Supporting Information

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SI Materials and Methods

Generation of DARPP-32 Conditional KO Mice. Floxed DARPP-32 mice were generated by Cell & Molecular Technologies. A targeting vector was generated in which loxP sites were inserted surrounding exons 1–4 of the mouse DARPP-32 gene. An FRT flanked neomycin cassette was inserted between exon 4 and the 3′ loxP site. The targeting vector included right and left homology arms, which were used to target the construct to the endogenous DARPP-32 locus. The targeted construct was electroporated into ES cells derived from C57BL/6 mice. Neomycin-resistant clones were screened by Southern blot analysis for correct homologous recombination using a 3′ external probe and a 5′ internal probe and EcoRV digestion. The neomycin cassette was excised in vitro by expression of FLPe recombinase to generate the final conditional floxed allele. A Southern blot strategy was used to detect cells with correct excision of the Neo cassette. These cells were used to generate chimeras by injection into C57BL/6 blastocysts using standard techniques. Germline transmission was confirmed by PCR genotyping, and heterozygous floxed DARPP-32 mice were interbred to generate homozygous floxed DARPP-32 mice.

Floxed DARPP-32 mice were bred to either D1R-Cre (EY262) or D2R-Cre (ER44) BAC transgenic mice (1) to generate DARPP-32 conditional KO mice. To generate experimental animals, homozygous floxed Cre-positive mice were bred to homozygous floxed Cre-negative mice. The resulting offspring were all homozygous floxed and either hemizygous or negative for D1R-Cre or D2R-Cre. Mice used in experiments were >90% C57BL/6 background as determined by single nucleotide polymorphism genotyping for strain-specific markers. The following primers were used for genotyping from tail DNA:

Floxed DARPP-32	For 5′-CTAGTTGTCCTAGGTGTCCTGTGCTCTGTC-3′
	Rev 5′-CCCTTCAACCTACTGTTAAGACCCTAGCTG-3′
	wt band 375 bp; floxed band 485 bp
D1R-Cre	For 5′-AGGGGCTGGGTGGTGAGTGATTG-3′
	Rev 5′-CGCCGCATAACCAGTGAAACAGC-3′
	451 bp band
D2R-Cre	For 5′-AGCATGCCTTGAAAACACTCCTG-3′
	Rev 5′-CACCGGCATCAACGTTTTCTTTTC-3′
	458 bp band

To verify cell type–specific deletion of DARPP-32, conditional KO mice were bred to BAC transgenic mice expressing EGFP under the control of the D1R or D2R promoters (2).

Western Blot Analysis. Mice were killed by focused microwave irradiation and striata were rapidly removed and frozen at −80 °C. Striata were sonicated and boiled in 1% SDS, and total protein concentration was determined using a BCA assay (Pierce). Then 15 μg of protein was loaded onto 10.5–14% Tris-HCl gels, separated by electrophoresis, and transferred to PVDF membranes. Membranes were blocked for 1 h in 5% milk in TBS/Tween-20 and incubated overnight at 4 °C with a primary antibody against DARPP-32 (3). Antibody binding was revealed by HRP-conjugated goat anti-mouse IgG and the ECL detection method (PerkinElmer) using Kodak BioMax film. Membranes were stripped (Reblot Plus strong stripping buffer; Chemicon) and reprobed using an antibody against actin (Abcam). Quantification of bands was done by densitometry, using National Institutes of Health Image software version 1.52.

Immunofluoresence. Mice were perfused transcardially with 1× PBS and 4% paraformaldehyde. Brains were postfixed in 4% paraformaldehyde for 1 h, cryoprotected in sucrose, and frozen at −80 °C until sectioning at 12 μm (Fig. 2 *A–D*). Alternatively, brains were postfixed overnight in the same solution and stored at 4 °C. Then 30-μm-thick sections were cut with a vibratome (Leica) and stored at −20 °C in a solution containing 30% (vol/vol) ethylene glycol, 30% (vol/vol) glycerol, and 0.1 M sodium phosphate buffer until they were processed for immunofluorescence (Fig. 2 *E–G*). Sections were blocked for 1 h in 2% normal serum and incubated overnight at 4 °C with antibodies against NeuN (Chemicon), DARPP-32 (Novus), and/or GFP (provided by Dr. Myriam Heiman, Rockefeller University, NY, NY). Slides were washed and incubated with Alexa Fluor 488– (Invitrogen), Cy2-, and/or Cy3- (Jackson ImmunoResearch) conjugated secondary antibodies. Fluorescent images were obtained with a Zeiss confocal microscope.

Electrophysiology. BAC mice expressing EGFP in D1R or D2R neurons (2) were bred to the $D32^{f/f}D1RCre^{+}$ and $D32^{f/f}D2RCre^{+}$ mice, respectively, to identify D1R and D2R MSNs for electrophysiology. Parasagittal corticostriatal slices were obtained from 19- to 30-d-old $D32^{wt/wt}D1RCre^{+}D1RGFP^{+}$ and $D32^{wt/wt}D2RCre^{+}D2RGFP^{+}$ (controls) or $D32^{F/F}D1RCre^{+}D1RGFP^{+}$ and $D32^{F/F}D2RCre^{+}D2RGFP^{+}$ (conditional KO) mice. Experiments were performed in the dorsal striatum at room temperature. Electrical access was achieved through the perforated-patch method using amphotericin B. EPSPs were evoked by focal extracellular stimulation with a small theta glass electrode positioned ∼100 μm from the recording electrode. Stimulation intensity (0.2 ms, 5–30 μA) was adjusted to evoke baseline single-component EPSPs. Back-propagating APs were evoked by direct somatic current injection (5 ms, 1–1.5 nA). Long-lasting synaptic plasticity was induced using a protocol consisting of subthreshold synaptic stimulation paired with bAPs at theta frequency (5 Hz). This protocol consisted of 10–15 trains of five bursts repeated at 0.1 Hz, with each burst composed of three bAPs preceded with three EPSPs at 50 Hz. To ensure induction of consistent synaptic plasticity, postsynaptic neurons were depolarized to −70 mV from their resting membrane potentials (typically −85 mV) during induction. $GABA_A$ receptors were blocked by the bath application of bicuculline methiodide (10 μM).

Open-Field Testing of Basal Locomotor Activity. Mice were brought into the testing room in their home cages at least 1 h before testing. The 8- to 12-wk-old female and male mice were kept separate and tested on different d. Mice were placed in the open-field chambers consisting of 38 cm (L) × 25 cm (W) × 17 cm (H) white plastic boxes covered with a clear Plexiglas lid left open at the edge for ventilation. Four mice were tested simultaneously using four identical side-by-side chambers. Locomotor activity was recorded using an overhead Panasonic digital camera. Mice were tracked using EthoVision software version 3.1 (Noldus), which sampled the *x/y* location of the mice in the chambers six times per second and recorded the distance traveled in centimeters in 3-min bins.

Locomotor Response to Cocaine. 8–12 wk-old male mice were injected with saline or cocaine (15 mg/kg i.p.) and immediately placed individually into a clean plastic mouse cage (18 cm × 28 cm)

similar to their home cage but containing only a small amount of Sani-chip bedding. Movement was monitored by five photobeams in one dimension (Photobeam Activity System; San Diego Instruments) for 2 h in a dark room, with the number of beam breaks recorded every 5 min. Mice were injected with saline for the first 3 testing d to habituate them to the injections, handling, and testing conditions.

Haloperidol-Induced Catalepsy. 8–12 wk-old mice were injected with haloperidol (1.5 mg/kg i.p.) and returned to their home cage for 60 min until testing. Testing was performed in a quiet room in which they would not be disturbed by extraneous noise or movement. Each mouse was individually placed so that its forelimbs rested on a wire bar (0.2 cm diameter) located 4.5 cm above the countertop with its hind limbs on the countertop. If the mouse moved immediately, then it was replaced on the bar for up to five attempts. A mouse that never remained on the bar in these five trials was given a score of 0 seconds. Once the mouse remained immobile after release, the time until the first removal of a front paw or sustained head movement was recorded. The test was repeated three times for each animal, and the average catalepsy time was calculated. Haloperidol (Sigma-Aldrich) was dissolved in 100 μL of glacial acetic acid, followed by saline, and the final pH was adjusted to 5.5–5.8.

6-Hydroxydopamine Lesion. 8–12 wk-old mice were anesthetized with a mixture of fentanyl citrate (0.315 mg/mL), fluanisone (10 mg/mL; VetaPharma), and midazolam (5 mg/mL; Hameln Pharmaceuticals), in water (1:1:2 in a volume of 10 mL/kg) and mounted in a stereotaxic frame (David Kopf Instruments) equipped with a mouse adaptor. The 6-OHDA-HCl was dissolved in 0.02% ascorbic acid in saline at a concentration of 3 μg of free-base 6-OHDA/μL. As described previously (4), each mouse received two unilateral injections of 6-OHDA (2 μL/injection) into the right striatum according to the following coordinates (in mm): AP +1, ML −2.1, DV −3.2 and AP +0.3, ML −2.3, DV −3.2. The mice were allowed to recover for 3 wk before drug treatment and behavioral evaluation.

AIMS. Mice were treated for 10 d with one injection per day of L-DOPA (20 mg/kg) along with benserazide hydrochloride (12 mg/kg). Abnormal involuntary movements (AIMS) were assessed after the last injection on day 10 using a previously established AIMS scale (5). Twenty minutes after L-DOPA administration, mice were placed in separate cages and individual AIMS were assessed for 1 min every 20 min over a 120-min period. AIMS were classified into four subtypes: locomotive (contralateral turns), axial (dystonic posturing of the upper part of the body toward the side contralateral to the lesion), limb (abnormal movements of the forelimb contralateral to the lesion), and orolingual (vacuous jaw movements and tongue protrusion). Each subtype was scored on a severity scale of 0–4: 0, absent; 1, occasional; 2, frequent; 3, continuous; and 4, continuous and not interruptible by outer stimuli. A lower total AIMS score indicates less dyskinetic behavior.

1. Gong S, et al. (2007) Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci* 27:9817–9823.
2. Gong S, et al. (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425:917–925.
3. Hemmings HC, Jr, Greengard P (1986) DARPP-32, a dopamine- and adenosine 3′:5′-monophosphate–regulated phosphoprotein: Regional, tissue, and phylogenetic distribution. *J Neurosci* 6:1469–1481.
4. Santini E, et al. (2007) Critical involvement of cAMP/DARPP-32 and extracellular signal–regulated protein kinase signaling in L-DOPA–induced dyskinesia. *J Neurosci* 27: 6995–7005.
5. Lundblad M, Picconi B, Lindgren H, Cenci MA (2004) A model of L-DOPA–induced dyskinesia in 6-hydroxydopamine lesioned mice: Relation to motor and cellular parameters of nigrostriatal function. *Neurobiol Dis* 16:110–123.

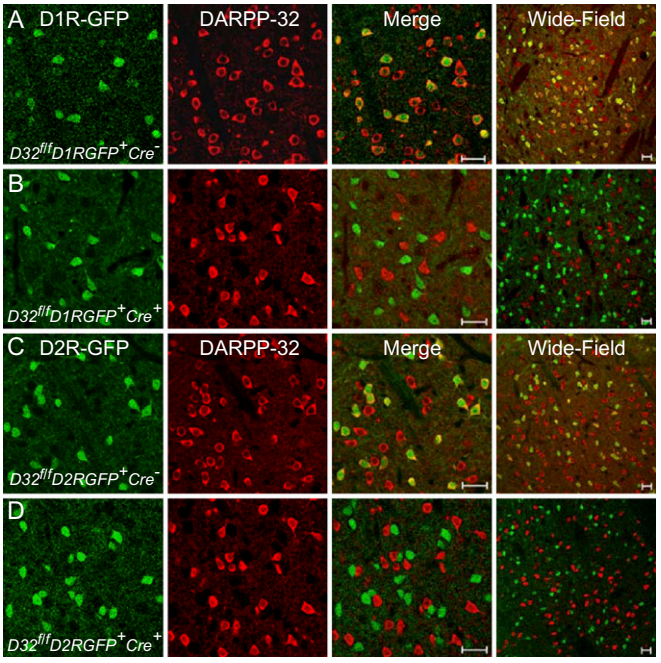

Fig. S1. Cell type-specific deletion of DARPP-32. DARPP-32 conditional KO mice were bred to BAC transgenic mice expressing EGFP from the D1R or D2R promoters. Striatal sections were immunostained with antibodies against GFP (green) and DARPP-32 (red). (Scale bar: 20 μm.) (*A* and *B*) Sections from a D32f/fD1RGFP^{+}Cre^{+} conditional KO mouse show selective loss of DARPP-32 in D1R-GFP–positive neurons. (*C* and *D*) Sections from a D32f/fD2RGFP^{+}Cre^{+} conditional KO mouse show selective loss of DARPP-32 in D2R-GFP–positive neurons.

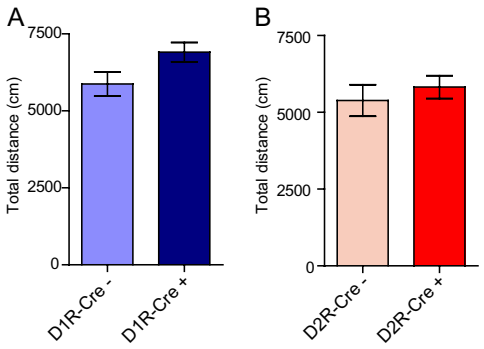

Fig. S2. Open-field locomotor activity of D1R- and D2R-Cre mice. Locomotor activity of BAC mice positive (+) or negative (–) for D1R-Cre (*A*) or D2R-Cre (*B*) (WT for the DARPP-32 gene) was measured by placing mice in an open-field chamber and recording the total distance traveled in centimeters over a 30-min period. *n* = 6–8 mice per genotype. Data are expressed as group mean; error bars represent SEM.

Table S1. Body weight at 8 wk, g

	$D32^{f/f}D1RCre^{-}$	$D32^{f/f}D1RCre^{+}$	$D32^{f/f}D2RCre^{-}$	$D32^{f/f}D2RCre^{+}$
Males	23.1 ± 0.3	23.6 ± 0.6	24.2 ± 0.3	24.6 ± 0.4
Females	19.6 ± 0.7	19.0 ± 0.7	20.0 ± 0.5	19.3 ± 0.6