Localization of virus injections.

(a) Schematic showing the approximate center of AAV-DIO-ChR2-YFP injection sites in the NAc of Dyn-cre mice (n=8 mice, 16 injections; caudate/putamen, CPu; nucleus accumbens core, NAcC; nucleus accumbens shell, NAcSh). (b) Representative GABA_B oIPSCs at different stimulation frequencies.
D2 MSNs project to the ventral pallidum, but not directly to the VTA.

(a) Schematic of experimental protocol. Cre-dependent ChR2-YFP was injected into the VTA of A2A-cre mice and whole-cell patch-clamp recordings were performed in VTA dopamine and GABA neurons and in ventral pallidal neurons. (b) Summary data of light-evoked GABA<sub>A</sub> oIPSCs in voltage clamp recordings (V<sub>m</sub>=-70 mV) of VTA dopamine (blue), VTA GABA (red), and ventral pallidal neurons (purple; n=10, 8, and 11 cells; one-way ANOVA, F<sub>28</sub>=8.606, p<0.01). (c) Representative traces of light-evoked GABA<sub>A</sub> IPSCs in VTA dopamine (blue), VTA GABA (red) and ventral pallidal neurons (purple). Data are shown as mean ± s.e.m.
Supplementary Figure 3

GABA\textsubscript{B}Rs are strongly expressed in dopamine neurons throughout the midbrain.

(a) Wide-field image showing TH immunoreactivity (brown) and GABA\textsubscript{B1} in situ (black; scale bar, 300 µm). (b) TH neurons co-express GABA\textsubscript{B1} mRNA (scale bar, 25 µm). (c) Percentage of TH neurons co-expressing GABA\textsubscript{B1} mRNA in the substantia nigra pars compacta (SNC) and VTA (n=3 mice, 4 sections per mouse, \(p=0.68, t(4)=0.4504\)). (d) Percentage of GABA\textsubscript{B1}+ cells that do not express TH (n=3 mice, 4 sections per mouse, \(p=0.30, t(4)=1.191\)). All data are shown as mean ± s.e.m.
Amplitudes of GABA_A oIPSCs correlate with electrophysiological properties of GABA neurons and amplitudes of GABA_B oIPSCs correlate with electrophysiological properties of dopamine neurons.

(a-c) Example traces demonstrating the AP width (a), firing rate (b), and h-current (c) of a representative GABA (red) and dopamine (blue) neuron. (d) Correlation of GABA_A oIPSCs with AP width (n=45 cells, 6 mice). (e) Correlation of GABA_A oIPSCs with firing rate (n=45 cells, 6 mice). (f) Correlation of GABA_A oIPSCs with h-current (n=45 cells, 6 mice). (g) Correlation of GABA_B oIPSCs with AP width (n=64 cells, 12 mice). (h) Correlation of GABA_B oIPSCs with firing rate (n=64 cells, 12 mice). (i) Correlation of GABA_B oIPSCs with h-current (n=64 cells, 12 mice).
Supplementary Figure 5

Nucleus accumbens inputs preferentially activate GABA\textsubscript{B}Rs in dopamine neurons and GABA\textsubscript{A}Rs in GABA neurons.

(a) Horizontal brain section containing biocytin-filled cells – two TH+ cells and two TH- cells (scale bar, 60 \(\mu\)m). (b) Representative GABA\textsubscript{B} oIPSCs from the cells shown in (a). (c) Summary data of GABA\textsubscript{B} oIPSCs in TH+ and TH- neurons (\(V_m=-55\) mV; \(n=8\) and 10 cells, respectively, 6 mice; unpaired t-test, \(p<0.01\), \(t_{(16)}=5.502\)). (d,e) Example (d) and summary (e) of GABA\textsubscript{A} oIPSCs in VTA dopamine (blue) and GABA (red) neurons using K-glucuronate internal solution (\(V_m=-55\) mV; \(n=15\) and 12 cells respectively, 4 mice each, unpaired t-test, \(p<0.01\), \(t_{(25)}=7.808\)). (f,g) Example (f) and summary (g) of GABA\textsubscript{B} oIPSCs in VTA dopamine and GABA neurons (\(V_m=-55\) mV; \(n=16\) and 11 cells respectively, 4 mice each, unpaired t-test, \(p<0.01\), \(t_{(25)}=7.989\)). All data are shown as mean ± s.e.m.
Evidence for synaptic release of GABA onto GABA<sub>B</sub> receptors.

(a) Time to onset (10% max current) of GABA<sub>B</sub> IPSCs while GABA (1M) was iontophoretically released (open circles) at different distances from the soma compared to the time to onset of electrically-evoked GABA<sub>B</sub> eIPSCs (closed circle, n=9 and 10 cells). (b) Experimental schematic for (c-e). GABA<sub>A</sub> oIPSCs were evoked in voltage clamp recordings (-70 mV) of VTA GABA neurons. (c) Representative traces of normalized NAc→VTA GABA<sub>A</sub> oIPSCs in normal artificial cerebrospinal fluid (aCSF) versus dextran-incubated slices. (d) Summary of time to onset for GABA<sub>A</sub> oIPSCs from normal ACSF versus dextran-incubated slices (n= 8 cells, two-tailed t-test, t<sub>(14)</sub>=0.18, p=0.86). (e) Summary of time to maximum current for GABA<sub>A</sub> oIPSCs from normal ACSF versus dextran-incubated slices (n= 8 cells, two-tailed t-test, t<sub>(14)</sub>=1.85, p=0.09). (f) Experimental schematic for (g-i). GABA<sub>B</sub> oIPSCs were evoked during voltage clamp recordings (-55 mV) of VTA dopamine neurons. (g) Representative traces of normalized NAc→VTA GABA<sub>B</sub> oIPSCs onto dopamine neurons in normal aCSF versus dextran-incubated slices. (h) Summary of time to onset for GABA<sub>B</sub> oIPSCs from normal ACSF versus dextran-incubated slices (n= 9 cells, two-tailed t-test, t<sub>(16)</sub>=0.32, p=0.75). (i) Summary of time to maximum current for GABA<sub>B</sub> oIPSCs from normal ACSF versus dextran-incubated slices (n=9 cells, two-tailed t-test, t<sub>(16)</sub>=0.58, p=0.55). (j) Representative iontophoretic GABA<sub>B</sub> current before and after dextran application. (k) Time to peak amplitude of iontophoretic GABA<sub>B</sub> current before and after dextran (n=3 cells, paired t-test, t<sub>(2)</sub>=7.647, p<0.05). (l) Peak amplitude of iontophoretic currents at different distances from the cell, normalized to the peak amplitude at 0 µm (n=7 cells each, two-way ANOVA, F<sub>(4,60)</sub>=2.723, p<0.05). (m) Decay time from the peak of iontophoretic currents (n=7 cells each, two-way ANOVA, F<sub>(4,55)</sub>=9.985, p<0.05) All data are shown as mean ± s.e.m.
Supplementary Figure 7

Nucleus accumbens inputs inhibit dopamine neurons via GABA\(_B\)Rs.

(a) Experimental schematic, stimulating NAc→VTA terminals and recording tonic firing in cell-attached mode from VTA dopamine neurons. (b-f) Effect of optical stimulation of NAc→VTA terminals on the normalized firing rate of dopamine cells at various frequencies (1, 2, 5, 10, or 20 Hz). Optical stimulation inhibited dopamine cell firing during baseline (blue, n=10 cells and 4 mice) and during GABA\(_A\) blockade with picrotoxin (100 µM, grey, n=8 cells and 4 mice), but not during GABA\(_B\) blockade with CGP 35348 (100 µM, black, n=8 cells and 4 mice). Data are shown as mean ± s.e.m.
Supplementary Figure 8

VTA GABA neurons inhibit dopamine neurons via GABA$_A$Rs.

(a) Experimental schematic, stimulating VTA GABA neurons and recording tonic firing in cell-attached mode from VTA dopamine neurons. (b-f) Effect of optical stimulation of VTA GABA neurons on the normalized firing rate of dopamine cells at various frequencies (1, 2, 5, 10, or 20 Hz). Optical stimulation inhibited dopamine cell firing during baseline (red, n=10 cells and 4 mice) and during GABA$_B$ blockade with CGP 35348 (100 µM, black, n=11 cells and 5 mice), but not during GABA$_A$ blockade with picrotoxin (100 µM, grey, n=11 cells and 5 mice). Data are shown as mean ± s.e.m.
Supplementary Figure 9

Deletion of GABA<sub>B</sub>Rs from dopamine neurons does not affect general or morphine-induced locomotion.

(a) Summary of locomotor activity in a 30 min open-field test for AAV-Control versus AAV-TH-iCre mice (n=8 mice each, unpaired t-test, t<sub>7</sub>=0.3768, p=0.71). (b) Summary of time spent in the center during the open-field test for AAV Control versus AAV TH-iCre mice (n=8 mice each, unpaired t-test, t<sub>7</sub>=0.88, p=0.40). (c) Locomotor activity in 15 min bins after 10 mg/kg morphine injection. (d) Locomotor activity in 15 min bins after 30 mg/kg morphine injection. All data are shown as mean ± s.e.m.