Supporting Information

Cui et al. 10.1073/pnas.1717106115

SI Materials and Methods

Animals. For in vivo behavioral studies, adult male C57BL/6J mice (8 wk old) were used. For in vitro studies, 7- to 10-wk-old C57BL/6J mice and FVB Drd2-EGFP BAC transgenic mice of both sexes were used. (Breeders were kindly provided by C. Savio Chan, Northwestern University, Evanston, IL.) The animals were bred and maintained by the Laboratory Animal Service Center of the Chinese University of Hong Kong (CUHK). The animal room was kept at a controlled temperature of 23 °C with controlled humidity on a 12-h light/dark cycle. All animals were handled in strict accordance with the CUHK guidelines on animal ethics.

Chemicals. CNQX, D-AP5, quinpirole, SKF38393, SKF81297, sulpiride, and TTX were purchased from Tocris Bioscience. 4-AP and 6-OHDA (H4381) were obtained from Sigma-Aldrich. DiI was a gift from Sun-On Chan, School of Biomedical Sciences, CUHK, Hong Kong.

Stereotaxic Surgery for in Vivo Studies. Microinjection needles were connected to a 0.5-μL Hamilton syringe (33-gauge) and were filled with purified, concentrated AAV (~1–2 × 10^{12} genome copies/mL) encoding ChR2-EYFP or NpHR-EYFP under control of the CaMKII promoter. C57BL/6J mice were anesthetized with pentobarbital sodium (40 mg /kg, i.p.) and placed gently in a stereotaxic frame (Narishige SR-5). Microinjection needles were unilaterally placed into the PrL [anteroposterior (AP): 1.8 mm; mediolateral (ML): 0.4 mm; dorsoventral (DV): 1.7 mm from dura] in either the left or right hemisphere, and 0.5 μL virus or 0.5 μL 0.9% saline in the sham group was injected over 5 min. The needle was left in place for an additional 10 min before retraction. The scalp incision was sutured, and postinjection analgesics were given for 3 d to aid recovery. Three weeks after virus injection, a total dose of 8 μg of 6-OHDA, dissolved in 2 μL of sterile 0.9% saline and 0.02% ascorbic acid (or 2 μL of 0.9% saline for vehicle injection in the sham group) was injected into the ipsilateral NAc core (AP: 1.6 mm; ML: 0.8 mm; DV: 4.0 mm from dura) by using another 2-μL Hamilton microsyringe (33-gauge) at a rate of 0.2 μL/min. After injection the needle was kept in place for an additional 10 min and then was slowly withdrawn. An optical fiber (200-μm core, NA = 0.48) or fluid-injection cannula (26-gauge guide cannula possessing a 32-gauge dummy cannula) was implanted directly above the ipsilateral NAc core (AP: 1.6 mm; ML: 0.8 mm; DV: 3.6 mm from dura). The sham control group received the same surgical procedure. The fiber or guide cannula, together with two stainless steel screws was secured to the skull using dental cement. Correct location of implanted fiber/cannula was confirmed postmortem. To determine the extent of lesion of dopaminergic terminals in the NAc and dorsal striatum, TH immunohistochemistry was performed as detailed below. Optical density from the core subregion of the NAc, dorsolateral striatum, and ventromedial striatum was measured using ImageJ software (NIH) and was compared between 6-OHDA–injected and vehicle-injected mice. The extent of dopamine depletion in the NAc core after 6-OHDA injection was also determined in vivo by standard HPLC. The impact of unilateral NAc dopamine on the general motor behavior was assessed by the open-field test and T-maze test. Specifically, mice at 2–3 wk following 6-OHDA injection or sham surgery were placed individually in a black plastic open field, and the spontaneous locomotor behavior was videotaped with a camera over the open-field arena for 5 min. Total distance traveled, total time mobile (%), and clockwise/anti-clockwise rotations were analyzed using Any-Maze software (version 4.70). For the T-maze test, mice were placed in the stem arm, and the left/right turn ratio was quantified in 10 trials.

Optogenetic Stimulation and in Vivo Drug Infusion. For the animal groups treated with optical stimulation, the light source consisted of a single, high-power LED (470 nm, 5.5 mW, or 590 nm, 3.2 mW for the ChR2 and NpHR experiments, respectively; ThorLabs) coupled to a 200-μm core, 0.53 NA standard multimode hard-cladding optical fiber (ThorLabs) that passed through a single-optical rotary joint (Doric Lenses). The intensity of light output was at least 25 mW/mm^2. The optical fiber implanted into the mice was connected just before starting each behavioral session. Continuous light stimulation was given in both the initial learning and strategy-switching phases, with a 1-h break between the tasks to minimize the carryover effect. For in vivo ChR2 experiments, mice were given phasic blue light (470 nm) illumination (10 pulses every 400 ms for each 10 s, 5 ms pulse width). For in vivo NpHR experiments, mice were exposed to 563 nm amber light (for each 10 s, the light was turned on for 8 s and off for 2 s). Ten minutes before behavioral task, in vivo drug infusion into the NAc core was made through the unilaterally implanted 26-gauge injection cannula attached to a 33-gauge Hamilton syringe. The concentrations of infused drugs were 300 μM SKF81297 and 200 μM sulpiride (Sigma-Aldrich), each delivered in a volume of 1 μL at a rate of 0.5 μL/min manually. For all behavioral experiments, mice were videotaped, and the behavior was evaluated offline.

Immunohistochemistry. To determine the extent of lesion of the dopaminergic terminals in the NAc, TH immunohistochemistry was performed after the behavior experiments or patch-clamp recording session, when applicable, as reported previously (1). Briefly, after behavioral experiments, mice were killed, and the brains were sectioned as patch-clamp recordings (see below). The slices containing the NAc from either behavioral experiments or patch-clamp recordings were fixed in 4% paraformaldehyde at room temperature overnight and then were rinsed in PBS and incubated with 30% H_2O_2 for 30 min. After another rinsing in PBS, the slices were blocked by 10% normal goat serum in PBS with 0.3% Triton X-100 for 30 min. Then the slices were incubated with anti-TH polyclonal antibody (1:400; AB152; Millipore) in the previous blocking solution for ~22 h. After thorough rinsing in PBS, the slices were incubated with polyclonal goat anti-rabbit IgG secondary antibody (1:100; P0448; DakoCytomation) in block solution. The sections were then rinsed in PBS and incubated with 3, 3′-diaminobenzidine-tetrahydrochloride (DAB) with peroxide (DAB substrate kit SK-4100; Vector Laboratories) for ~5–6 min, washed, collected on Superfrost Plus slides (Thermo Scientific), coverslipped, and examined with a Nikon Eclipse TE2000-U microscope. The images including the NAc were captured. The optical density from the core subregion of the NAc was measured using ImageJ software (NIH). The optical density from the infralimbic cortex was not affected by 6-OHDA lesion and was used for normalization of the optical density of the NAc of the same slice. For each area, the optical density was measured at three separate sites, and the values from the three sites were averaged. The normalized optical densities from the slices of the same side belonging to the same mouse were then averaged again. The final optical densities were compared between 6-OHDA–injected and vehicle-injected mice for bilaterally injected mice

or between the lesion side and unlesioned side for unilaterally injected mice.

To detect D1 and D2 receptor expression in control and knockdown groups, immunohistochemistry was performed after the behavior experiment. After deep anesthesia, mice were perfused with ice-cold saline followed by 4% paraformaldehyde in 0.1 M PBS, pH 7.4. The brains were removed, postfixed overnight in 4% paraformaldehyde at 4 °C, and transferred to 30% sucrose in 0.1 M PBS, pH 7.4. Coronal cryosections (40 μm) were prepared and stored in 0.1 M PBS. The sections were incubated in blocking buffer containing 5% normal goat serum in 0.2% Triton X-100/PBS (PBST) for 1 h at room temperature and then with primary antibodies in blocking buffer overnight at 4 °C (rabbit anti-D1 receptor, 1:200; ab20066; Abcam; rabbit anti-D2 receptor, 1:300; AB5084P; Merck Millipore). After three washes with PBST, sections were incubated with Alexa Fluor 546-conjugated secondary antibodies at room temperature for 1 h. After another three washes in PBST, sections were mounted with mounting medium (SLBT8376; Sigma). Images were captured by a Nikon Eclipse Ti-E inverted confocal microscope. Fluorescence intensity was analyzed with ImageJ software.

Tracing Study. Drd2-EGFP BAC transgenic mice aged 6–7 wk were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus. A red fluorescent tracing dye, DiI (0.2 μL), was injected into the PrL (AP: 2.0 mm, ML: 0.3 mm, DV: 2.2 mm ventral to the skull surface). Three weeks later, the animals were perfused with 4% paraformaldehyde transcardially, and the brains were taken out. Oblique slices (100 μm) ∼10° to the coronal plane to preserve the PrL–NAc projection were prepared as detailed below. Then the slices were rinsed in PBS, put on the slides, and coverslipped. Images were collected with a Nikon D-Eclipse C1 laser-scanning confocal unit attached to a Nikon Eclipse Ti fluorescence microscope.

Preparation of Prefrontal Cortex–NAc Brain Slices. Drd2-EGFP BAC transgenic mice and C57BL/6J mice were used for the preparation of acute brain slices. After decapitation, the brains were immediately removed in ice-cold artificial CSF (ACSF) (in mM: 120 NaCl, 2.0 KCl, 26 $NaHCO_3$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, and 11 glucose). For the preparation of PrL–NAc projections, the brains of Drd2-EGFP BAC transgenic mice were manually cut at angle of ∼10° to the coronal plane, based on a previous report (2), and were glued onto the stage of an Intergraslice 7550MM vibrotome (Campden Instrument) as well as fixed by agar blocks. The stage was then immersed in ice-cold ACSF continuously bubbled with 95% O_2/5% CO_2. Oblique slices (250 μm) were cut and transferred to a holding chamber containing ACSF continuously bubbled with 95% O_2/5% CO_2. After equilibration in 35 °C ACSF for 30 min, the slices were returned to room temperature before subsequent recordings.

Whole-Cell Patch-Clamp Recordings. Slices were transferred to a recording chamber mounted on an upright microscope (Zeiss Axioskop) and were perfused with ACSF at a rate of ∼1.0 mL/min at room temperature. Neuronal soma and proximal dendrites of neurons were directly visualized by a combination of differential-interference contrast optics and contrast-enhanced video microscopy. Bright green fluorescent MSNs (D2-MSNs) identified via epifluorescence microscopy, as well as negatively identified putative D1-MSNs, unidentified prelimbic cortical neurons, and NAc neurons, were recorded using a patch-clamp amplifier (Multiclamp 700B; Axon Instruments). The identity of D2-MSNs had been confirmed by a single-cell RT-PCR experiment previously (3, 4). Recording pipettes typically had a resistance of 3–6 MΩ. For most of the recordings, the internal solution contained (in mM): 130 K-gluconate, 10 KCl, 10 Hepes, 1 EGTA, 2 $MgCl_2$, 2 Na_2-ATP, and 0.4 Tris-GTP, and the pH was adjusted to 7.25–7.30 with 1 M KOH. For recording the current–voltage relationship of eEPSCs, the internal solution consisted of (in mM): 125 $CsMeSO_3$, 10 Na_2phosphocreatine, 5 Hepes, 5 tetraethylammonium-Cl, 2 Mg_2ATP, 1 QX314-Cl, 0.5 Na_3GTP, 0.25 EGTA, and 0.2% (wt/vol) biocytin, pH adjusted to 7.25–7.30 with CsOH. With monitoring through a television connected to the camera, a pipette was placed on the soma of a selected neuron, and conventional whole-cell recordings in voltage-clamp and current-clamp configurations were made. EPSCs in identified MSNs in the NAc were evoked by delivering stimuli (0.2 ms) at 20-s intersweep intervals to the PrL using a parallel bipolar stimulating electrode. Drugs were applied directly to the perfusate. Normally no series resistance compensation was applied, but the cell was rejected if the series resistance changed significantly (>20%) during recording. Signals were sampled at 10 kHz, filtered at 3 kHz, and acquired using the DigiData pCLAMP system (Axon Instruments). The data of evoked responses from the whole-cell patch-clamp recordings were analyzed offline using Clampfit 10.2 (Molecular Devices). For experiments with drug application, a stabilized part of drug effect was taken for comparison with baseline.

Cre-Dependent AAV-shDRD1a and AAV-shDRD2.

Plasmid construction. D1- and D2-overexpressing AAVs were constructed first. Total mRNAs were extracted from mouse cortical tissues and were converted to cDNAs by reverse transcription using RNAiso Plus and PrimeScript Reverse Transcriptase (Takara Bio, Inc.) according to the manufacturer's instructions. *Drd1a* and *Drd2* genes were cloned from the mouse cDNAs by PCR using PrimeSTAR HS (Premix) (Takara Bio, Inc.). DNA sequencing results showed that the *Drd2* gene cloned was in the D2L isoform. The paavCAG-iCre plasmid (Addgene no. 51904) was first modified by replacing the iCre gene with short oligonucleotides encoding the HA-tag together with the downstream restriction sites NheI and XhoI. The short oligonucleotide sequence was ligated into the plasmid at the BamHI and HindIII restriction sites, replacing the original iCre gene from the original plasmid, and was named "pAAV-CAG-HA." The PCR products of the *Drd1a* and *Drd2* genes were ligated into the pAAV-CAG-HA plasmid at the NheI and XhoI restriction sites and were named "pAAV-CAG-D1" and "pAAV-CAG-D2," respectively. The pAAV-hSyn-Cre plasmid was constructed by ligating the iCre gene from the paavCAG-iCre plasmid (Addgene no.51904) into pAAV-hSyn-EGFP plasmid (Addgene no. 50465) at the BamHI and HindIII restriction sites, replacing the EGFP gene from the pAAV-hSyn-EGFP plasmid. To construct the Cre-dependent D1- and D2-knockdown AAV plasmids, the pSico plasmid (Addgene no. 11578) was used. shRNA sequences targeting D1 and D2 were ligated into the pSico plasmid at the HpaI and XhoI restriction sites separately, and shRNA sequences showing high knockdown efficacies were identified in vitro. The sequence between the XbaI and the second HindIII restriction sites was cloned by PCR. The cloned DNA fragments were ligated into an AAV plasmid (Addgene no. 50465) at the XbaI and HindIII restriction sites, replacing the promoter and EGFP gene from the original AAV plasmid.

Primers for shDRD1a.

F: 5′-TGCCATTATGATCGTCACTTACCTCGAGGTAAGTGACGATCATAATGGCTTTTTC

R: 5′-TCGAGAAAAAGCCATTATGATCGTCACTTACCTCGAGGTAAGTGACGATCATAATGGCA

Primers for shDRD2.

F: 5′-TCCGTTATCATGAAGTCTAATGCTCGAGCATTAGACTTCATGATAACGGTTTTTC

R: 5′-TCGAGAAAAACCGTTATCATGAAGTCTAATGC-TCGAGCATTAGACTTCATGATAACGGA

Primers for pAAV-CAG-HA construction.

F: 5′-GATCCATGTACCCATACGATGTTCCAGATTAC-GCTCTAGCTAGCTAGTTGGGTCGAGATCCGCTCGA-GCGGAAAATCTCCAGTA

R: 5′-AGCTTACTGGAGATTTTCCGCTCGAGCGGATC-TCGACCCAACTAGCTAGCTAGAGCGTAATCTGGA-ACATCGTATGGGTACATG

Primers for DRD1a overexpression AAVs.

F: 5′-CTAGCTAGCGCCACCATGGCTCCTAACACTTC-TACC

R: 5′-CCGCTCGAGTCAGGTTGAATGCTGTCCGCT

Primers for DRD2 overexpression AAVs.

F: 5′-CTAGCTAGCGCCACCATGGATCCACTGAACCT-GTCC

R: 5′-CCGCTCGAGTCAGCAGTGCAGGATCTTCATG

AAV packaging. HEK293T cells were triple-transfected with AAV helper plasmid, AAV DJ plasmid, and the AAV vector plasmid at a 1.6:1:1 ratio using polyethylenimine (PEI). Seventy-two hours after transfection, cell pellets of the HEK293T cells were collected and were resuspended with cold hypotonic buffer [10 mM Hepes (pH 7.4), 1.5 mM M_gCl_2, 10 mM KCl, and 1.7 mM spermine] and were incubated on ice for 10 min. Then 10× restore buffer [10 mM Hepes (pH 7.4), 1.5 mM M_gCl_2, 10 mM KCl, 1.7 mM spermine, and 1.8 mM sucrose] was added. The cell suspensions were homogenized on ice and were sonicated. The contents were centrifuged in iodixanol gradients at 400,000 × *g* for 1 h at 18 °C. The viral solution was extracted and purified by repeated washing with PBS using an Amicon Ultra-15 Centrifugal Filter Unit with an Ultracel-100 membrane (Millipore). The AAV was aliquoted and stored at –80 °C.

AAV titer. AAV titer was determined by qPCR using the SYBR Green system (5). Plasmid DNA with a known concentration (in molecules per microliter) of the corresponding AAV was used in the qPCR reaction to generate a standard curve for estimation of the viral titer. Viral particles were treated with DNase I and incubated for 30 min at 37 °C to eliminate any external DNA. Plasmid DNA and AAV particles underwent serial dilution with nuclease-free water and acted as the template in the qPCR reaction for standard curves generation or titer determination, respectively. qPCR was performed. Primers targeting the AAV inverted terminal repeat region were

F: 5′-GGAACCCCTAGTGATGGAGTT

R: 5′-CGGCCTCAGTGAGCGA

The titers for AAVs used were AAV-CAG-D1: 2.23×10^{12} viral genomes (vg)/mL; AAV-CAG-D2: 3.21×10^{12} vg/mL; AAV-CAG-Cre: 5.38×10^{12} vg/mL; AAV-hSyn-Cre: 3.56×10^{12} vg/mL; AAV-shDRD1a: 2.32×10^{12} vg/mL; AAV-shDRD2: 2.81×10^{12} vg/mL.

In vivo validation. C57BL/6J mice (6 wk old) were injected with 1 μL AAV-hSyn-Cre virus stereotaxically and unilaterally at the PrL. Ten days after the injection of Cre virus, the mice received Cre-dependent D1- or D2-knockdown AAVs at the same sites. Control mice were injected with D1 or D2 shRNA AAV without receiving the prior injection of AAV-hSyn-iCre virus. The mice were allowed to recover from surgery for an additional 21 d. The PrL tissues were dissected and processed with real-time qPCR to confirm the knockdown efficacy of D1 or D2 shRNA AAV.

Real-Time qPCR. Lysates obtained from cell culture or cortical tissues were treated with RNAiso Plus (Takara Bio, Inc.) according to the manufacturer's instructions. Total mRNAs were extracted and quantified with a NanoDrop 2000 spectrophotometer (Sigma-Aldrich). cDNA was prepared from the extracted RNA by PrimeScript Reverse Transcriptase (Takara Bio, Inc.) according to the manufacturer's recommended protocol. RT-PCR was performed with SYBR Premix Ex Taq II (Tli RNase H Plus) (Takara Bio, Inc.) used according to the manufacturer's recommendations. The PCR was monitored by iQ5 Multicolor Real Time RT PCR Detection system and iQ5 Optical System Software version 2.1 (Bio-Rad).

1. Dehorter N, et al. (2009) Dopamine-deprived striatal GABAergic interneurons burst and generate repetitive gigantic IPSCs in medium spiny neurons. *J Neurosci* 29: 7776–7787.
2. Yang CR, Seamans JK, Gorelova N (1996) Electrophysiological and morphological properties of layers V-VI principal pyramidal cells in rat prefrontal cortex in vitro. *J Neurosci* 16:1904–1921.
3. Wang Z, et al. (2006) Dopaminergic control of corticostriatal long-term synaptic depression in medium spiny neurons is mediated by cholinergic interneurons. *Neuron* 50: 443–452.
4. Day M, et al. (2006) Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models. *Nat Neurosci* 9:251–259.
5. Aurnhammer C, et al. (2012) Universal real-time PCR for the detection and quantification of adeno-associated virus serotype 2-derived inverted terminal repeat sequences. *Hum Gene Ther Methods* 23:18–28.

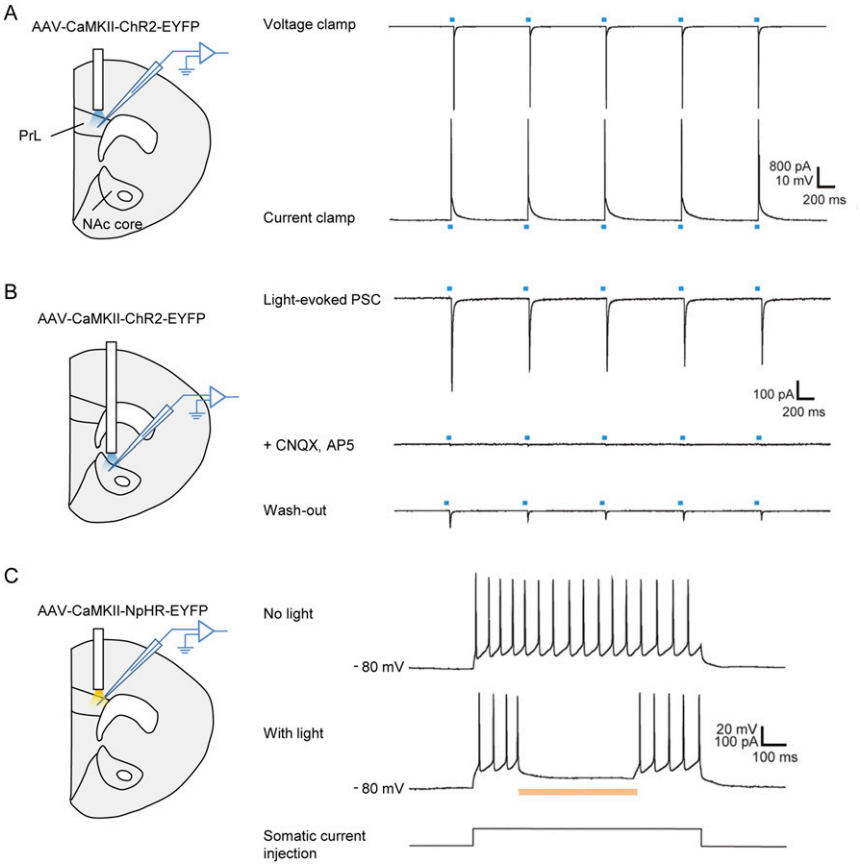

Fig. S1. Verification of the effectiveness of the optogenetic stimulation and inhibition protocols based on brain-slice preparation. Adult mice were injected with AAV expressing ChR2 or NpHR according to the schematics shown in Fig. 1. Four weeks after virus injection, brain slices containing the PrL and/or the NAc were prepared. (*A*) Whole-cell recordings were made from AAV-ChR2–infected PrL neurons. Delivery of blue-light stimuli (470 nm, 10 ms, 1 Hz, 2 mW) could robustly evoke inward currents in voltage-clamp (*Upper Trace*) or membrane depolarization in current-clamp (*Lower Trace*) configurations. The same phenomenon can be found in four other neurons. (*B*) When recordings were made from NAc MSNs, delivery of blue-light stimuli (470 nm, 20 ms, 1 Hz, 5 mW) onto the NAc to activate nerve terminals of the PrL–NAc pathway could evoke inward-going postsynaptic currents (PSCs) that can be blocked by CNQX and D-AP5, which are an AMPA receptor antagonist and a NMDA receptor antagonist, respectively. The light-evoked postsynaptic current can be partially restored after washout of the antagonists. (*C*) In PrL neurons infected with AAV-NpHR, injection of depolarizing current via the patch-pipette evoked robust firing (*Upper Trace*), which could be suppressed after activation of NpHR by delivery of amber light stimuli (500 ms, 0.15 mW) (*Lower Trace*).

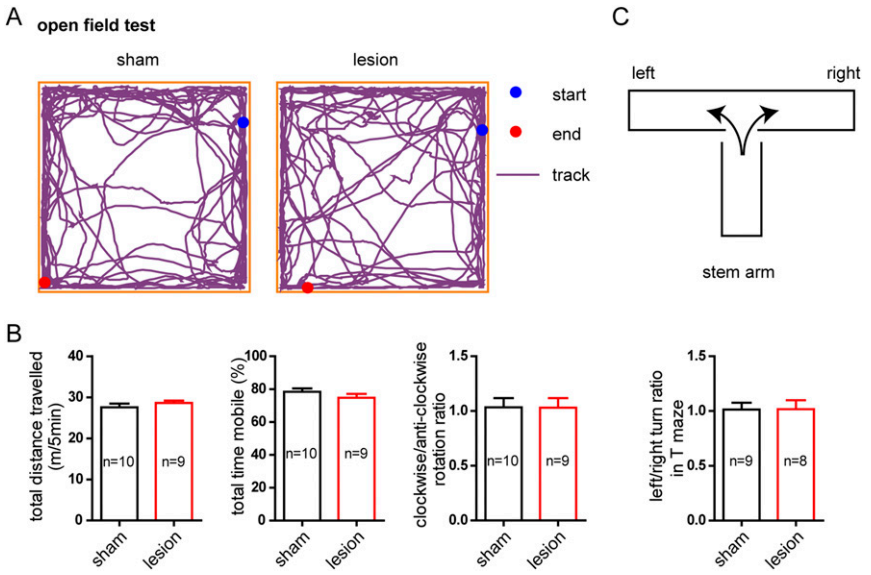

Fig. S2. General motor ability and turn bias are not affected by unilateral dopamine depletion in the NAc. (*A*) Examples of the performance in an open-field arena from one sham mouse and one lesioned mouse. (*B*) Mobility, as measured by total distance traveled and total percentage of time mobile in a 5-min open-field test, was not impaired in lesioned mice compared with sham control. In addition, no rotational bias (rotation ratio at ∼1) was observed in these unilaterally dopamine-depleted mice. (*C*) Consistently, in the T-maze test there was no overall turn bias in lesioned mice, similar to the sham group. Data are presented as mean ± SEM.

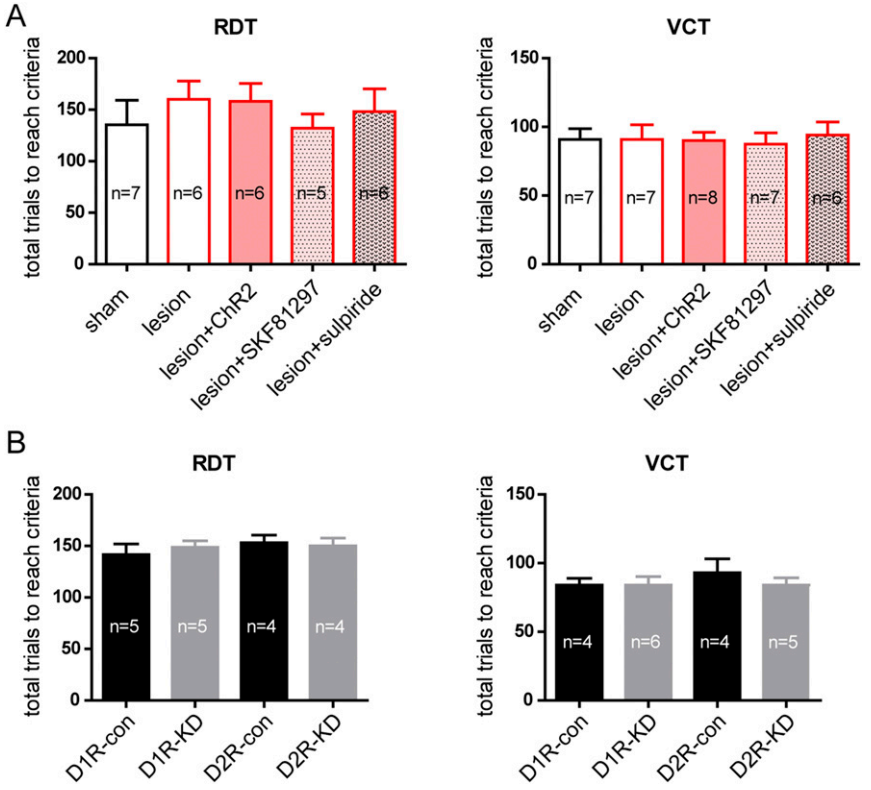

Fig. S3. Lack of effect of 6-OHDA lesion, 6-OHDA lesion in combination with optogenetic and pharmacological manipulation, or dopamine receptor knockdown on learning of the RDT and the VCT per se. (*A*) In both the RDT (*Left*) and VCT (*Right*), there were no differences in the total number of trials needed to reach the criterion among the sham group, the 6-OHDA lesioned group, or the lesioned groups with ChR2 stimulation or pretreatment with the D1-type agonist SKF81297 or the D2-type antagonist sulpiride (one-way ANOVA). (*B*) Similarly, knockdown of D1 receptors or D2 receptors did not impact the total number of trials needed to reach the criterion in both the RDT and the VCT compared with the respective controls. Data are presented as mean ± SEM.

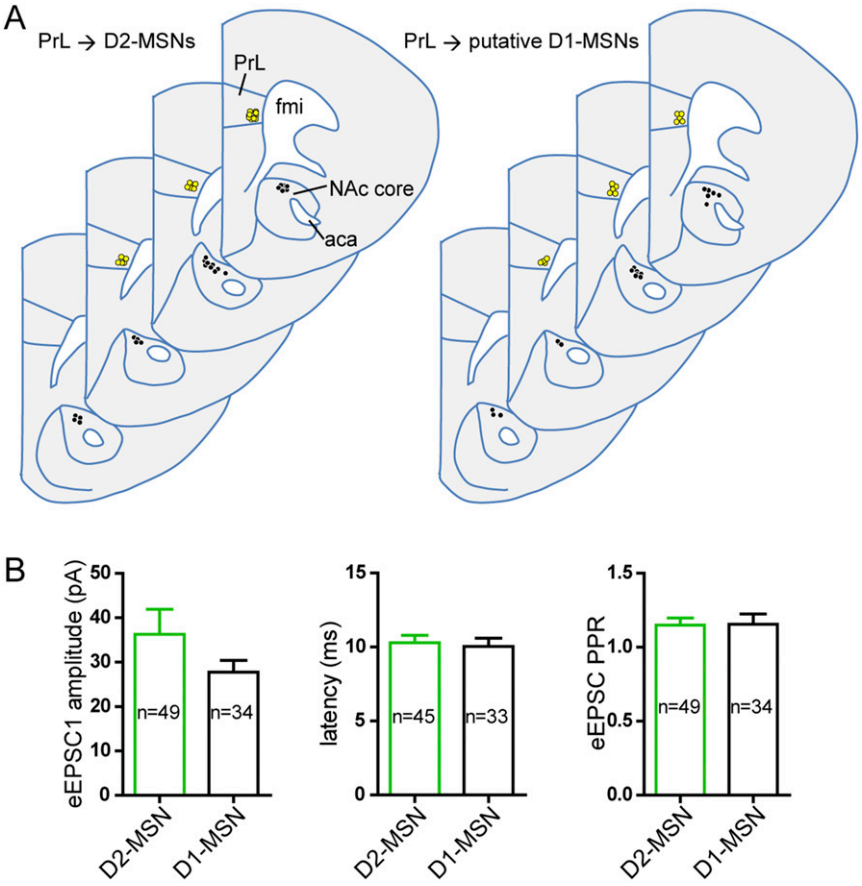

Fig. S4. The PrL sends glutamatergic projections to both D2-MSNs and putative D1-MSNs in the NAc core subregion. (*A*) Innervation of the PrL to D2-MSNs and putative D1-MSN (nonfluorescent soma in Drd2-EGFP BAC transgenic mice) demonstrated by recorded eEPSCs following focal stimulation in the PrL in brain slices. Distribution of the effective stimulation sites in the PrL (yellow) and recording sites in the NAc core (black) are shown. aca, anterior commissure, anterior part; fmi, forceps minor of the corpus callosum. (*B*) No statistical differences were found in the amplitudes of the eEPSCs between the D2-MSNs and putative D1-MSNs. Data are presented as mean ± SEM.

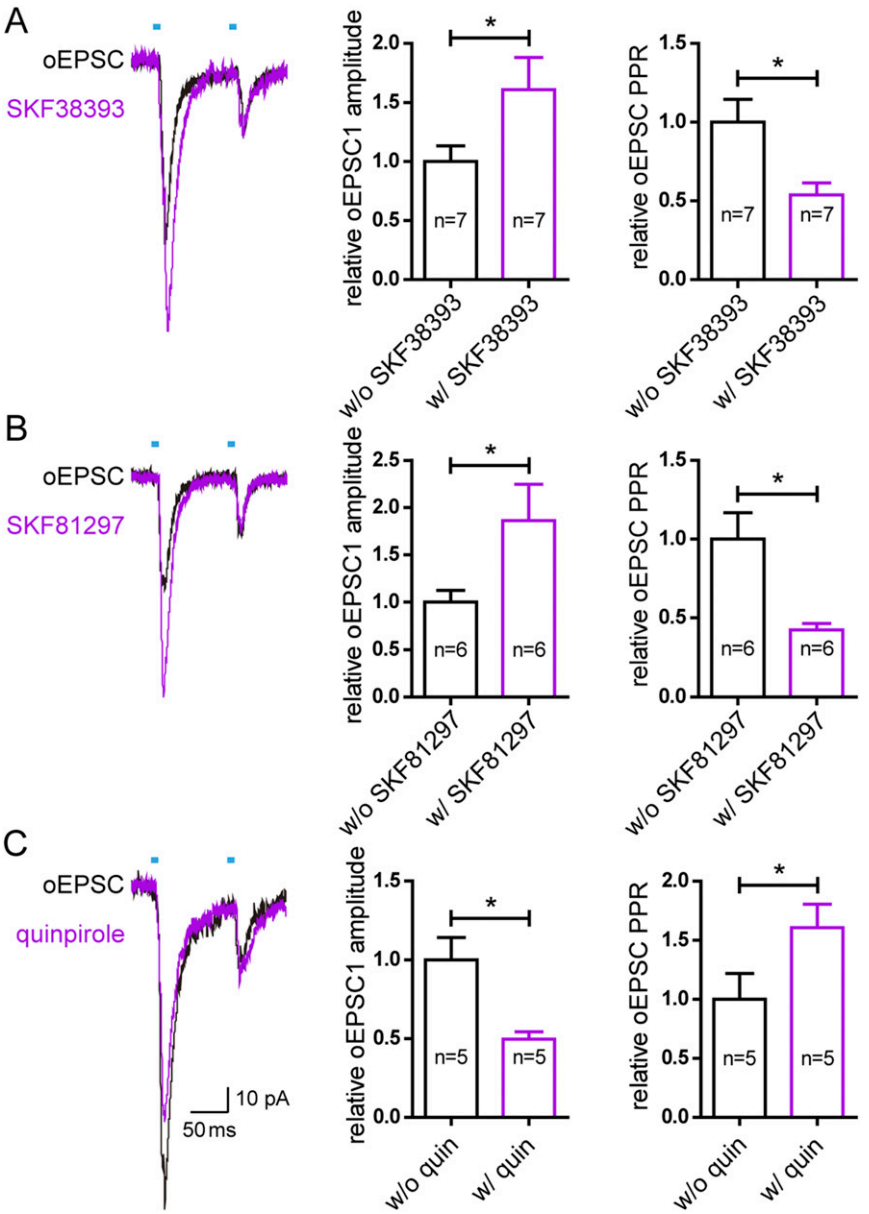

Fig. S5. Presynaptic dopamine receptor-mediated modulation of PrL-NAc transmission is maintained with optogenetic stimulation-evoked glutamate release from the PrL. Effects of the D1-type receptor partial agonist SKF38393 (*A*), the D1-type receptor full agonist SKF81297 (*B*), and the D2-type receptor agonist quinpirole (*C*) on the normalized amplitude and PPR of oEPSCs evoked by optical stimulation of ChR2-expressed prelimbic terminals in the NAc. The concentrations of the drugs used were the same as those used in the experiments shown in Fig. 5. *$P < 0.05$, paired *t* test. Data are presented as mean ± SEM.

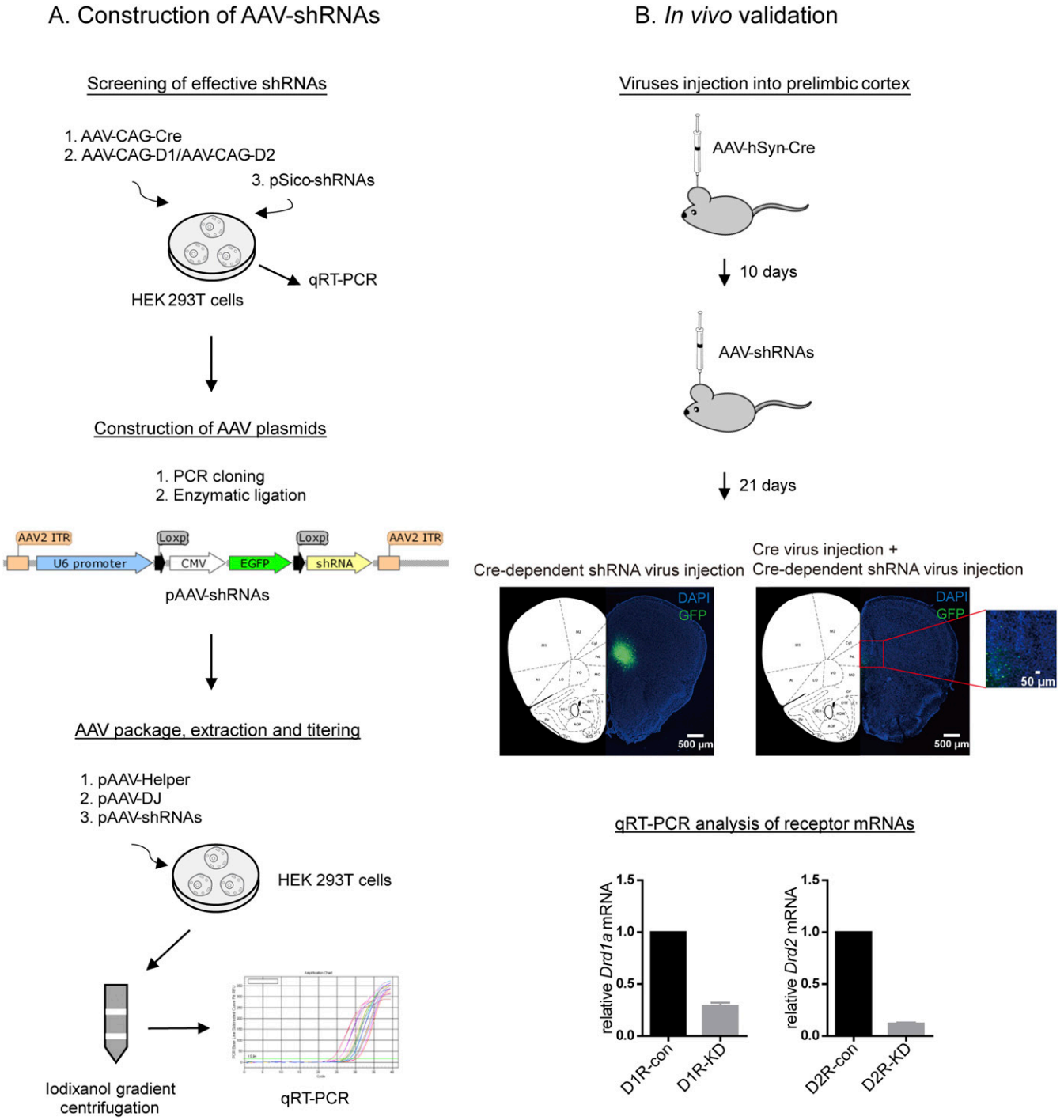

Fig. S6. Summary of the construction and validation of D1R-shRNA and D2R-shRNA. (*A*) Procedures of screening of effective shRNA, construction of AAV plasmids, as well as AAV packaging, extraction, and titering, are illustrated. (*B*) Validation of the cre-dependent AAV-shRNAs was performed by injecting the virus into the mice that had been preinjected with AAV-hSyn-Cre at the same site in the PrL. Control mice received injections of AAV-shRNA but not AAV-hSyn-Cre. Three weeks after AAV-shRNA injection, the target of the injections was confirmed with histology. Prelimbic cortical tissue was collected and subject to RT-qPCR analysis to confirm the efficacy of the knockdown. The experiments were repeated in triplicate. Data are presented as mean ± SEM.