SUPPLEMENTAL FIGURES

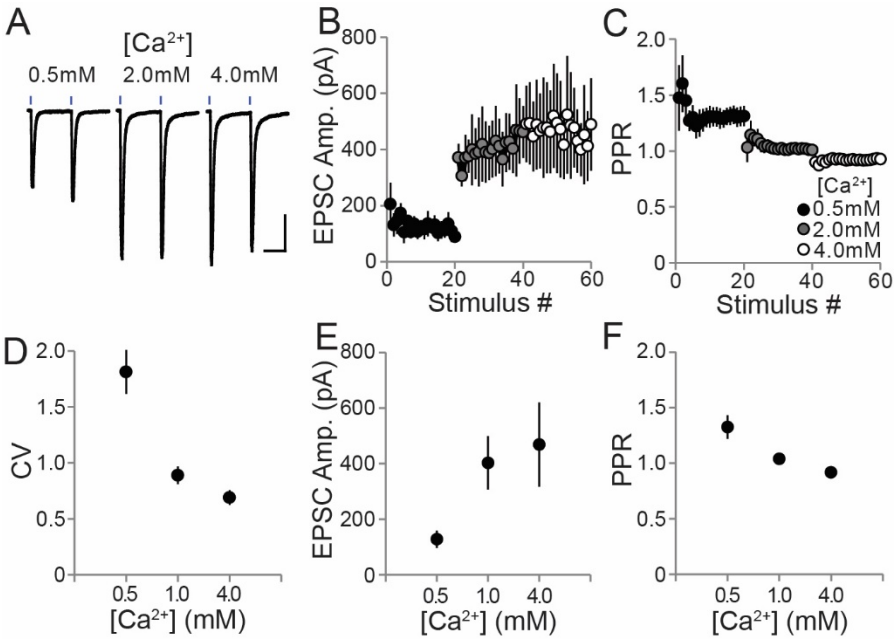

Figure S1, related to Figure 3. Dependence of paired pulse ratio on presynaptic release probability.

(A) Example responses in a MSN to thalamostriatal stimulation in a VGLUT2-Cre mouse expressing ChR2-EYFP at varying external calcium concentrations.

(B) EPSC amplitudes plotted as a function of stimulus number. Circle color represents different calcium concentrations: black 0.5 mM, grey 2.0 mM, white 4.0 mM.

(C) Paired pulse ratio plotted as a function of stimulus number across different calcium concentrations.

(D) Average coefficient of variation for each calcium concentration.

(E) Average EPSC amplitude for each calcium concentration.

(F) Average PPR for each calcium concentration. $p = 0.004$, one-way ANOVA, $n = 6$ cells.

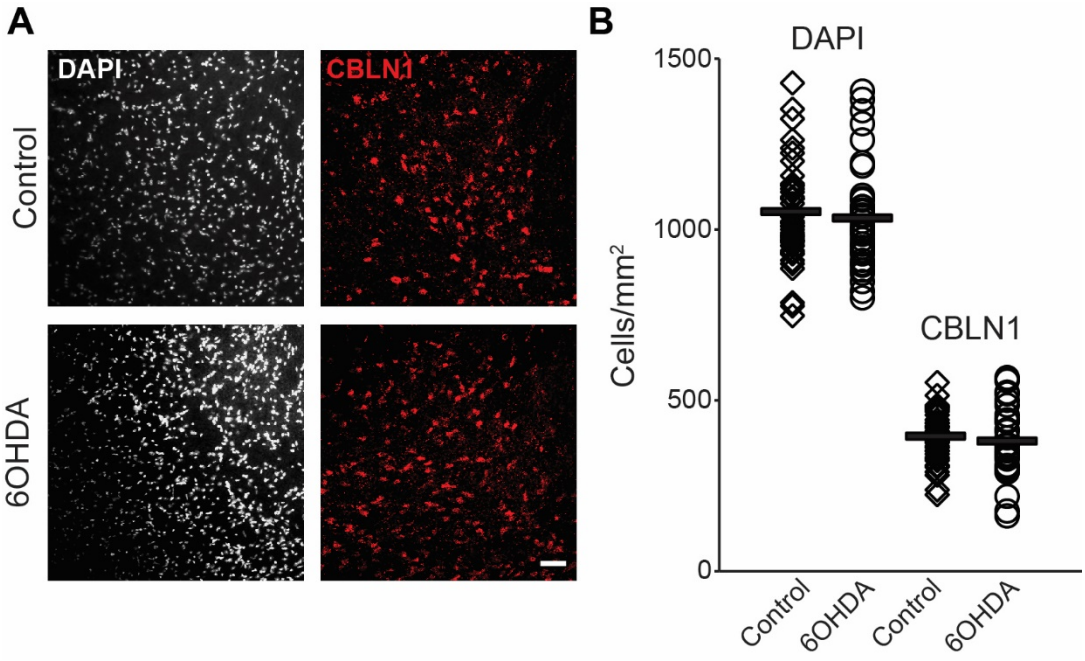

Figure S2, related to Figure 3. Quantification of Pf cell number in control and 6OHDA animals.

(A) Example confocal images of DAPI and anti-Cerebellin-1 (CBLN1) staining within Pf in control (top) and 6OHDA (bottom) animals.

(B) Quantification of cell density for each optical section analyzed. No significant differences were seen in DAPI or CBLN1 staining between control and 6OHDA animals (control n = 5 animals, 52 sections; 6OHDA = 3 animals, 38 sections; DAPI p = 0.618, CBLN1 p = 0.513).

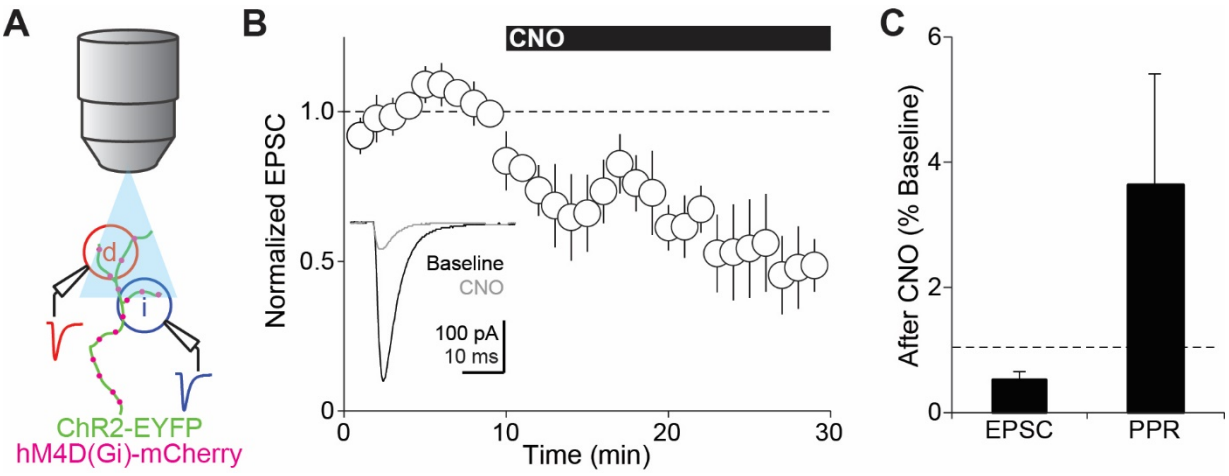

Figure S3, related to Figure 4. Bath application of CNO reduces ChR2-evoked thalamostriatal EPSCs in striatal slices.

(A) Schematic of recording setup. Thalamic neurons were transduced with ChR2- and hM4D-expressing viruses. EPSCs from thalamostriatal stimulation evoked by 470 nm LED pulses through the objective were recorded simultaneously from one dMSN (red) and one iMSN (blue). hM4D receptors in thalamostriatal axons were activated by bath application of clozapine N-oxide (CNO).

(B) Averaged data from MSNs (n = 6) showing reduction in thalamostriatal EPSC amplitude after bath application of CNO. Inset: traces from an iMSN showing synaptic responses to ChR2-mediated thalamostriatal stimulation before (baseline) and after (CNO) bath application of CNO.

(C) EPSC amplitude and PPR after CNO application expressed as a percentage of the baseline value. EPSC, $p = 0.025$; PPR, $p = 0.136$.

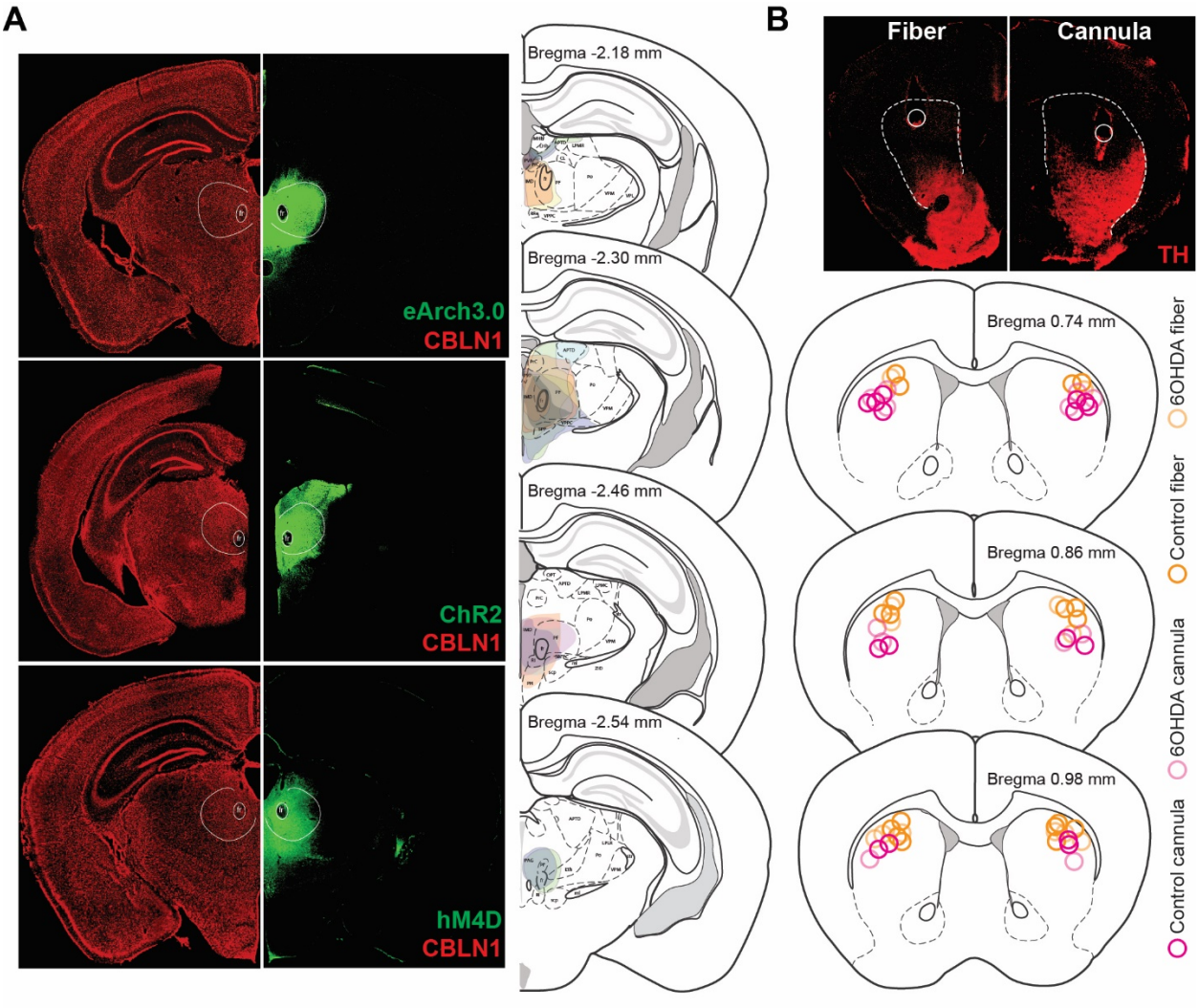

Figure S4, related to Figure 4. Characterization of viral expression and cannula/fiber implantation.

(A) Left: example images of viral injections with counterstaining against cerebellin-1 (CBLN1) to aid regional identification. Borders of fasciculus retroflexus (fr) and Pf were approximated by comparing CBLN1 staining to Franklin and Paxinos (2007). Right: characterization of viral expression patterns in the thalamus. Each color represents an individual injection.

(B) Top: example of a fiber (left) and cannula (right) tract in animals with DLS 6OHDA lesions. Bottom: characterization of fiber (orange) and cannula (magenta) tip locations for control (dark) and 6OHDA (light) animals. Stereotaxic images adapted from Franklin and Paxinos (2007).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animals

All experiments were conducted with the approval of the Institutional Animal Care and Use Committee at the University of California, San Francisco. VGLUT2-Cre, Emx1-Cre, and D1-Tomato mice were purchased from Jackson Laboratories (stock numbers 016963, 005628, and 016204 respectively). The two Cre lines were crossed to the D1-Tomato line and bred in-house.

Surgeries

Six- to ten-week-old mice were anesthetized (isoflurane 2%) and placed in a stereotaxic frame. Using bregma as a reference, a mounted drill was used to create holes in the skull, and a 33-gauge needle (Plastics One) was inserted for viral injections into cortex (AP +0.5/1.5, ML +/-2.0, DV -1.0 from dura) or thalamus (AP -2.3, ML +/-0.5, DV -3.25 from dura). A volume of 0.75 µl/site of ChR2, hM4D, and/or eArch3.0 (AAV5-EF1a-DIO-ChR2(H134R)-EYFP, AAV5-CaMKII-ChR2(H134R)-EYFP, AAV8-EF1a-DIO-hM4D-mCherry, AAV5-CaMKII-hM4D-mCherry, AAV5-CaMKII-eArch3.0-EYFP, titer ~ 2 x 10^{12}, UNC Vector Core) was injected at a rate of 0.2 µl/min. The needle was left in place for 5 min before being withdrawn and the scalp was sutured. Two weeks later, the scalp was reopened and 1 µl of 5 µg/µl 6-hydroxydopamine (6OHDA; Sigma-Aldrich) was injected unilaterally into the medial forebrain bundle (MFB; AP -1.0, ML -1.0, DV -4.9 from dura) for electrophysiology experiments, or bilaterally into the DLS (AP +0.8, ML +/- 2.2, DV -2.5 from dura) for behavioral experiments. Cannulae (Plastics1) or optical fiber implants

(400 μm fiber, Thorlabs; stainless alloy ferrules, Precision Fiber Products) were lowered into the same DLS site and secured with dental acrylic.

Electrophysiology

One to two weeks after 6-OHDA/saline injection, acute slices (300 μm) were cut through the DLS in sucrose-based artificial cerebrospinal fluid (ACSF) using a vibratome (Leica VT1200S). Slices recovered in NaCl-based ACSF for 30 min at 32°C and then were held at room temperature until being transferred into the recording chamber at 32°C. ACSF [consisting of (in mM): NaCl 125, $NaHCO_3$ 26, NaH_2PO_4 1.25, KCl 2.5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 12.5, and 50 μM picrotoxin to suppress GABAergic responses] was perfused over the slices at ~2 ml/min while simultaneous voltage clamp recordings were made from one D1-Tomato+ and one D1-Tomato- MSN using 2-4 MΩ patch pipettes pulled from borosilicate glass on a Sutter P-97 and filled with internal solution [consisting of (in mM): $CsMeSO_3$ 120, CsCl 15, NaCl 8, EGTA 0.5, HEPES 10, QX-314 5, TEA-Cl 10, Mg-ATP 2, and Na-GTP 0.3]. Light pulses (470 nm, 0-2 mW/mm^2, 0.5-5 ms duration) were delivered through a 40x immersion objective at 0.15 Hz using a high-intensity LED (Thorlabs LED4C driven by a Prizmatix BLCC-2). MSNs were held at -80 mV and depolarized to +40 mV to measure NMDAR-mediated currents (measured at 50 ms after stimulus onset when AMPA-mediated currents were negligible). For hM4D experiments, clozapine-N-oxide (CNO, 10 μM; Sigma-Aldrich) was added to the bath solution after a ten-minute baseline recording.

Open Field Behavior

One to two weeks after 6-OHDA/saline injection, activity in the open field was tracked using ETHOVISION 9 software (Noldus). For hM4D experiments, half of each group (control, 6OHDA) received intraperitoneal (i.p.) saline injections or DLS infusions while the other half received i.p. CNO injections or DLS infusions (i.p.: 1 mg/kg CNO in saline; DLS: 0.4 µl of 50 µM CNO in saline at 0.1 µl/min) 30 min before open field measurements. Two days later, the experiment was repeated and the saline/CNO cohorts were switched. For eArch3.0 experiments, 10-15 mW/mm^2 of 532 nm laser light was coupled via 400 µm fibers to each optical fiber implant, and light was delivered during 30 s intervals spaced 65 s apart (95 s cycles) for 10 repetitions. Ambulation was defined as movement of the mouse center-point >1.75 cm/s. Fine movement was defined as movement of the mouse center point <1.75 cm/s with >2% change in image pixels. Freezing was defined as movement of the mouse center-point <1.75 cm/s with <2% change in image pixels. Statistical significance was evaluated using a two-way ANOVA and post-hoc Bonferroni-corrected paired student's t-test. Animals were excluded if incomplete bilateral loss of TH+ axonal labeling in DLS was achieved (6OHDA group), quantified as <30% loss of total striatal TH staining, or if the location of the cannula/fiber tip was outside of DLS (both groups).

Histology

Mice were transcardially perfused with PBS followed by 4% paraformaldehyde (PFA), brains were rapidly dissected out and placed into 4% PFA overnight. The next day they were rinsed in PBS and switched to 30% sucrose-containing PBS for 48 h, after which

slices (30 μm) were cut on a sliding microtome (Leica SM2010R). After blocking with 10% donkey serum and permeabilization with 0.1% Triton-X-100 for 30 min at room temperature, slices were incubated in primary antibody (rabbit anti-tyrosine hydroxylase 1:500, Pel-Freeze; mouse anti-RFP 1:500, Rockland Immunochemicals; rabbit anti-cerebellin1, 1:250, Abcam) overnight at 4°C. Slices were then incubated in secondary antibody (donkey anti-mouse Alexa568, donkey anti-rabbit Alexa647, 1:1000, Invitrogen) for 1 h at room temperature and mounted onto slides for imaging with a Nikon 6D epifluorescence microscope or Olympus Fluoview FV1000 confocal microscope.