaka, 2001; Apicella et al., 1991, 2011; Apicella, 2007). In response to reward, the peak of the initial phase coincides with the rise in dopamine neuron activity. We speculate that the initial rise phase of cholinergic interneuron firing rate and subsequent ACh-Glu release could act as a priming event, exciting medium spiny neurons and boosting dopamine release originating from the midbrain, whereas the transition to the pause in cholinergic interneuron activity may allow for the hypothesized contrast enhancement of the midbrain signal (Zhang and Sulzer, 2004; Cragg, 2006; Nicola et al., 2004). Moreover, activation of nAChRs promotes long-term depression of corticostriatal glutamatergic transmission via regulation of dopamine release (Partridge et al., 2002), and thus our findings provide evidence of a link between cholinergic interneuron activity and synaptic plasticity implicated in reinforcement learning. Our results generate another conceptual framework with which to interpret the regulation of accumbal dopamine release and its role in reward-directed behaviors.

EXPERIMENTAL PROCEDURES

Subjects

Male ChAT-Cre mice were used. They were single-housed in a room under a 12 hr light/dark cycle and food/water was available ad libitum.

Stereotaxic Virus Injection

An AAV-ChR2 vector was injected bilaterally (500 nl/side) into the nucleus accumbens of mice that were allowed to recover for 4 to 8 weeks after virus injection, before any subsequent intervention.

Histology

At least 4 weeks after viral injection, mice were perfused transcardially with 4% paraformaldehyde. Brains were extracted, sliced, and processed with an anti-ChAT polyclonal antibody and a fluorescein-conjugated secondary antibody. Sections were then mounted and visualized by epifluorescence with a Leica DM LB microscope to identify ChAT-labeled and eYFP-positive neurons.

In Vitro Optical Stimulation and Fast-Scan Cyclic Voltammetry

Four weeks after virus injection, mice were killed by decapitation, and the brain was quickly removed and incubated in modified Krebs buffer. Coronal slices containing the nucleus accumbens (250 μm thick) were obtained, incubated, and then transferred to the recording chamber, perfused at 1 ml/min with 34°C oxygenated Krebs buffer.

Using a cylindrical carbon fiber, voltammetric recordings (versus an Ag/AgCl reference electrode) were performed using Demon Voltammetry and Analysis Software. Optical and electrical stimulations were delivered through an optical fiber in apposition with the brain slice and through a bipolar tungsten electrode in contact with the slice, respectively.

Whole-Cell Electrophysiological Recordings

After incubation, brain striatal slices were transferred to the recording chamber, superfused with artificial cerebrospinal fluid, and maintained at 30 ± 1°C. Current- and voltage-clamp recordings of cholinergic interneurons were performed in unmodified artificial cerebral spinal fluid while voltage clamp recordings of medium spiny neuron excitatory postsynaptic potentials were performed in artificial cerebral spinal fluid containing 50 μM picrotoxin to block $GABA_A$-mediated currents. Optical stimulation was delivered via the epifluorescence light path. Clampex 10.3 software was used for data acquisition.

In Vivo Optical Stimulation and Fast-Scan Cyclic Voltammetry

Four weeks after virus injection, access to the nucleus accumbens was obtained under stereotaxic surgery. An optrode (carbon fiber/optical fiber) was implanted and voltammetric recordings (versus an Ag/AgCl reference electrode) performed using Tar Heel CV software.

Statistical Analysis

Unless otherwise indicated, two-way ANOVA followed by Bonferroni corrections was performed using Prism (GraphPad Software, La Jolla, CA, USA).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and two figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.05.011.

LICENSING INFORMATION

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