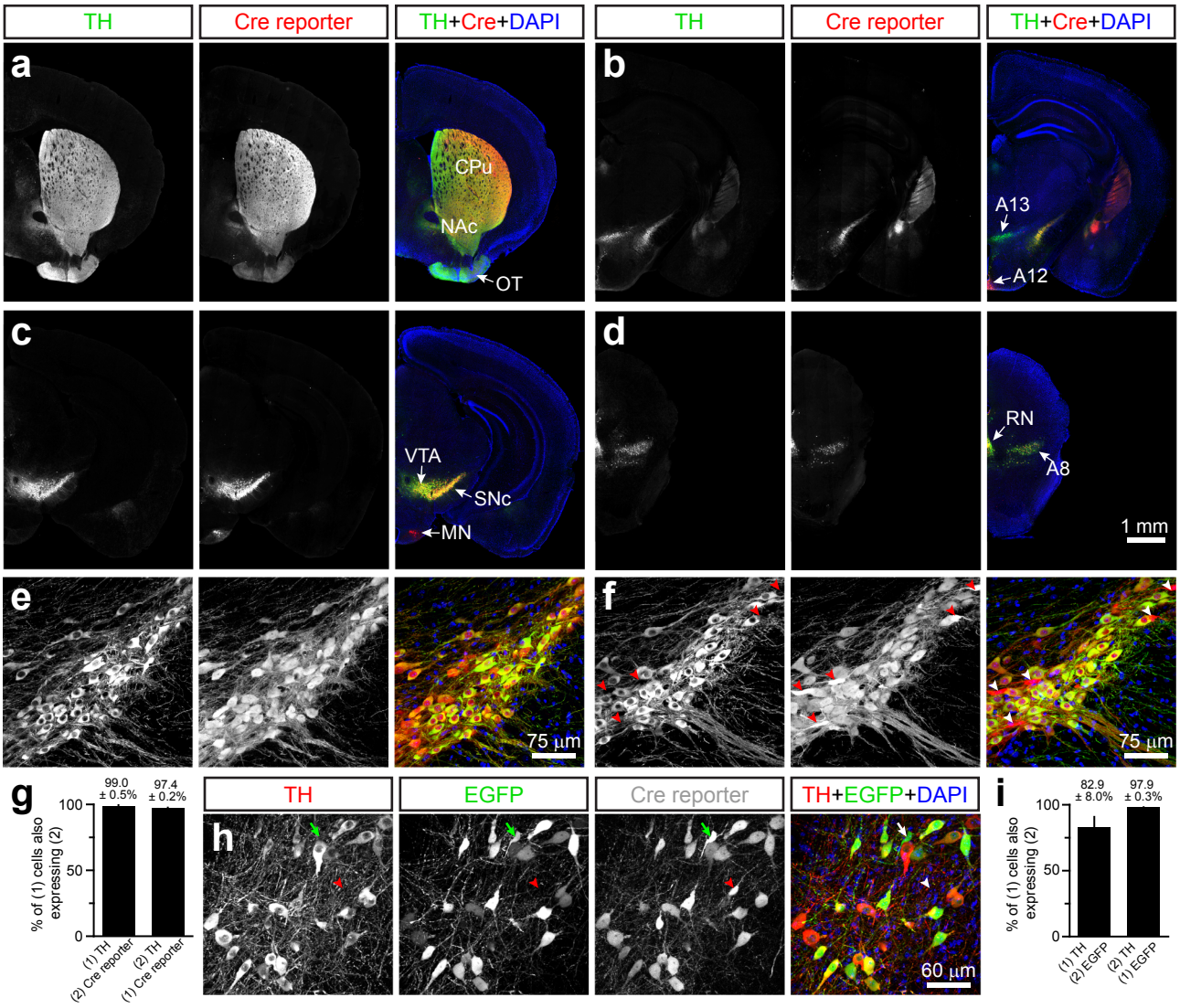
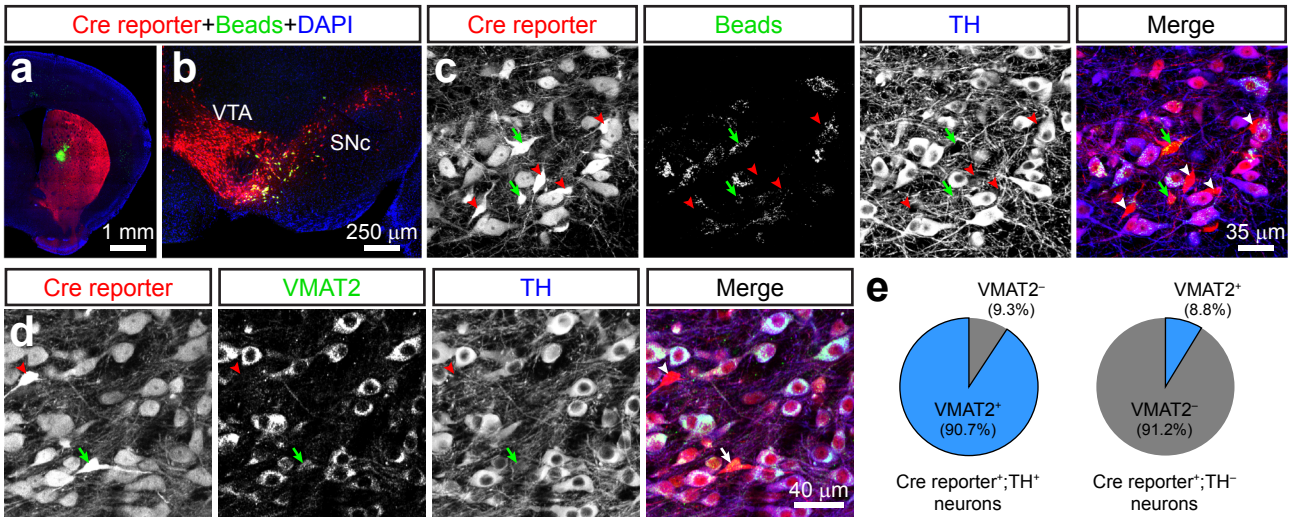
SUPPLEMENTARY FIGURES

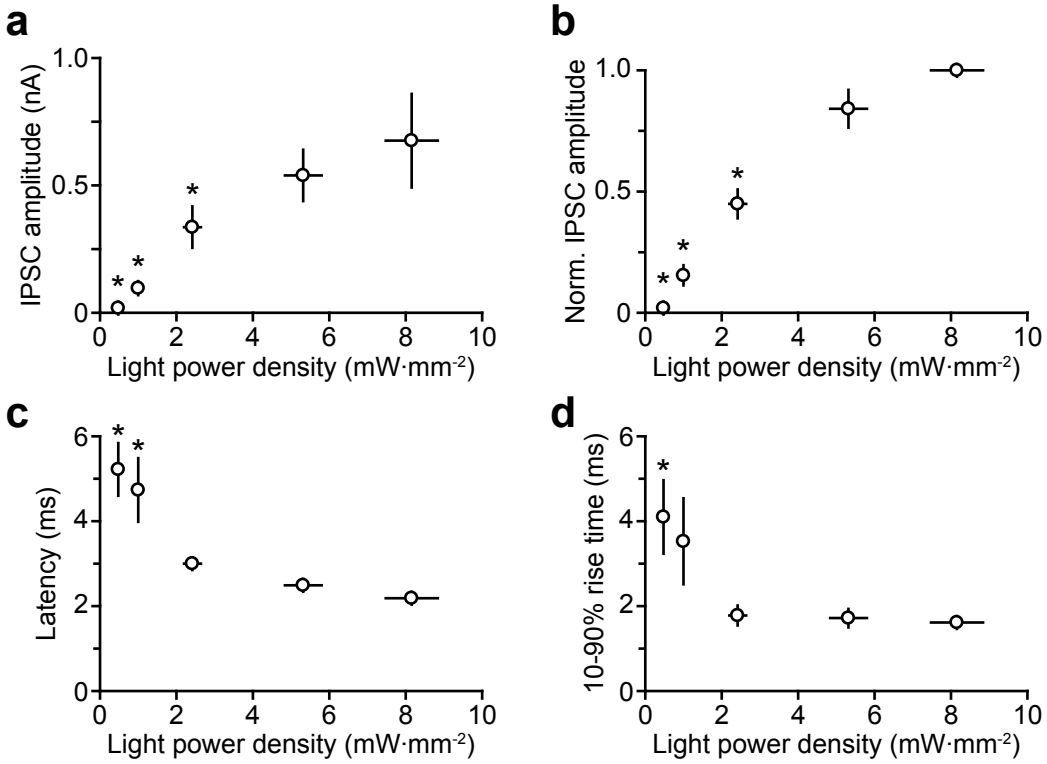


Supplementary Figure 1. *Slc6a3*$^{\text{IRES-Cre/wt}}$ characterization.
a-d. Representative coronal sections of a P55 *Slc6a3*$^{\text{IRES-Cre/wt}}$; *Rosa26*$^{\text{lsl-tdtomato/wt}}$ mouse brain immunolabeled for TH (green) at levels +0.5 (**a**), -1.4 (**b**), -3.1 (**c**) and -4.1 mm (**d**) from Bregma. TdTomato fluorescence demarcates Cre-expressing cells (Cre reporter, red). DAPI-stained nuclei in blue. A8/A12/A13, dopaminergic cell groups; CPu, dorsal striatum; MN, mammillary nuclei; NAc, ventral striatum; RN, raphe nuclei, SNc, substantia nigra pars compacta; VTA, ventral tegmental area. Raphe nuclei are partly composed of dopaminergic neurons[39]. Note that the A13 dopaminergic cell group does not express Cre and that the non-catecholaminergic mammillary nucleus does, consistent with DAT mRNA expression profiles in adult mice[40]. **e,f**. Representative high magnification confocal images of SNc in the same animal showing high specificity and near complete penetrance of the Cre transgene in DA neurons. Although TdTomato fluorescence was restricted to TH$^+$ cells in most cases (**e**), some sections did reveal a subpopulation of putatively non-dopaminergic TdTomato$^+$ cells expressing little or no TH (**f**, arrowheads). **g**. Summary histogram (mean ± s.e.m.) of Cre transgene penetrance (left) and specificity (right) for TH$^+$ DA neurons in SNc and VTA (n = 3 mice, 490-750 cells in each). **h**. Cre-dependent viral expression of EGFP in SNc/VTA is restricted to Cre-containing (TdTomato$^+$; Cre reporter) cells, including some TdTomato$^+$/TH$^-$ cells (arrow), indicating that these cells expressed Cre at the time of infection. Arrowhead points to a non-transduced TdTomato$^+$/TH$^-$ cell. **i**. Summary histogram (mean ± s.e.m.) of viral transduction efficiency (left) and specificity (right) for TH$^+$ DA neurons in SNc and VTA (n = 3 mice). 2.1% of transduced cells had low or non-detectable TH immunolabeling.

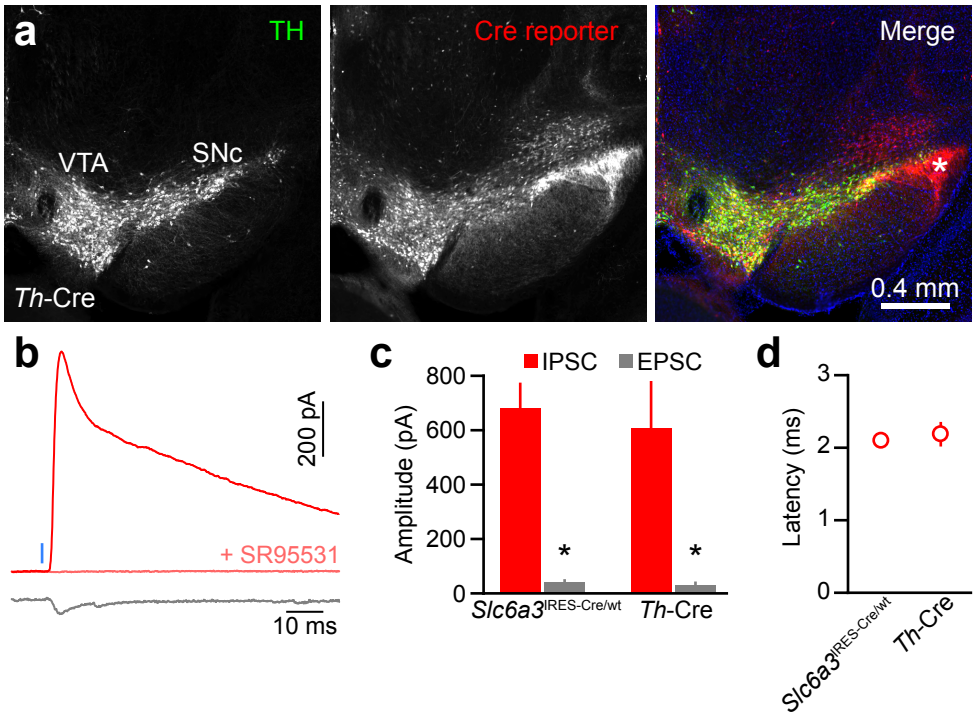


Supplementary Figure 2. Cre-expressing TH⁻ cells are unlikely to contribute to VMAT2-dependent GABA release in striatum.

a-c. Retrograde labeling of dorsal striatum-projecting DA neurons in *Slc6a3*$^{IRES\text{-}Cre/wt}$; *Rosa26*$^{lsl\text{-}tdtomato/wt}$ mice. **a**. Fluorescent retrobead injection site in dorsal striatum. **b**. Retrogradely-transported fluorescent beads (green) in ventral midbrain distribute exclusively to TdTomato⁺ cells (Cre reporter) within SNc/VTA. DAPI (blue), nuclear stain. **c**. High magnification confocal image of bead-labeled neurons in medial SNc. Consistent with previous reports[41], some retrogradely-labeled neurons are immunonegative for TH (arrows), indicating that TdTomato⁺/TH⁻ cells project to striatum. Non-labeled TdTomato⁺/TH⁻ cells are denoted with arrowheads. **d,e**. The vast majority of dopaminergic (TdTomato⁺/TH⁺) SNc/VTA neurons are immunopositive for VMAT2, whereas TdTomato⁺/TH⁻ neurons are immunonegative for VMAT2. **d**. Confocal images showing two SNc TdTomato⁺/TH⁻ neurons, one VMAT2⁺ (arrow) and one VMAT2⁻ (arrowhead). **e**. Summary chart of the proportion of dopaminergic ($n = 712$ cells) and TdTomato⁺/TH⁻ neurons ($n = 80$ cells) immunopositive for VMAT2. Together, these data indicate that although Cre⁺/TH⁻ cells project to striatum, they only represent 2.1% of transduced neurons and <10% of them are immunopositive for VMAT2, making them unlikely candidates for the large, VMAT2-dependent IPSCs readily observed in dorsal striatum SPNs.

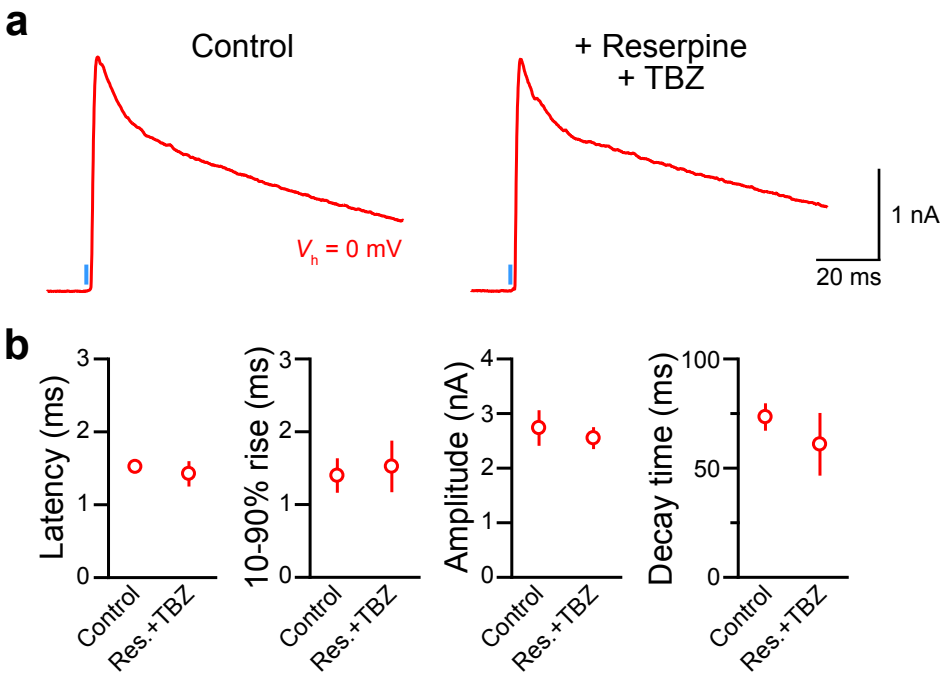


Supplementary Figure 3. Properties of ChR2-evoked IPSCs in SPNs scale with light stimulation power.
Light-evoked IPSCs were recorded at V_h = 0 mV in four SPNs at 32–34°C under control conditions at varying light intensities (0.2–10.0 mW·mm^{-2}). Each SPN was presented with brief light flashes (1ms) at 1 or 2 different illumination powers in each of five irradiance bins (<0.7; 0.8–1.7; 1.8–3.6; 3.7–6.4 and 6.5–10.0 mW·mm^{-2}) in a semi-random order. A minimum of two IPSCs were obtained and averaged for each light power density. **a–d**. Mean peak IPSC amplitudes (**a**), IPSC amplitudes normalized to responses evoked at 6.5–10.0 mW·mm^{-2} (**b**), latency between onset of light presentation and IPSC onset (**c**) and 10–90% IPSC rise time (**d**) as a function of light power density. IPSC amplitudes scale with irradiance and do not appear fully saturated at the maximal intensities sampled. At minimum light intensities, IPSCs progressively ran into baseline noise without giving rise to measurable step-like responses interleaved with clearly-identifiable synaptic failures. This observation suggests that light-evoked IPSCs in SPNs represent compound responses to numerous small-amplitude GABAergic inputs as opposed to a few large ones, in agreement with anatomical evidence of multiple dopaminergic innervation of SPNs[42,43]. Throughout this manuscript, we limited our analyses to IPSCs evoked with maximal light stimulation (6.5–10.0 mW·mm^{-2}) to minimize IPSC variability arising from slow and inconsistent stimulation of dopaminergic axons associated with lower light powers. Asterisks in **a–d** indicate $P < 0.05$ vs. IPSCs at 6.5–10.0 mW·mm^{-2} (Kruskal-Wallis). Error bars represent s.e.m.



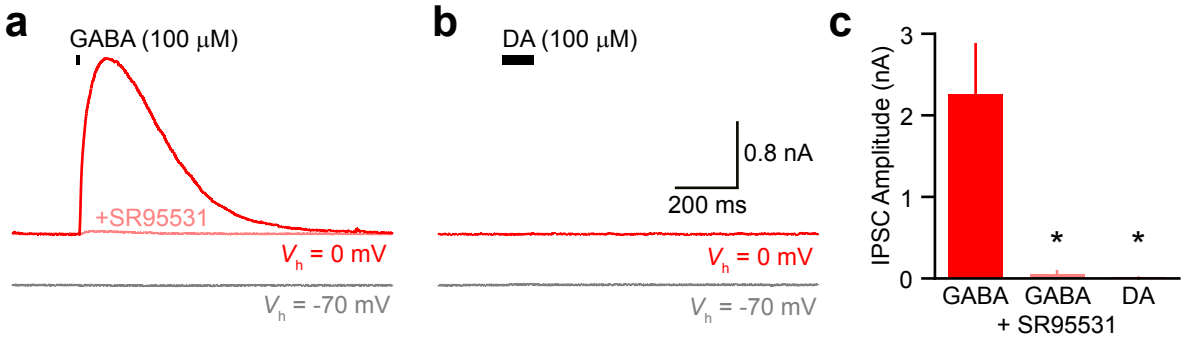
Supplementary Figure 4. ChR2-activation of Cre-containing ventral midbrain neurons in *Th*-Cre mice evokes glutamatergic and GABAergic currents in dorsal striatum SPNs.

a. Coronal ventral midbrain section of a P48 *Th*-Cre; *Rosa26*$^{lsl\text{-}tdtomato/wt}$ mouse revealing Cre expression (*middle*, Cre reporter) in VTA/SNc DA neurons immunolabeled for TH (*left*). *Right*, merged image. Nuclear stain with DAPI indicated in blue. Note that Cre expression is not exclusively limited to TH$^+$ DA neurons in this mouse. Asterisk indicates a TH$^-$ fiber tract coursing lateral to SNc. **b**. Current response of a dorsal striatum SPN from a *Th*-Cre mouse recorded in voltage-clamp (red/pink traces: V_h = 0 mV; gray trace: V_h = -70 mV) upon optogenetic activation (1 ms, blue line) of local ChR2$^+$ axons. **c**. Summary histogram of mean (± s.e.m.) absolute IPSC (red) and EPSC (gray) amplitudes recorded in striatal slices from *Slc6a3*$^{IRES\text{-}Cre/wt}$ (n = 24 SPNs) and *Th*-Cre mice (n = 8 SPNs). Asterisk, $P < 0.05$ vs. IPSC (Kruskal-Wallis). **d**. Mean (± s.e.m.) delay separating light flash and IPSC onsets measured in SPNs from *Slc6a3*$^{IRES\text{-}Cre/wt}$ and *Th*-Cre mice. No significant difference between both groups was observed.



Supplementary Figure 5. Reserpine and tetrabenazine do not affect VGAT-dependent inhibitory synaptic transmission.

SPNs form GABAergic axon collateral synapses with each other that depend on VGAT for vesicular packaging of GABA (see **Fig. 4** and refs. 44,45); dSPN-to-iSPN synapses therefore constitute a suitable model to investigate whether reserpine and tetrabenazine affect VGAT-dependent inhibitory synaptic transmission. **a**. Representative whole-cell voltage-clamp recordings (holding potential, V_h = 0 mV) from iSPNs upon optogenetic stimulation of dSPN axon collaterals in *Drd1a*-Cre; *Drd2*-EGFP BAC transgenic mice transduced in dorsal striatum with AAV-DIO-ChR2. Prior to recording, mice were either injected with saline (*left*) or treated with both reserpine and TBZ (*right*; 5 mg·kg^{-1} i.p. injections of reserpine and TBZ 24 h and 2 h before slicing, respectively, followed by slice incubation in 1 µM reserpine and 50 µM TBZ, as specified in methods). Traces represent averages of 3 consecutive stimulations. **b**. Mean (± s.e.m.) ChR2-evoked IPSC onset latency, 10–90% rise time, amplitude and decay time did not differ between control mice (n = 4 cells) and in mice treated with reserpine and TBZ (n = 4 cells).



Supplementary Figure 6. DA does not activate $GABA_A$ receptors on SPNs.
a. Focal application of GABA (100 μM dissolved in ACSF; 10 ms duration) on a whole-cell voltage-clamped iSPN using a Picospritzer (5 psi) evoked a large SR95531-sensitive outward current at 0 mV (red and pink traces) that reversed at ECl (V_h = -70 mV; gray trace). **b**. Focal application of DA (100 μM; 10–100 ms duration) did not evoke any detectable current at 0 mV or -70 mV. **c**. Summary histogram of average (± s.e.m.) peak IPSC amplitude (measured at V_h = 0 mV) upon pressure ejection of GABA (n = 3 SPNs) or DA (n = 8 SPNs). Asterisk, $P < 0.05$ vs. GABA (two-sample t-test).

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