The substantia nigra pars compacta and ventral tegmental area contain the two largest populations of dopamine-releasing neurons in the mammalian brain. These neurons extend elaborate projections in the striatum, a large subcortical structure implicated in motor planning and reward-based learning. Phasic activation of dopaminergic neurons in response to salient or reward-predicting stimuli is thought to modulate striatal output through the release of dopamine to promote and reinforce motor action. Here we show that activation of dopamine neurons in striatal slices rapidly inhibits action potential firing in both direct- and indirect-pathway striatal projection neurons through vesicular release of the inhibitory transmitter GABA (γ-aminobutyric acid). GABA is released directly from dopaminergic axons but in a manner that is independent of the vesicular GABA transporter VGAT. Instead, GABA release requires activity of the vesicular monoamine transporter VMAT2, which is the vesicular transporter for dopamine. Furthermore, VMAT2 expression in GABAergic neurons lacking VGAT is sufficient to sustain GABA release. Thus, these findings expand the repertoire of synaptic mechanisms used by dopamine neurons to influence basal ganglia circuits, show a new substrate whose transport is dependent on VMAT2 and demonstrate that GABA can function as a bona fide co-transmitter in monaminergic neurons.

The striatum integrates inputs from cortex, hippocampus, thalamus, amygdala and ventral tegmental area/substantia nigra pars compacta (VTA/SNC) to instruct the selection of appropriate motor actions. Inputs from midbrain dopamine (DA) neurons play an important role in this process, as evidenced by the psychomotor deficits that arise after loss of these cells in Parkinson’s disease, or by the occurrence of compulsive and addictive behaviours upon potentiation of dopaminergic signalling. Through release of DA, these neurons promote activation of direct-pathway striatal projection neurons (dSPNs), which express G_{D_1}coupled D_{1} receptors, and inhibit indirect-pathway SPNs (iSPNs), which express G_{D_2}coupled D_{2} receptors. However, midbrain DA neurons also express neuropeptides and a subset releases glutamate, suggesting that the net effects of activity in these cells may not be limited to the actions of DA.

To investigate how DA neurons influence neuronal activity in striatum, we expressed the light-activated channel channelrhodopsin-2 (ChR2) in SNC neurons using Cre recombinase-dependent adeno-associated viruses (AAVs). In SNC1 Cre IRES–Cre/wt mice, Cre expression shows high penetrance and specificity for midbrain DA neurons (Supplementary Fig. 1). We validated SNC targeting using an AAV encoding Cre-dependent enhanced green fluorescent protein (EGFP). Fluorescence was restricted to Cre-containing neurons in SNC, and most EGFP-expressing cells (97.9%, _n_ = 1587 cells; three mice) were immunopositive for the catecholaminergic enzyme tyrosine hydroxylase, demonstrating specific expression in DA neurons (Fig. 1a, b and Supplementary Figs 1 and 2). Moreover, EGFP axons densely innervated dorsal striatum (Fig. 1c), consistent with their nigrostriatal identity.

Carbon-fibre amperometry confirmed the ability to evoke DA release from ChR2-expressing axons in slices of dorsal striatum. Brief flashes of blue light (1 ms) reliably evoked DA transients, which were sensitive to the tyrosine hydroxylase antagonist α-methyl-tyrosine and...
DA neurons directly release GABA onto SPNs. a, Evoked current responses from an iSPN held at indicated potentials (V_e) to optogenetic activation of nigrostriatal axons (1 ms, blue) upon sequential bath application of SR95531 and NBQX/CPP. b, As in a for a dSPN with antagonists applied in reverse order. c, Mean IPSC (red) and EPSC (grey) absolute amplitudes in dSPNs (n = 8) and iSPNs (n = 21). *P < 0.05 versus IPSC (Mann–Whitney rank sum test). d, Normalized IPSCs and EPSCs from a (dark) and b (light) shown on an expanded timescale. Blue, light presentation. e, Average (n = 29 SPNs) IPSC (red) and EPSC (grey) latencies from light onset to current onset (circle), half maximal amplitude (triangle) and peak amplitude (square). IPSCs are not delayed compared with EPSCs. f, g, Mean (n = 3–18) EPSC (f) and IPSC (g) amplitudes under control conditions (ACSF) or in indicated antagonists normalized to baseline. *P < 0.05 versus ACSF (Mann–Whitney rank sum test). h, Amperometric DA transients (black) and current responses of a voltage-clamped iSPN (V_e = 0 mV, red; V_e = −70 mV, grey) to DA neuron stimulation under baseline conditions (left), in TTX (middle) and after co-application of TTX and 4-aminopyridine (4AP, right). i, Extracellular DA concentration (black), IPSC amplitude (red) and EPSC amplitude (grey) evoked by ChR2 stimulation across conditions normalized to baseline. Data from ACSF condition same as in Figs 1e and 2f, g. *P < 0.05 versus ACSF; ** < 0.05 versus TTX (Mann–Whitney rank sum test). Error bars, s.e.m.

The VMAT2 antagonists reserpine and tetrabenazine (TBZ) (Fig. 1d, e). Moreover, DA release required Ca^{2+} channels, as it was blocked by cadmium, and activation of D2 receptors with quinpirole reduced DA release, consistent with the effect of DA autoreceptors in nigrostriatal axons. To determine the net effect of DA neuron stimulation on striatal output, we performed whole-cell current-clamp recordings from dSPNs and iSPNs in brain slices obtained from Slc6a3^{RES−/Cre/wt;Drd2-EGFP} mice. Whereas action potentials evoked by current steps occurred at regular intervals under baseline conditions, light presentation reliably paused firing and rapidly hyperpolarized SPNs of both populations (dSPNs: 6.8 ± 1.9 mV, n = 10; iSPNs: 7.0 ± 1.4 mV, n = 7) (Fig. 1f, g). The light-evoked pause and hyperpolarization were unaffected by a cocktail of antagonists targeting DA receptors (dSPNs: 6.6 ± 2.2 mV, n = 4; iSPNs: 9.5 ± 2.5 mV, n = 4; both P > 0.05 versus light only, Mann–Whitney rank sum test). Instead, they were abolished by the GABA_A receptor antagonist SR95531 (n = 4 dSPNs, seven iSPNs) (Fig. 1f, g), which does not alter DA release (Fig. 1e). These data indicate that DA neurons exert a rapid and strong inhibitory influence on SPNs through activation of GABA_A receptors.

Previous attempts at characterizing DA receptor-independent effects of DA neurons on SPNs showed a small, but rapid excitatory influence mediated by co-release of glutamate. However, these experiments were performed in the presence of GABA receptor antagonists, precluding the detection of inhibitory influences. To observe conductances recruited after DA neuron stimulation, we performed whole-cell voltage-clamp recordings from dSPNs and iSPNs in dorsal striatum without pharmacological blockers. When SPNs were held at −70 mV (E_C, the reversal potential for chloride), nigrostriatal fibre stimulation evoked fast inward currents in approximately 75% of dSPNs (n = 6/8) and iSPNs (n = 16/21) (Fig. 2a–e). These currents showed similar properties in both cell types (Fig. 2a–c) and were consequently pooled for analysis: they exhibited peak amplitudes of 40 ± 5 pA (n = 22), rise times of 2.6 ± 0.4 ms and decay time constants of 6.8 ± 1.5 ms. Moreover, they reversed at approximately 0 mV and were sensitive to the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and NMDA (N-methyl-d-aspartate) receptor blockers NBQX and CPP (Fig. 2a, b, f), indicating that they are glutamatergic excitatory postsynaptic currents (EPSCs). Although glutamate release by midbrain DA neurons was proposed to be limited to mesolimbic fibres innervating ventral striatum, the currents reported here in dorsal striatum show similar properties and are sensitive to the same pharmacological agents as DA release (Figs 1e and 2f), suggesting a dopaminergic origin. Moreover, EPSCs were unaffected by DA receptor antagonists (Fig. 2f), indicating that glutamate release is not secondary to DA receptor activation.

To isolate conductances that mediate SPN inhibition, the membra ne potential was held at the reversal potential of ionotropic glutamate receptors (~0 mV). Under these conditions, DA neuron stimulation evoked large outward currents in all recorded dSPNs (n = 8) and iSPNs (n = 21) (Fig. 2a–c and Supplementary Fig. 3). Collectively, these currents had peak amplitudes of 617 ± 94 pA (n = 29; range = 0.11–1.93 nA), rise times of 20 ± 0.2 ms and decay time constants of 56 ± 4 ms. They reversed at E_Cl and were blocked by SR95531 and bicuculline, but not by the GABA_C receptor antagonist TPMPA (Fig. 2a, b, g), indicating that they represent GABA_A receptor-mediated inhibitory postsynaptic currents (IPSCs). Similar observations were made in mice expressing Cre under control of a tyrosine hydroxylase promoter (Supplementary Fig. 4). Consistent with previous reports, no current remained with both glutamate and GABA_A receptors blocked. Thus, these data show that (1) activation of dopaminergic terminals rapidly activates ionotropic glutamate and GABA receptors in SPNs, (2) the resulting currents do not differentially affect dSPNs and iSPNs, and (3) GABAergic conductances mediate the net inhibitory effect of DA neurons on striatal output.

DA neurons may activate GABA_A receptors on SPNs either by recruiting a population of GABAergic interneurons—a mechanism akin to feed-forward inhibition—or by directly releasing GABA. Several lines of evidence suggest the latter. First, the latency between light and IPSC onset averaged 2.2 ± 0.1 ms (n = 29; Fig. 2d, e), which may be too short to accommodate two synaptic transmission steps at 32–34 °C (ref. 16). Second, GABAergic conductances preceded or occurred synchronously with EPSCs (Fig. 2d, e), which arise after...
glutamate co-release from DA terminals10–12 (Fig. 2h, i). Third, a population of dopaminergic SNC neurons expresses messenger RNA for glutamic acid decarboxylase (GAD-65)17, indicating that they may synthesize GABA. We reasoned that if GABA originates from dopaminergic terminals, optically evoked IPSCs, EPSCs and extracellular DA should be similarly affected by pharmacological agents and persist under conditions that prevent disynaptic transmission. Indeed, IPSCs in SPNs were reduced by quinpirole, eliminated by cadmium, but were insensitive to glutamate and DA receptor inhibitors (Fig. 2g), indicating that they require Ca2+-dependent release of a transmitter other than glutamate or DA. Moreover, light-evoked IPSCs, EPSCs and DA release were abolished in the presence of the voltage-gated Na+ channel blocker tetrodotoxin (TTX), showing that ChR2-mediated depolarization is not sufficient to trigger transmitter release from nigrostriatal axons (Fig. 2h, i). Neurotransmission can be rescued from directly illuminated ChR2-expressing terminals in the presence of TTX by providing extra depolarization with the voltage-gated K+ channel blocker 4-aminopyridine18. Accordingly, IPSCs, EPSCs and DA release recovered upon co-application of TTX and 4-aminopyridine (Fig. 2h, i), indicating that GABA and DA are directly released from dopaminergic terminals.

The vesicular GABA transporter VGAT (encoded by SLC32A1) is the only transporter known to package GABA into synaptic vesicles and is considered indispensable for inhibitory synaptic transmission19. We generated conditional knockout (cKO) mice in which VGAT is specifically deleted from DA neurons (Slc6a3lox/lox;Slc32a1lox/lox mice), predicting that ChR2-evoked IPSCs would be abolished. However, light-evoked IPSCs and EPSCs were unaffected in these mice (Fig. 3a, b, g, j), indicating that VGAT is not responsible for vesicular loading of GABA in DA neurons. We instead hypothesized that GAD-65 may synthesize GABA from its metabolite precursor glutamate once inside synaptic vesicles. If correct, preventing glutamate loading into synaptic vesicles by genetically ablating the vesicular glutamate transporter 2 (VGLUT2; encoded by Slc17a6) from DA neurons (Slc6a3lox/lox;Slc17a6lox/lox mice) should eliminate IPSCs and EPSCs20. However, conditional deletion of VGLUT2 abolished light-evoked IPSCs in SPNs without affecting IPSC amplitude or latency (Fig. 3c, g, j), excluding this possibility.

These results indicate that GABA release originates in nigrostriatal terminals but is independent of VGAT and VGLUT2, suggesting the existence of an alternative vesicular transporter with previously unidentified function for GABA. Consistent with this, IPSCs were eliminated in slices obtained from mice treated with the VMA2 antagonists reserpine, Ro4-1284 or TBZ and largely recovered upon Ro4-1284 and TBZ washout (Fig. 3d–g). The same manipulation did not affect EPSCs or VGLUT2-dependent GABA release from SPNs (Supplementary Fig. 5), and we confirmed that DA itself does not function as a GABAA receptor agonist in SPNs (Supplementary Fig. 6). Moreover, DA depletion with α-methyl-tyrosine (Fig. 1e) had no effect on IPSCs or EPSCs (Fig. 3g). We therefore conclude that VMAT2, but not DA, is required for the release of GABA by DA neurons.

If GABAergic IPSCs depend on VMAT2 solely for GABA transport into synaptic vesicles, IPSCs should be restored in reserpine-treated mice by expressing VGAT in DA neurons (Fig. 3h). Accordingly, in Slc6a3lox/lox;Slc17a6lox/lox mice injected with AAV encoding Cre-dependent VGAT (AAV-DIO-VGAT) and treated with reserpine, optogenetic stimulation of nigrostriatal axons elicited large SR95531-sensitive IPSCs exhibiting synaptic latencies (Fig. 3g–i) and rise times (1.8 ± 0.3, n = 10; P > 0.05 versus control, Mann–Whitney rank sum test) indistinguishable from IPSCs observed in untreated mice. Together, these data indicate that presynaptic DA terminals contain GABA, the synaptic packaging of which requires VMAT2 but not VGAT.

VMAT2 transports a variety of substrates29, including catecholamines, serotonin and histamine. Although GABA does not bear structural resemblance to known VMAT2 substrates, our findings suggest that VMAT2 may function as a vesicular GABA transporter. To test this possibility, we asked if VMAT2 can substitute for VGAT to sustain GABA release in a population of non-monoaminergic GABAergic neurons. Specifically, we attempted to restore GABA release in iSPNs devoid of VGAT by exogenously expressing VMAT2. We conditionally excised the gene encoding VGAT in iSPNs and virally expressed ChR2 in these cells to allow monitoring of GABA release from iSPN axon collaterals onto neighbouring dSPNs as light-evoked IPSCs (Fig. 4a). Whereas optogenetic stimulation of iSPNs in control mice (Adora2a-Cre;Slc32a1lox/lox;Drd2-EGFP) reliably evoked large IPSCs in dSPNs, IPSCs were almost entirely abolished in iSPNs from VGAT cKO mice (Adora2a-Cre;Slc32a1lox/lox;Drd2-EGFP) (Fig. 4b, c, e), confirming the independence of vesicular GABA transport in SPNs.
Figure 4 | VMAT2 functions as a vesicular GABA transporter.

a, Experimental setup: ChR2 was selectively expressed in Cre-containing iSPNs of mice with one (control; Adora2a-Cre;Slc32a1lox/lox;Drd2-EGFP mice) or both alleles of the gene encoding VGAT flanked by lox sites (VGAT cKO; Adora2a-Cre;Slc32a1lox/lox;Drd2-EGFP mice). VGAT cKO + VMAT2, an AAV encoding Cre-dependent VMAT2 (AAV-DIO-VMAT2), was co-injected with AAV-DIO-ChR2 in the striatum of VGAT cKO mice to rescue GABA release from iSPNs. b–d, Voltage-clamp recordings (Vh = 0 mV) of axon-collateral IPSCs in dSPNs evoked by optogenetic stimulation (1 ms, blue) of iSPNs in the absence (red) or presence (pink) of SR95531 in control (b), VGAT cKO (c) and VGAT cKO + VMAT2 (d) mice. Insets: iSPN presynaptic terminal schematic illustrating experimental conditions. Red triangles, GABA.

e, Summary histogram (mean ± s.e.m.) of experiments in b–d (n = 10–15 dSPNs).

*p < 0.05 versus control and VGAT cKO + VMAT2 (Kruskal–Wallis analysis of variance); **P < 0.05 versus iSPC without SR95531 (Mann–Whitney rank sum test).

Whole-cell current- and voltage-clamp recordings were obtained from pathway-identified dorsal striatum SPNs in acute parasagittal slices of mature (P40–218) mice at 32–34°C using standard techniques. Constant-potential amperometry (+400 to 600 mV versus Ag/AgCl) was performed using carbon-fibre microelec-
trodes. ChR2-expressing fibres were stimulated using brief full-field flashes of blue laser light (1 ms; 473 nm; 6.5–10.0 mW mm−2) at intervals of 30 s or more. Traces are the average of three to five consecutive acquisitions. For pharmacological analyses, the peak amplitude of three consecutive light-evoked responses 3–4 min after drug perfusion onset were averaged, normalized to baseline and compared with values obtained at corresponding times in control preparations bathed in artificial cerebrospinal fluid (ACSF). Data in text and figures are reported as mean ± standard error of the mean (s.e.m.). Statistical tests are noted in the text (significance: P < 0.05).

Full Methods and any associated references are available in the online version of the paper.

Received 15 May; accepted 27 July 2012.

Published online 3 October 2012.

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Supplementary Information is available in the online version of the paper.

Acknowledgements The authors thank A. Saunders and Y. Kozorovitskiy for generating and characterizing the AAV-DIO-EGFP and AAV-DIO-VGAT constructs, D. Sulzer and H. Zhang for assistance with amperometry, R. Shah and C. Johnson for technical support, and members of the laboratory for discussions. This work was supported by a Nancy Lurie Marks Family Foundation postdoctoral fellowship (N.X.T.) and by grants from the National Institutes of Health (NS046579 to B.L.S. and 4R00NS075136 to J.B.D.).

Author Contributions N.X.T., J.B.D. and B.L.S. designed the experiments. N.X.T. performed the experiments described in the figures and text and analysed the data. J.B.D. performed experiments that initiated this study, devised the injection coordinates, established amperometric recordings and participated in their acquisition. N.X.T. and B.L.S. wrote the manuscript with contributions from J.B.D.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to B.L.S. (bsabatini@hms.harvard.edu).
METHODS

Mice. Knock-in mice bearing an internal ribosome entry site (IRES)-linked Cre recombine gene downstream of the gene Slc6a3, which encodes the plasma membrane DA transporter DAT (referred to as Slc6a3\textsubscript{Cre/Cre} mice)\textsuperscript{12} were obtained from the Jackson Laboratory (stock number 006660). Homozygous (Slc6a3\textsubscript{Cre/Cre}) and heterozygous (Slc6a3\textsubscript{Cre/+/+}) animals were bred with Drd2-EGFP transgenic mice (GENSAT, founder line S118), which express EGFP under control of a bacterial artificial chromosome containing the D2 receptor genomic locus to permit distinction between direct- and indirect-express EGFP under control of a bacterial artificial chromosome containing the D2 receptor genomic promoter\textsuperscript{35}. Conditional deletion of EGFP mice, or the D2 receptor genomic locus to permit distinction between direct- and indirect-express EGFP under control of a bacterial artificial chromosome containing the D2 receptor genomic promoter\textsuperscript{35}.

For intracranial injection, a pulled glass pipette at a rate of 100 nl min\textsuperscript{−1} hole was drilled and AAVs were injected (0.5–1 µl) using a UMP3 microsyringe (World Precision Instruments). Injection coordinates were 0.8 mm anterior to the recording site at 30 s intervals under control of the acquisition software. For current-clamp recordings, depolarizing current steps evoking 10–20 Hz trains of action potentials were applied at regular intervals (10–15 s) either alone or in combination with 3–5–3 mV depolarizing steps (1 s) for voltage-clamp recordings, or with a K\textsuperscript{+}+-based low Cl\textsuperscript{−} internal solution composed of (in mM) 135 KMeSO\textsubscript{4}, 10 HEPEs, 1 EGTA, 3.3 QX-314 (Cl\textsuperscript{−} salt), 4 Mg\textsuperscript{2+}, 0.9 Na\textsuperscript{2} -Na\textsuperscript{+}–phosphocreatine (pH 7.3 adjusted with KO\textsubscript{2}HPO\textsubscript{4}, 295 mOsm kg\textsuperscript{−1}) for voltage-clamp recordings, or with a K\textsuperscript{+}+-based low Cl\textsuperscript{−} internal solution composed of (in mM) 135 KMeSO\textsubscript{4}, 10 HEPEs, 1 EGTA, 0.1 Ca\textsuperscript{2+}, 4 Mg\textsuperscript{2+}, 0.3 Na\textsuperscript{2} -Na\textsuperscript{+}–phosphocreatine (pH 7.3 adjusted with KO\textsubscript{2}HPO\textsubscript{4}, 295 mOsm kg\textsuperscript{−1}) for current-clamp recordings. Bath solutions for whole-cell recordings did not contain drugs unless specified otherwise. For all voltage-clamp experiments, errors due to the voltage drop across the series resistance (<20 M\textOmega) were left uncompensated. Membrane potentials were corrected for a ~8 mV liquid junction potential. To activate ChR2-expressing fibres, light from a 473 nm laser (Optoengine) was focused on the back aperture of the microscope on a wide-field objective, and the illumination of the fibre was pulsed at 1 Hz with a 50–100 µm diameter spot of light (1 ms duration, 6.5–10.0 mW mm\textsuperscript{−2} under the objective) were delivered at the recording site at 30 s intervals under control of the acquisition software. For current-clamp recordings, depolarizing current steps evoking 10–20 Hz trains of action potentials were applied at regular intervals (10–15 s) either alone or in combination with a 1 ms flash of blue light.

Amperometric recordings. Constant-potential amperometry was performed using homemade glass-encased carbon-fibre microelectrodes (7 µm diameter, 50–100 µm length) placed approximately 50 µm within dorsal striatum slices and held at a constant voltage of ~400 to 600 mV versus Ag/AgCl by a Multiclamp 700B amplifier (Molecular Devices). Electrodes were calibrated with fresh 5 µM dopamine standards in ACSF using fast-scanning cyclic voltammetry (from −0.5 V to 0.9 V, and back to −0.5 V at a rate of 280 mV s\textsuperscript{−1} every 100 ms, with the electrode held at 0 V between scans) to determine the optimal oxidation potential, followed by constant-potential amperometry of dopamine flow-in to allow conversion of current amplitude to extracellular dopamine concentration. Dopaminergic terminals surrounding the electrode were stimulated with 1 ms flashes of blue laser light (6.5–10.0 mW mm\textsuperscript{−2}) delivered at 2–3 min intervals.

Reagents. Drugs (all from Tocris, unless specified otherwise) were applied by bath perfusion: SR95531 (10 µM), (-)-bicuculline (20 µM), 3,3'-dipropylthiouridine-4'-phosphonic acid (2,3-dialkylamino-6-phenyl-benzoxiquinoline (NRBQ), 10 µM), R5-3-2-(2-carboxypropyl)-4-ylpropyl-1-phosphonic acid (CPP, 10 µM), tetrodoxin (TTX, 1 µM), 4-aminoypyridine (4AP, 0.1–1.0 mM), DcI5a (Sigma, 30 µM) and (-)-quinipiline (10 µM). The cocktail of antagonists used broadly to target D1 and D2 receptor families (D1\textsubscript{D2}R

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antagonists) consisted of SCH23390 (1 μM), SKF83566 (1 μM), (−)-sulpiride (10 μM) and L-741,626 (1 μM). To inhibit monoaminergic vesicular transport and deplete transmitter-filled vesicles, Slc6a3IRESCre mice were injected intraperitoneally with either the irreversible VMAT inhibitor reserpine (5 mg kg⁻¹) 24 h before slicing, the reversible VMAT antagonist Ro4-1284 (Sigma, 15 mg kg⁻¹) 1 h before slicing or the competitive and selective VMAT2 antagonist tetrabenazine (TBZ; 5 mg kg⁻¹) 2 h before slicing. To deplete presynaptic terminals of dopamine, Slc6a3IRESCre mice were administered the tyrosine hydroxylase antagonist α-methyl-DL-tyrosine methyl ester hydrochloride (Sigma, 250 mg kg⁻¹ intraperitoneally) 3 h and 1 h before slicing. Brain sections from these animals were prepared as described above, but were recovered and incubated in ACSF containing 1 μM reserpine, 10 μM Ro4-1284, 50 μM TBZ or 30 μM α-methyl-tyrosine, respectively. Half of the slices obtained from Ro4-1284- and TBZ-treated mice were kept at least 1 h in regular ACSF before recording to allow for drug washout and resumption of neurotransmitter transport into synaptic vesicles (washout condition in Figs 1e and 3g).

Data acquisition and analysis. Membrane currents and potentials were amplified and low-pass filtered at 3 kHz using a Multiclamp 700B amplifier (Molecular Devices), digitized at 10 kHz and acquired using National Instruments acquisition boards and a custom version of ScanImage written in MATLAB (Mathworks)⁹⁶. Amperometry, electrophysiology and imaging data were analysed offline using Igor Pro (Wavemetrics) and ImageJ (National Institutes of Health). In figures, amperometry and voltage-clamp traces represent the averaged waveform of three to five consecutive acquisitions. Detection threshold for IPSCs and EPSCs was set at 10 pA. Averaged waveforms were used to obtain current latency, peak amplitude, 10–90% rise time and decay time. Current onset was measured using a threshold set at three standard deviations of baseline noise. Peak amplitudes were calculated by averaging over a 2 ms window around the peak. For pharmacological analyses in Figs 1e and 2f, g, i, the peak amplitudes of three consecutive light-evoked responses 3–4 min after drug perfusion onset were averaged, normalized to baseline averages and compared statistically with values obtained at corresponding times in control preparations bathed in ACSF. Data (reported in text and figures as mean ± s.e.m.) were compared statistically using the following: Mann–Whitney rank sum test, Kruskal–Wallis analysis of variance (ANOVA) with Dunn’s multiple comparison test, and two-way ANOVA followed by Bonferroni post-hoc tests, as indicated in the text. P values less than 0.05 were considered statistically significant.

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