Inhibition of Nigrostriatal Dopamine Release by Striatal GABA<sub>A</sub> and GABA<sub>B</sub> Receptors

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Nigrostriatal dopamine (DA) is critical to action selection and learning. Axonal DA release is locally influenced by striatal neurotransmitters. Striatal neurons are principally GABAergic projection neurons and interneurons, and a small minority of other neurons are cholinergic interneurons (ChIs). ChIs strongly gate striatal DA release via nicotinic receptors (nAChRs) identified on DA axons. Striatal GABA is thought to modulate DA, but GABA receptors have not been documented conclusively on DA axons. However, ChIs express GABA receptors and are therefore candidates for potential mediators of GABA regulation of DA. We addressed whether striatal GABA and its receptors can modulate DA release directly, independently from ChI regulation, by detecting DA in striatal slices from male mice using fast-scan cyclic voltammetry in the absence of nAChR activation. DA release evoked by single electrical pulses in the presence of the nAChR antagonist dihydro-β-erythroidine was reduced by GABA or agonists of GABAA or GABAB receptors, with effects prevented by selective GABA receptor antagonists. GABA agonists slightly modified the frequency sensitivity of DA release during short stimulus trains. GABA agonists also suppressed DA release evoked by optogenetic stimulation of DA axons. Furthermore, antagonists of GABAA and GABAB<sub>B</sub> receptors together, or GABAB<sub>B</sub> receptors alone, significantly enhanced DA release evoked by either optogenetic or electrical stimuli. These results indicate that striatal GABA can inhibit DA release through GABAA and GABAB<sub>B</sub> receptors and that these actions are not mediated by cholinergic circuits. Furthermore, these data reveal that there is a tonic inhibition of DA release by striatal GABA operating through predominantly GABAB<sub>B</sub> receptors.

Key words: dopamine; GABA; presynaptic; striatum; voltammetry

Significance Statement
The principal inhibitory transmitter in the mammalian striatum, GABA, is thought to modulate striatal dopamine (DA) release, but definitive evidence for GABA receptors on DA axons is lacking. Striatal cholinergic interneurons regulate DA release via axonal nicotinic receptors (nAChRs) and also express GABA receptors, but they have not been eliminated as potentially critical mediators of DA regulation by GABA. Here, we found that GABAA<sub>A</sub> and GABAB<sub>B</sub> receptors inhibit DA release without requiring cholinergic interneurons. Furthermore, ambient levels of GABA inhibited DA release predominantly through GABAB<sub>B</sub> receptors. These findings provide further support for direct inhibition of DA release by GABA receptors and reveal that striatal GABA operates a tonic inhibition on DA output that could critically influence striatal output.

Introduction
The striatum plays key roles in promoting motivated behaviors and learned actions. Nigrostriatal dopamine (DA) neurons release DA from immensely arborized structures, with each neuron forming ~10<sup>5</sup> en passant varicosities and reaching ~2.7% of striatum in rat (Matsuda et al., 2009). DA output is gated by numerous striatal neuromodulators (Sulzer et al., 2016). Cholinergic interneurons (ChIs) play a particularly powerful role in gating and driving DA release through nicotinic acetylcholine receptors (nAChRs) on DA axons (Jones et al., 2001; Zhou et al., 2001; Rice and Cragg, 2004; Zhang and Sulzer, 2004; Thrrell et al., 2012). ChIs make up 1–2% of striatal neurons (Oorschot, 2001).
Striatal GABAergic interneurons include the principal spiny projection neurons (SPNs) (~95%) as well as interneurons (~2–3%) including fast-spiking interneurons (FSIs) and low-threshold spiking interneurons (LTS), among others (Gittis and Kreitzer, 2012). Furthermore, DA axons might provide a source of co-released GABA (Tritsch et al., 2012, 2014; Kim et al., 2015). Striatal GABA<sub>A</sub> and GABA<sub>B</sub> receptors have been shown to modulate DA release, but information about their localization to DA axons is lacking. A role for ChIs as potential mediators of GABA regulation of DA has not been excluded.

Striatal administration of pregnanolone, a positive allosteric modulator of the GABA<sub>A</sub> receptor, or biccuculline, a GABA<sub>A</sub> antagonist, respectively decreased and increased extracellular dopamine in intact rats measured by microdialysis (Smolders et al., 1995) and muscimol, a GABA<sub>A</sub> agonist, inhibited DA release from striatal synaptosomes (Ronken et al., 1993). A GABA<sub>A</sub>-mediated enhancement of DA release has been reported in guinea pig striatal slices during prolonged electrical pulse trains, but this is thought to arise indirectly via inhibition of H<sub>2</sub>O<sub>2</sub> release from striatal neurons during prolonged stimuli (Avshalumov et al., 2003). Striatal perfusion of the GABA<sub>B</sub> agonist baclofen or the antagonist phaclofen respectively decreased and increased extra-cellular dopamine in intact rats (Smolders et al., 1995) and baclofen decreased electrically evoked DA release in acute slices of mouse caudate putamen (Schmitz et al., 2002) and nucleus accumbens (Pitman et al., 2014). Findings in slice preparations are less confounded by potential effects on long loop circuits in vivo that could regulate DA via changes in DA neuron firing and therefore more directly support a local mechanism of action. However, whereas both GABA<sub>A</sub> and GABA<sub>B</sub> receptors are densely expressed throughout striatum (Ng and Yung, 2000; Waldvogel et al., 2004), only the GABA<sub>B</sub> receptor has been indicated on structures that resemble DA axons. Ultrastructural studies report GABA<sub>B</sub> receptors in striatal neuropil in monkey and rat (Charara et al., 2000; Yung et al., 1999), which is consistent with, but not definitive evidence for, GABA<sub>B</sub> receptors on DA axons.

Both GABA<sub>A</sub> and GABA<sub>B</sub> receptors are present on ChIs (Waldvogel et al., 1998; Yung et al., 1999). Because ChIs operate strong control over DA release and can mediate effects of other neuromodulators on DA, including opioids, nitric oxide, glutamate, and insulin (Britt and McGehee, 2008; Hartung et al., 2011; Stouffer et al., 2015; Kosillo et al., 2016), ChIs emerge as the potential mediators of GABAergic inhibition of DA release. ChIs need to be excluded as potential mediators before direct regulation of DA by GABA<sub>A</sub> and/or GABA<sub>B</sub> receptors seems more plausible.

Here, we explored the control of DA release by GABA<sub>A</sub>, GABA<sub>B</sub>, receptors, and GABA<sub>B</sub> receptors in the absence of ACh input to nACHRs. We assessed GABA receptor regulation of DA release when evoked electrically in the presence of nAChR antagonist dihydro-β-erythroidine (DHβE) and also when DA release was evoked by targeted activation of DA axons using optogenetics when DA release is not under nAChR control (Threlfell et al., 2012; Melchior et al., 2015). We found that GABA<sub>A</sub> and GABA<sub>B</sub> receptors can inhibit DA release independently from nAChR activation, providing further support for a direct mechanism of action. Furthermore, we found that endogenous striatal GABA provides a tonic inhibition of DA release.

Materials and Methods

Animal preparation and surgery. Animals were either wild-type C57BL6 male mice at postnatal day 35 (P35) to P70 (RRID:IMSR_JAX:000664) or DAT-Cre mice injected with an adeno-associated virus encoding Cre-dependent ChR2. For experiments with light activation, DAT-Cre mice were bred from homozygous for DAT-internal ribosome entry site (IRES)-Cre, obtained from Jackson Laboratories (B6SJLN-Slc6a3<sup>tm1(cre)klom/J</sup>, stock 006660) (RRID:IMSR_JAX:006660). P25–P35 DAT-Cre mice were anesthetized with isoflurane, placed in a small animal stereotaxic frame (David Kopf Instruments), and injected with an adeno-associated virus (~10<sup>12</sup> genome copies per milliliter; UNC Vector Core Facility, Chapel Hill, NC) encoding Cre-dependent ChR2 (AAV5-EF1α-DIO-hChR2(H134R)-eYFP) (RRID:SCR_002448). A total volume of 1 μl of virus solution was injected bilaterally (500 nl per hemisphere/injection) into substantia nigra pars compacta (SNc, AP: −3.1 mm, ML ±1.2 mm from bregma, DV: −4.25 mm from dura mater). Virus solution was injected at an infusion rate of 50 nl/min with a 32 gauge Hamilton syringe and withdrawn 5–10 min after the end of injection. Virus-injected mice were used for experiments >4 weeks after viral injection.

Slice preparation. Wild-type C57BL6 animals (P35–P70) or DAT-Cre mice (P53–P63) were decapitated after cervical dislocation and the brains were extracted. Coronal slices, 300 μm thick, were prepared in ice-cold HEPES-buﬀered artiﬁcial CSF (aCSF) containing the following (in mM): 120 NaCl, 20 NaHCO<sub>3</sub>, 6.7 HEPES acid, 5 KCl, 2 CaCl<sub>2</sub>, 2 MgSO<sub>4</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, and 10 glucose. Slices were then maintained in HEPES-buﬀered artiﬁcial CSF at room temperature for at least 1 h before recording. All procedures were performed according to institutional guidelines and conformed to the UK Animals (Scientiﬁc Procedures) Act of 1986.

Fast-scan cyclic voltammetry. Evoked DA release was detected in acute slices using fast-scan cyclic voltammetry (FCV). Slices were superfused with a bicarbonate-buﬀered aCSF saturated with 95% O<sub>2</sub>/ 5% CO<sub>2</sub> at 31−32°C containing the following (in mM): 124 NaCl, 26 NaHCO<sub>3</sub>, 3.8 KCl, 2.4 CaCl<sub>2</sub>, 1.3 MgSO<sub>4</sub>, 1.3 KH<sub>2</sub>PO<sub>4</sub>, and 10 glucose. All experiments with electrical stimulation were conducted in the presence of 1 μM DHβE to prevent the effects of nAChR activation on DA release (Zhou et al., 2001; Rice and Cragg, 2004). Extracellular DA concentration ([DA<sub>e</sub>]) was monitored using FCV with 7-μm-diameter carbon fiber microelectrodes (tip length 50–100 μm) and a Millar voltammeter (Julian Millar, Barts and the London School of Medicine and Dentistry) as described previously (Threlfell et al., 2010). In brief, the scanning voltage was a triangular waveform (~0.7 V to +1.3 V range versus Ag/AgCl) at a scan rate of 800 V/s and sampling frequency of 8 Hz. Signals were attributable to DA by the potentials for peak oxidation and reduction currents (oxidation peak: +500−600 mV, reduction peak: ~−200 mV). Electrodes were calibrated post hoc with 2 μM DA in experimental medium. None of the drugs altered electrode sensitivity to DA. Data were acquired and analyzed using Axoscope 10.6 (Molecular Devices) and locally written Excel macros.

Mean ± SEM. n refers to the number of experiments. Each experiment was repeated at least three times (n ≥ 3). Statistical analysis was performed using one-way ANOVA followed by Tukey post hoc tests (Threlfall et al., 2010).

Optical stimulation. Optimal wavelengths for light stimulation were determined using a Maxspectry 6400 (Molecular Devices) as described previously (Threlfall et al., 2010). The initial wavelengths determined for light stimulation were 470 nm and 532 nm. To determine the optimal wavelength for light stimulation, slices were illuminated with 470 nm blue light at ~500 mW. A GrabiScope microscope (Olympus BX50WI) with fluorescence optics for visualizing eYFP.
periment was performed at a single recording site in one brain slice. For each experiment at a given recording site, data for each variable were obtained in at least triplicate before averaging to obtain the value for that individual parameter. The number of animals in each dataset was \( \geq 3 \). Data are expressed as extracellular concentration of dopamine ([DA]) or as [DA] normalized to mean peak [DA] evoked by single pulses in control conditions. In all cases, [DA] displayed typical kinetics to peak and decay, indicative of good slice quality and were obtained from recording sites that maintained sufficiently stable levels of release over time. No data were excluded after acquisition.

Data acquired immediately before drug application were used as predrug control data and were compared with data acquired after drug effects had equilibrated after \(-10–20\) min of application. Ratios for [DA] evoked by 5p/1p were obtained by dividing each 5p-evoked [DA] value with an average 1p-evoked [DA] value in the same condition at that recording site. In graphs representing peak [DA] evoked by 1p over time, missing data points were time points when data were acquired using different stimulation parameters interspersed among 1p stimulations. Comparisons for statistical significance were assessed by one- or two-way ANOVA, paired \( t \) tests, or Mann–Whitney \( U \) tests where data were not normally distributed using GraphPad Prism (RRID:SCR_002798).

Drugs. DHBE, saclofen, and biccuculline were obtained from Tocris Bioscience. Baclofen, picrotoxin, and GABA were obtained from Sigma-Aldrich. Muscimol and CGP 53845 hydrochloride were obtained from Abcam. Stock aliquots of drugs were prepared at \( 800–10,000 \times \) final concentrations in deionized water, aqueous acid (bacclofen), or DMSO (picrotoxin) and stored at \(-20^\circ \)C. DHBE was present throughout all experiments with electrical stimulation.

Results

GABA\(_A\) and GABA\(_B\) receptors inhibit striatal DA release

We assessed whether GABA can modulate striatal DA release in the absence of nAChR activity using the antagonist DHBE (1 \( \mu \)M) to inhibit nAChRs as described previously (Rice and Cragg, 2004; Threlfell et al., 2012). We first confirmed that the DHBE concentration was supramaximal for inhibition of DA release by confirming the concentration–response relationship for DHBE (1 \( \mu \)M to 1 \( \mu \)M) on [DA] evoked by a single electrical pulse. DHBE concentration–dependently inhibited evoked [DA], (Fig. 1A; sigmoidal concentration–response curves, \( R^2 = 0.93 \); Hill slope, \(-1.92, I_{50} = 11 \) nm, \( n = 9 \)) and 1 \( \mu \)M DHBE was supramaximal for inhibition of [DA].

GABA was applied to striatal slices in the presence of the nAChR antagonist DHBE (1 \( \mu \)M). Bath application of GABA (100 \( \mu \)M, 5 \( \mu \)M) concentration dependently reduced DA release evoked by single electrical pulses by \(-15–50\%\) (Fig. 1B,C; two-way ANOVA: \( F_{(1,10)} = 48.32, p < 0.0001, 100 \mu \)M GABA, \( U = 0, p = 0.016, n = 7; U = 6, 5 \mu \)M, \( p = 0.0079, n = 5 \), Mann–Whitney tests), indicating that GABA is capable of inhibiting DA release independently from any effects on ChI input to nAChRs.

To identify which GABA receptors can inhibit DA release, we tested the effect of GABA\(_A\) or GABA\(_B\) agonists. Activation of GABA\(_A\) receptors with muscimol (20 \( \mu \)M) suppressed DA release evoked by single electrical pulses by \(-30\%\) (Fig. 1D,E; \( U = 0, p = 0.0079, n = 5 \), Mann–Whitney test) and this effect was prevented by prior application of GABA\(_A\) channel blocker picrotoxin (100 \( \mu \)M), confirming an effect via GABA\(_A\) receptors (Fig. 1F; \( U = 11, p = 0.85, n = 5 \), Mann–Whitney test). Activation of GABA\(_B\) receptors with baclofen (10 \( \mu \)M) also suppressed DA release evoked by single electrical pulses by \(-25\%\) (Fig. 1H,I; \( U = 0, p = 0.0006, n = 7 \), Mann–Whitney test) and this effect was confirmed to be due to GABA\(_B\) receptors by prior application of the GABA\(_B\) antagonist saclofen (100 \( \mu \)M), which prevented the effect of baclofen (Fig. 1J,K; \( U = 5, p = 0.11, n = 5 \), Mann–Whitney test).

GABA receptors on frequency sensitivity of DA release

We investigated whether GABA receptor activation inhibited DA release during trains of stimuli and across a range of frequencies. Muscimol significantly decreased [DA] evoked by 5-pulse trains at all frequencies tested (Fig. 2A,B; two-way ANOVA: drug effect: \( F_{(3,48)} = 67.55, p = 0.0025 \), frequency effect: \( F_{(3,48)} = 259.6, p < 0.0001, n = 7 \)) and, furthermore, there was a significant interaction between stimulation frequency and drug effect (Fig. 2B; two-way ANOVA: \( F_{(3,48)} = 5.485, p = 0.0025 \)), which was borne out by an increase in the 5p/1p ratio examined at 100 Hz (Fig. 3C; paired \( t \) tests: \( t_{(48)} = 3.09, p = 0.014 \). In other words, activation of GABA\(_A\) receptors can marginally promote the contrast in DA signals released by different firing patterns or rate.

Baclofen significantly decreased evoked [DA] (Fig. 2E; two-way ANOVA: \( F_{(3,48)} = 6.271, p = 0.016 \); frequency effect: \( F_{(3,48)} = 144.5, p < 0.0001, n = 7 \)). We did not detect a...
significant interaction between stimulation frequency and drug effect (Fig. 2E; two-way ANOVA: $F_{(3,48)} = 1.58, p = 0.215$; effects of frequency: $F_{(5,240)} = 128.4, p < 0.0001, n = 5$) or interact with stimulation frequency (Fig. 4C: two-way ANOVA: $F_{(3,44)} = 0.115, p = 0.95$ or the 5p:1p ratio (Fig. 4H: $t_{(27)} = 1.217, p = 0.263$, paired $t$ test). The GABA<sub>A</sub> antagonist saclofen significantly increased evoked [DA]<sub>n</sub> (Fig. 4E,F,F; two-way ANOVA: effect of drug: $F_{(1,24)} = 4.87, p = 0.0571$; effects of frequency, $F_{(1,24)} = 57.8, p < 0.0001$). There was no significant interaction with stimulation frequency (Fig. 4C: two-way ANOVA: $F_{(3,24)} = 0.205, p = 0.89$) or 5p:1p ratio (Fig. 4F, $t_{(3)} = 1.49, p = 0.233$, paired $t$ test).

**Discussion**

Here, we show that GABA, GABA<sub>A</sub> receptors, and GABA<sub>B</sub> receptors in dorsal striatum inhibit DA release and that these actions are not mediated via regulation of striatal ACh acting at nAChRs. By eliminating ChIs as a necessary site of action of GABA, a direct action on DA axons becomes plausible as a site for GABA action on DA transmission. Furthermore, we show that GABA receptor antagonists increase DA release evoked by single optogenetic
stimuli, revealing that there is tonic inhibition of DA release by endogenous striatal GABA arising from a tonically active source or ambient level.

**GABA receptor influence on DA output does not require striatal ACh**

We found that both GABA_A and GABA_B receptor ligands can inhibit DA release whether evoked electrically in the presence of nAChR antagonist or optogenetically by targeted activation of ChR2-expressing DA axons in DAT-Cre mice. Light-activated DA release is not under tonic control by striatal nAChRs in these stimulation conditions (Threlfell et al., 2012; Melchior et al., 2015). Because GABA receptor effects were seen in the absence of nAChR activation, they are unlikely to require GABA receptors on Chls. These findings are consistent with a study in NAc indicating that GABA_B-mediated control of DA is independent of ACh input (Pitman et al., 2014). We note that muscarinic M5 receptors on DA axons have been suggested in some studies to regulate DA release (Bendor et al., 2010; Shin et al., 2015), but we found that electrically evoked DA release in the presence of DHβE and optogenetically evoked DA release were not attenuated by muscarinic receptor antagonists (Threlfell et al., 2010, 2012), so these effects of GABA and GABA receptors are unlikely to be mediated by alternative actions of ACh at M5 mAChRs on DA axons.

Activation of GABA receptors had limited effects on the frequency sensitivity of evoked DA release. Activation of GABA_A receptors, but not GABA_B receptors, interacted in a statistically significant manner with frequency and number of stimulus pulses to slightly promote the ratio of [DA]_o evoked by high-frequency trains over single pulse release, but the effect size was modest. Therefore, GABA receptor activation primarily limits the overall amplitude of DA output with only a minor additional enhancement in the frequency filtering, unlike ACh, which profoundly changes the relationship of DA output to presynaptic activity (Rice and Cragg, 2004; Zhang and Sulzer, 2004).

**Tonic inhibition**

We found that GABA receptor antagonists can enhance DA release evoked by a short single optogenetic stimulation of ChR2-expressing DA axons in DAT-Cre mice, suggesting that DA release is under tonic inhibition by GABA. Light activation is targeted to DA axons and should not activate GABA release from other striatal neurons. Furthermore, the stimulus is sufficiently short (2 ms) that evoked DA release should not be under the control of any GABA that might be coreleased by this stimulus. Mesostriatal DA neurons can apparently synthesize, store, and corelease GABA (Tritsch et al., 2014; Kim et al., 2015), which can evoke inhibitory currents in postsynaptic medium spiny neurons, but it is unlikely that any GABA coreleased with DA could simultaneously gate the concurrent release of DA evoked by the same single 2 ms stimulus. Rather, it is more likely that DA release is under tonic inhibition by a preexisting striatal GABA tone. A tonic inhibition is consistent with previous reports that there is an ambient GABA tone in striatum detected as a GABA_A receptor-mediated current in postsynaptic neurons even in ex vivo preparations (Ade et al., 2008; Kirmse et al., 2008, 2009; Santhakumar et al., 2010; Cepeda et al., 2013). Our findings in-
icate that tonic inhibition of DA release can readily be detected, at least for GABA<sub>B</sub> receptors.

An ambient GABA tone that can act on DA axons to limit DA output should not be entirely surprising. At least one type of GABAergic interneuron, the LTS interneuron, is capable of autonomous firing in striatal slices (Beatty et al., 2012). Furthermore, striatal neurons are ~95% GABAergic, so even low levels of GABA release per neuron could potentially summate for significant impact. No GABAergic axoaxonic synapses have been identified on DA axons, but this need not preclude an interaction.

**Figure 4.** GABA receptor antagonists increase DA release. A, B, Mean peak [DA]<sub>o</sub> (± SEM) versus time (A) and mean [DA]<sub>o</sub> (± SEM) versus time (B) evoked by 1 light pulse before and in the presence of 3.5 μM CGP 55845 and 10 μM bicuculline (GABA<sub>B</sub> antagonists). **p < 0.01, Mann–Whitney test. Shown is the mean C, E, Mean peak [DA]<sub>o</sub> (± SEM) versus time in control conditions (black), 10 μM bicuculline (D) (red), or 100 μM saclofen (F) (blue). G, I, Mean peak [DA]<sub>o</sub> (± SEM) versus stimulation frequency in control conditions (black), with bicuculline (G), or with saclofen (I). Data are normalized to peak 1p-evoked [DA]<sub>o</sub> before drug application; two-way ANOVA, effect of drug *p < 0.05. H, J, Ratio of peak [DA]<sub>o</sub> released by 5p versus 1p (100 Hz) in control conditions, bicuculline (H), or with saclofen (J).
GABA can spillover for extrasynaptic actions in many neurons (Farrant and Nusser, 2005) and might act on DA axons through extrasynaptic effects. The volume of striatum reached by the extensive axonal arbor of a single DA neuron (Matsuda et al., 2009) can be calculated from striatal neuron counts (Oorschot, 1996) to encompass ~70,000 GABAergic neurons (plus additional nonneuronal cells that might provide a source of GABA). This large number of potential GABA sources might readily spillover and/or otherwise summate to provide an ambient GABA tone that can act at GABA receptors to limit DA output. Striatal GABA might therefore be in a position to influence striatal output, not only through direct actions on output neurons and local interneurons and input networks, but also by governing DA transmission.

Direct versus indirect actions of GABA receptors

The site of GABA receptor localization remains undefined. DA neurons in substantia nigra express GABA_A and GABA_B receptors and these are certainly functional in somatodendritic compartments (Bowery et al., 1987; Nicholson et al., 1992; Boyes and Bolam, 2003; Brazhnik et al., 2008), but their trafficking and localization to DA axons has not yet been demonstrated. GABA_B receptors have been immunocytochemically detected in striatal neuropil, consistent with localization of these receptors on DA axons (Charara et al., 2000), but GABA_A receptors have not yet been shown on DA axons. It can be difficult to verify the ultrastructural location of membrane bound receptors using classic immunocytochemical methods, which depend on appropriate fixation methods and conditions for antibody penetration; absence of proof is not proof of absence.

Key candidates for alternative sites of action can however be eliminated. Here, we exclude actions of GABA receptors on Chls acting through downstream nAChRs. Furthermore, GABAergic interneurons are unlikely as intermediaries: for example, if GABA_A receptors are acting indirectly through an intermediary GABA circuit that modulates DA via downstream GABA_B receptors, then GABA_A receptor agonists would be expected to inhibit the intermediary GABA circuit and therefore increase DA release, which is the opposite of what was seen here. The remaining candidate locations for the GABA receptors that regulate DA are therefore either an undisclosed neuron or other cell type with currently unknown actions on DA or, more parsimoniously, DA axons themselves. Responses to GABA have been described for axons of several other neurons (Kullmann et al., 2005; Trigo et al., 2008; Bucher and Gailliard, 2011). For example, in other CNS neurons, GABA_A receptors can reduce axonal spike amplitude and propagation and promote spike failures (Zhang and Jackson, 1995; Ruiz et al., 2003; Verdier et al., 2003), whereas GABA_B receptors inhibit voltage-gated Ca^{2+} channels (Sun and Chiu, 1999). Similar mechanisms could account for GABAergic inhibition of DA release from DA axonal arbors.

In conclusion, we show here not only that GABA_A and GABA_B receptors can gate DA output, but that there is a tonic inhibition by endogenous GABA through an apparent ambient GABA tone. In addition to directly regulating striatal output, striatal GABA tone might therefore also govern striatal integration via dampening DA output. We exclude cholinergic interneurons acting through nAChRs on DA axons as intermediaries for GABA regulation of DA, thereby adding support to the hypothesis that GABA is acting directly on DA axons. With the assistance of new genetic tagging and imaging tools, future studies should revisit the potential localization of GABA_A and GABA_B receptors to DA axons.

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