# Supporting Information

## Ding et al. 10.1073/pnas.1006511108

### SI Materials and Methods

**Aphakia Mice.** Homozygous *Pitx3*$^{ak/ak}$ and heterozygous *Pitx3*$^{ak/+}$ aphakia mice were obtained by crossing homozygous *Pitx3*$^{ak/ak}$ with heterozygous *Pitx3*$^{ak/+}$ mice. Heterozygous *Pitx3*$^{ak/+}$ were obtained by crossing homozygous *Pitx3*$^{ak/ak}$ mice congenic in the C57BL/6 genetic background with wild-type C57BL/6 mice. Homozygous *Pitx3*$^{ak/ak}$ and heterozygous *Pitx3*$^{ak/+}$ mice were separated based on the phenotype of small, closed eyes in homozygous littermates because of abnormal lens development (1). Both age- and sex-matched mice were used initially starting at 6 to 10 wk of age for repeated treatment with either saline or L-DOPA (see below). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Chicago and the animals were maintained at pathogen-free environment with free access to food and tap water.

**The 6-Hydroxydopamine Lesion.** Heterozygous *Pitx3*$^{ak/+}$ mice (4–5 mo old) were used for unilateral 6-OHDA (6-hydroxydopamine) lesion as there were no biochemical differences between wild-type C57BL/6 and heterozygous *Pitx3*$^{ak/+}$ mice (Fig. S2) and *Pitx3*$^{ak/+}$ mice do not exhibit L-DOPA–induced dyskinesia (Fig. S3). This process allowed us to match the general gene background to homozygous *Pitx3*$^{ak/ak}$ mice. The surgical procedure used was the same as in our previous publication (2). *Pitx3*$^{ak/+}$ mice received one dose of either 1.125, 2.25, or 4.5 μg (repeated L-DOPA group) or 3.0 μg (acute L-DOPA group) of 6-OHDA–free base in 1.5 μL of 0.05% ascorbic saline into the left medial forebrain bundle at the following coordinates: AP (−1.3 mm), Lateral (+1.1 mm), ventral to skull surface (−5.4 mm) (3). Desipramine (25 mg/kg) was given 30 min prior the infusion of 6-OHDA to protect norepinephrine neurons.

**Drug Treatment and Behavioral Tests.** Homozygous *Pitx3*$^{ak/ak}$ and heterozygous *Pitx3*$^{ak/+}$ mice were treated repeatedly with either saline or L-DOPA (25 mg/kg) together with benserazide (6 mg/kg; Sigma-Aldrich), twice daily (at 8:00 AM and 4:00 PM) intraperitoneally for various time periods. The dose of L-DOPA used (25 mg/kg) did not interfere with feeding because the body weight of aphakia mice at the end of 7 wk repeated administration of 25 mg/kg L-DOPA was significantly increased by 3.6 g (SEM = 0.4), compared with initial body weight before drug treatment, and was comparable to the weight gain (4.1 ± 0.4) of the untreated *Pitx3*$^{ak/ak}$ mice ($P$ = 0.34). On days when behavioral testing was conducted, mice only received one injection of vehicle or drug. All mice and experimental conditions were coded immediately before videotaping for all cylinder tests and were decoded when all behavioral analyses were completed. Abnormal paw movements exhibited in the cylinder were scored as previously described (4). Mice reared against the wall of the cylinder and made abnormal sliding movements with both front paws, which increased over treatment time and dose of L-DOPA. Over time, the hind paw was also involved in sliding movements on the cylinder wall, which we termed as three-paw dyskinesia (4). The sum of front paw and three paw dyskinesia was used to score total dyskinesia. Behavior was evaluated 15 min after a challenge injection of 10 or 25 mg/kg of L-DOPA. Mice were perfused immediately following behavioral testing for immunohistochemical staining of phosphorylated ERK1/2 (pERK). Dyskinesia peaks at 15 min and lasts about 2 h. Because peak level correlates with the total area under the curve, 15-min data were used to represent the dyskinesia score for each condition so that ERK activation data could be obtained from the same animals. Specific mitogen-activated protein kinase kinase (MEK) inhibitors SL-327 and U0126, as well as the muscarinic receptor antagonist, dicyclomine, were also purchased from Sigma-Aldrich. The effect of dicyclomine on general locomotor activity of *Pitx3*$^{ak/+}$ mice was assessed in activity chambers using Med Associates Activity Monitor 5 software. Mice were injected with saline (vehicle), 15 or 45 mg/kg dicyclomine, and then placed in the activity chamber (27 × 27 × 20.3 cm high), where horizontal distance traveled was measured in 5-min time blocks over a 30-min period. For the assessment of locomotor activity in *Pitx3*$^{ak/ak}$ mice following A2A receptor antagonism, mice were injected with either vehicle (8%Tween-80/saline), 1 or 3 mg/kg of the A2A antagonist, KW-6002, and then directly placed into an activity chamber (40 × 40 × 30 cm high; Med Associates), where distance traveled was measured over a 3-h period.

Three to 4 wk after unilateral 6-OHDA lesion, mice allocated to the repeated L-DOPA treatment group were injected with 1 mg/kg of L-DOPA i.p., twice a day, as the maintenance dose; the acute L-DOPA treatment group was repeatedly injected with saline. For behavioral testing, a challenge dose of 3 mg/kg L-DOPA was used. Stepping tests were performed on a treadmill, as described previously (2, 5), and animals were videotaped for presence of dyskinesia at various time periods during repeated L-DOPA administration as detailed in *Results* or the figure legends. For dyskinesia, mouse behavior was videotaped for 3 min every 20 min during a 2-h period after L-DOPA challenge. The L-DOPA–induced limb and axial dyskinesias were evaluated from recorded videos by a blind observer using a previously described rating scale (6). Because no noticeable orolingual dyskinesia was observed after L-DOPA, only limb and axial dyskinesias were analyzed. Forepaw steps were recorded from one cycle of treadmill before and 40 min after L-DOPA injection. At the end of the repeated L-DOPA treatment, the animals were perfused 20 min after the last test dose of L-DOPA (3 mg/kg) for immunocytochemical analyses. The group of 6-OHDA–lesioned *Pitx3*$^{ak/+}$ mice treated repeatedly with saline were perfused 20 min after acute L-DOPA challenge (3 mg/kg) for immunocytochemical examination as well. Two lesioned mice were excluded from data analysis as these animals did not respond to L-DOPA and were subsequently found to have incomplete lesions by tyrosine hydroxylase immunostaining [~22% dopamine (DA) denervation].

**HPLC.** The dorsal, DA-depleted striatal area of *Pitx3*$^{ak/ak}$ mice and the corresponding striatal area of age-matched wild-type C57BL/6 mice (Charles River), as well as the matched dorsal striatal area of *Pitx3*$^{ak/+}$ mice, were dissected as described (4). The preparation and analysis of DA by HPLC were carried out as previously published (7). Because there was no significant difference in DA content in the dorsal corresponding area between C57BL/6 wild-type and *Pitx3*$^{ak/+}$ mice (Fig. S1), *Pitx3*$^{ak/+}$ littermates of homozygous *Pitx3*$^{ak/ak}$ mice were used as control animals.

**Immunohistochemistry.** Animals were perfused with 4% paraformaldehyde following behavioral testing for each experiment, as described in *Results* and the figure legends. Immediately after perfusion, brains were removed from skull and postfixed in 4% paraformaldehyde overnight at 4 °C, then transferred into 30% sucrose before slicing. Protein phosphatase inhibitors were not included prior or after perfusion with fixative. Coronal serial brain sections were made at 20 μm with a sliding microtome (Leica Instruments) and immunostaining was performed as de-

scribed (4). After blocking in Tris buffer saline (TBS) containing 10% normal serum and 0.3% Triton X-100 for 1 h at room temperature, sections were either directly transferred (for nonphospho-protein detection) or transferred following a 5-min rinse in TBS (for phospho-protein detection including pERK, phospho-p38 and phospho-JNK) to appropriately diluted primary antibody containing 0.3% Triton X-100 with either 10% normal serum (for nonphospho-protein) or 3% BSA (for phospho-protein) and incubated at 4 °C for 48 to 72 h. Primary antibodies used were rabbit anti–DARPP-32 (1:500), rabbit anti–phospho-ERK (pERK, 1:200), rabbit anti–phospho-p38 (1:100), and rabbit antiphospho-JNK (1:100) (Cell Signaling Technology), goat anticholine acetyltransferase (anti-ChAT) [1:500; (Millipore Corporation) or 1:1,000 (BD Bioscience)], rabbit anti-FosB (1:500; Santa Cruz Biotechnology), and rabbit anti-tyrosine hydroxylase (TH) (1:500; Pel-Freez Biologicals), mouse antiparvalbumin (1:500; Sigma) and mouse anticalretinin (1:200; Santa Cruz Biotechnology). Secondary antibodies were diluted in either 3% BSA (for detection of phospho-proteins) or 5% appropriate serum (for nonphospho-protein detection). Biotinylated secondary antibody (1:200; Vector Laboratories) was used for the avidin: biotin (ABC) immunoperoxidase procedure with a diaminobenzidine (DAB) chromogenic substrate, and Alexa Fluor-488 and Fluor-594 conjugated secondary antibodies (1:500; Invitrogen Corporation) or Dylight-488 and Dylight-594 conjugated secondary antibodies (1:500; Kirkegaard and Perry Laboratories, Inc.) were applied for fluorescent microscopy. Goat anti-Rabbit IRDye@ 800 CW (LI-COR Biosciences) was applied as secondary antibody for TH. Sections were incubated in secondary antibodies for 1 h at room temperature. TBS containing 0.1% Triton X-100 was applied to all washes. For every immunohistochemical staining experiment, omission of the primary antibody during immunostaining was used as a negative control. In addition, multiple groups from several different treatments were usually included in the same reaction set, which covers both positive and negative groups that would serve as internal controls.

**Immunoblot.** Denervated striatal area of *Pitx3*$^{ak/ak}$ mice was dissected from 0.5-mm thick coronal brain slices and homogenized in lysis buffer (8) containing phosphatase inhibitor mixture (Roche PhosSTOP/cOmplete; Roche Applied Science). Cell lysates were centrifuged for 10 min at 4 °C, the supernatant collected, and protein quantification was carried out using the bicinchoninic acid method (Pierce Biotechnology). Immunoblot was first carried for pERK (Cell Signaling Technology), and then the membrane was stripped and carried out again for β-actin (Sigma-Aldrich). The pERK signal was normalized to β-actin.

**Image Analysis.** For *Pitx3*$^{ak/ak}$ mice, numbers of total pERK-positive neurons in immunoperoxidase-labeled sections were counted from images taken at 20× magnification (area size of $1.168 \times 10^5$ μm$^2$ per image; ≈33 images per mouse brain) under bright field from a region of dorsal striatum lying adjacent to the external capsule using MetaMorph software (version 5.01). The pERK neurons were quantitated using Image J from four sequential, coronal sections (220-μm apart) from the right side of the brain comprising a total striatal sectional area of ≈$3.854 \times 10^6$ μm$^2$ per mouse. The pERK-positive neurons with long diameter of ≥20 μm were considered "large-size." Analysis of fluorescent-immunostained pERK-labeled neurons was based on images taken with a DSU confocal microscope at 40× (area size of $1.049 \times 10^5$ μm$^2$ per image) in the dorsal denervated striatal area from at least two different sections of the rostral striatum (between Bregma 0.86–1.18 mm) of each individual *Pitx3*$^{ak/ak}$. For 6-OHDA–lesioned mice, the total number of immunoperoxidase-stained pERK neurons were counted from three sequential coronal sections (220-μm apart, between Bregma 0.26–1.10 mm) using Image J on images taken at 20× magnification covering the whole dorsal striatum on the lesion side. The extent of striatal DA denervation produced by 6-OHDA was evaluated from TH-immunostained coronal sections of striatum using the infrared Odyssey Image Analysis system (LI-COR Biosciences). Because the percent-reduction in striatal DA innervation produced by 6-OHDA was similar for all three doses (4.5 μg, 94.2 ± 0.3%, $n$ = 13; 2.25 μg, 95.5 ± 0.1%, $n$ = 6; 1.125 μg, 94.1 ± 0.5%, $n$ = 7), quantitation of immunolabeled cells and behavioral data from repeated L-DOPA–treated lesioned mice were combined for analysis. Neurotoxin lesion of the acute L-DOPA treatment group resulted in a comparable degree of striatal DA denervation (95.7 ± 0.2%, $n$ = 4).

**Electrophysiological Recordings.** Age- and sex-matched heterozygous *Pitx3*$^{ak/+}$ and homozygous *Pitx3*$^{ak/ak}$ mice were first treated with either saline or L-DOPA (25 mg/kg, twice a day, i.p.) together with benserazide (6 mg/kg, twice a day, i.p.) for at least 7 wk. Then, the animals were removed individually for electrophysiological recording; the rest of the mice continued to receive repeated L-DOPA or saline treatment until being randomly picked for in vitro recording.

***Slice preparation.*** Parasagittal brain slices (250-μm thick) were obtained from adult mice. The mice were anesthetized with isoflurane (Abbott Laboratories) and rapidly decapitated. Their brains were transferred to cold, sucrose-artificial cerebrospinal fluid (ACSF) containing the following (in mM): 200 sucrose, 25 $NaHCO_3$, 20 glucose, 10 ascorbic acid, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$ and 1 $NaH_2PO_4$, pH 7.4, saturated with 95% $O_2$ and 5% $CO_2$ (9, 10). Striatal brain slices were made on a vibratome (VT100S; Leica Instruments). Slices were incubated for at least 1 h in bath circulated at 20 mL/min with normal, 32 °C ACSF containing (in mM): 125 NaCl, 25 $NaHCO_3$, 20 glucose, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 1 $NaHCO_3$, and 1 ascorbic acid, pH 7.4, saturated with 95% $O_2$ and 5% $CO_2$ (10). During recording, slices were superfused (2 mL min$^{-1}$) with this same ACSF at ~34 °C.

***Electrophysiology.*** On-cell current-clamp recordings from cholinergic interneurons located in the dorsal lateral striatum were obtained under visual control on a differential interference contrast, upright microscope (Axioskop; Carl Zeiss). Recordings were obtained using 2.5- to 5-MΩ resistance pipettes made from borosilicate glass capillary tubing (G150-4; Warner Instruments, Inc.) that was pulled on a Flaming/Brown micropipette puller (Model P-97; Sutter Instrument Company) and filled with ACSF. Action potentials were measured using a Multiclamp 700A amplifier (5-kHz low-pass Bessel filter) with a DigiData 1322A interface (10 kHz digitization) and pCLAMP 9.2 software (Molecular Devices Corp.). Cholinergic interneurons in the dorsal lateral striatum were identified by their large size and spontaneous firing rate (between 0.1 and 7 Hz). Data are expressed as mean ± SEM.

**Statistics.** The statistical programs Sigma Stat version 2.0.3 or StatView version 5.0.1 were used for data analysis. The data represent the mean ± SEM. See details in the figure legends about the specific statistical method used for the data analysis of individual experiments.


1. Varnum DS, Stevens LC (1968) Aphakia, a new mutation in the mouse. *J Hered* 59: 147–150.
2. Chen L, et al. (2008) Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. *J Neurosci* 28:425–433.
3. Paxinos G, Franklin KBJ (1997) *The Mouse Brain in Stereotaxic Coordinates* (Elsevier Science, San Diego, CA).
4. Ding Y, et al. (2007) Chronic 3,4-dihydroxyphenylalanine treatment induces dyskinesia in aphakia mice, a novel genetic model of Parkinson's disease. *Neurobiol Dis* 27:11–23.

5. Chang JW, Wachtel SR, Young D, Kang UJ (1999) Biochemical and anatomical characterization of forepaw adjusting steps in rat models of Parkinson's disease: Studies on medial forebrain bundle and striatal lesions. *Neuroscience* 88:617–628.
6. Winkler C, Kirik D, Björklund A, Cenci MA (2002) L-DOPA–induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: Relation to motor and cellular parameters of nigrostriatal function. *Neurobiol Dis* 10:165–186.
7. Potashkin JA, et al. (2007) MPTP administration in mice changes the ratio of splice isoforms of fosB and rgs9. *Brain Res* 1182:1–10.
8. Lin W, Kang UJ (2008) Characterization of PINK1 processing, stability, and subcellular localization. *J Neurochem* 106:464–474.
9. Britt JP, McGehee DS (2008) Presynaptic opioid and nicotinic receptor modulation of dopamine overflow in the nucleus accumbens. *J Neurosci* 28:1672–1681.
10. Fagen ZM, Mitchum R, Vezina P, McGehee DS (2007) Enhanced nicotinic receptor function and drug abuse vulnerability. *J Neurosci* 27:8771–8778.

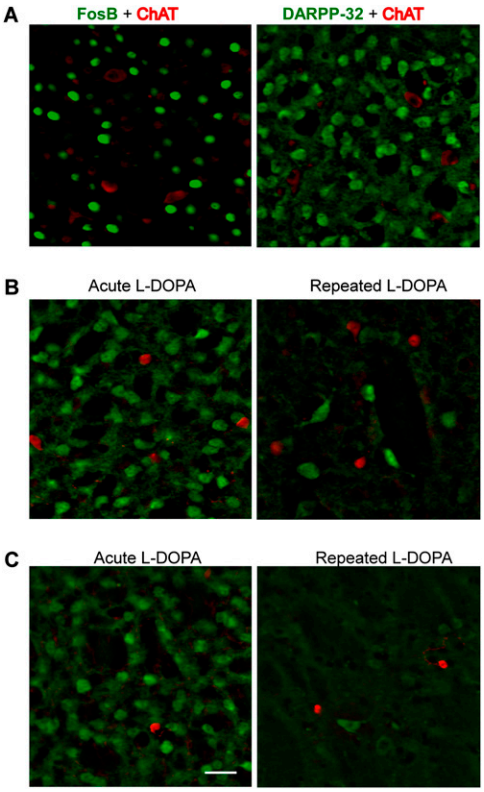


**Fig. S1.** Striatal cholinergic neurons do not express DARPP-32 or FosB in *Pitx3*$^{ak/ak}$ mice repeatedly treated with L-DOPA and pERK is not induced in striatal parvalbumin or calretinin-expressing interneurons. (*A*) *Pitx3*$^{ak/ak}$ mice were treated with L-DOPA (25 mg/kg, twice a day, i.p.) for 7 wk and perfused for double-immunostaining 15 min after the last dose of L-DOPA. Cholinergic neurons (in red) in dorsal striatum do not colocalize with FosB (*Left*, green) or DARPP-32 (*Right*, green). (*B* and *C*) Neither acute L-DOPA (*Left*, 25 mg/kg, i.p.) or repeated L-DOPA treatment (*Right*, 25 mg/kg, twice a day for 7 wk, i.p.) induced pERK (green) in dorsal striatal parvalbumin (*B*, red) or calretinin (*C*, red)-expressing interneurons. (Scale bar, 30 μm.)

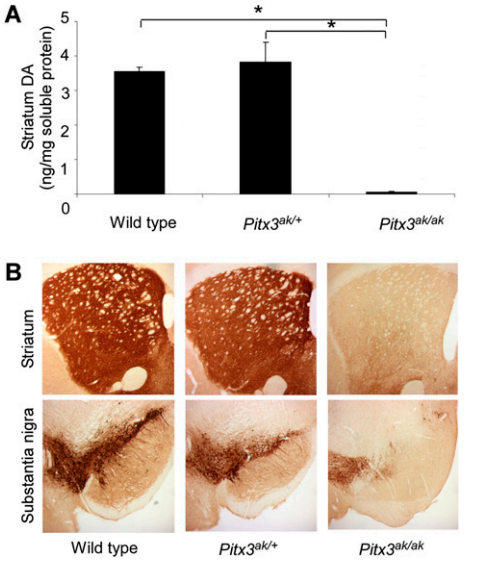


**Fig. S2.** Dopaminergic markers in wild-type, heterozygous *Pitx3*$^{ak/+}$ and homozygous *Pitx3*$^{ak/ak}$ mice. (*A*) Striatal DA content of *Pitx3*$^{ak/+}$ mice is similar to that of wild-type mice, whereas it is dramatically reduced in *Pitx3*$^{ak/ak}$ animals ($n = 4$ per group, mean ± SEM; $^*P < 0.05$, one-way ANOVA with post hoc Bonferroni *t* test). (*B*) Dopaminergic fibers and neurons, assessed by tyrosine hydroxylase labeling, are significantly reduced in the striatum and substantia nigra, respectively, of *Pitx3*$^{ak/ak}$ mice compared with heterozygotes or wild-type animals.

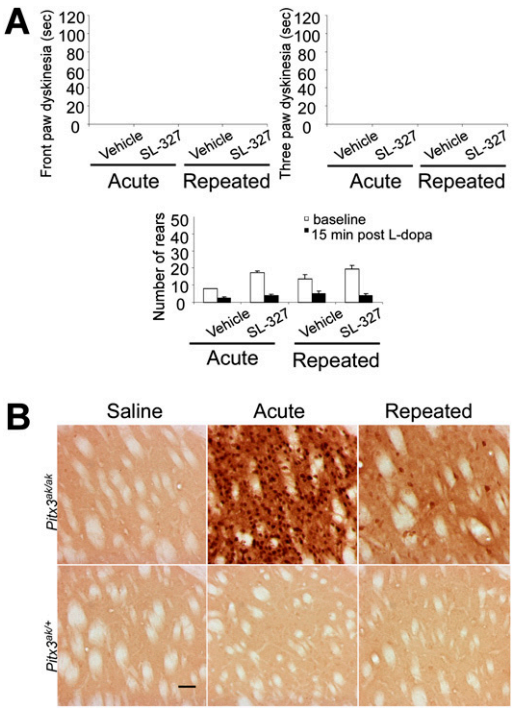


**Fig. S3.** Heterozygous *Pitx3*$^{ak/+}$ mice do not express dyskinetic behavior or striatal ERK activation in response to L-DOPA. *Pitx3*$^{ak/+}$ mice were treated acutely or repeatedly with L-DOPA or saline similarly to that described for *Pitx3*$^{ak/ak}$ mice in Fig. 4 and evaluated for striatal pERK labeling or for rearing activity and expression of dyskinesia after L-DOPA challenge with or without MEK1/2 inhibitor, SL-327, pretreatment ($n = 4$/group). (*A*) Front- and three-paw dyskinesias were not observed in *Pitx3*$^{ak/+}$ mice treated acutely or repeatedly with L-DOPA. Rearing activity in heterozygotes was not enhanced by acute or repeated administration of L-DOPA, or prior treatment with vehicle or SL-327, unlike that observed with *Pitx3*$^{ak/ak}$ mice (Fig. 4*C*). The data represent the mean ± SEM. (*B*) Acute or repeated L-DOPA administration activates ERK in *Pitx3*$^{ak/ak}$, but not *Pitx3*$^{ak/+}$ mice. (Scale bar, 50 μm.)

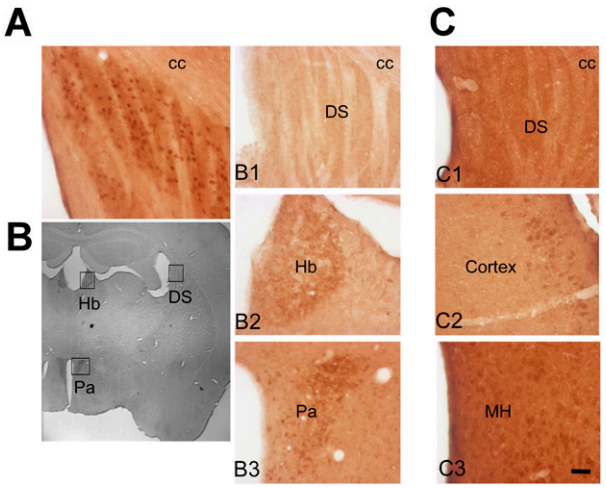


**Fig. S4.** L-DOPA activates ERK, but not phospho-p38 or phospho-JNK MAP kinases in the dorsal striatum of *Pitx3*$^{ak/ak}$ mice. Mice were treated acutely with L-DOPA (25 mg/kg) and perfused for immunohistochemistry for pERK, phospho-p38, and phospho-JNK 15 min later. (*A*) Strong ERK activation was present in the most dorsal region of dorsal striatum at the level of the hypothalamic paraventricular nucleus (Pa). (*B*) Low-power magnification depicting brain regions examined for phospho-p38 labeling. Immunostaining for phospho-p38 was not detected in dorsal striatum (DS, *B*1), but was observed in the habenula (Hb, *B*2) and Pa (*B*3) region. (*C*) Phospho-JNK positive staining was not detected in dorsal striatum (DS, *C*1), but was present in the cortex (*C*2) and in medial hypothalamus (MH, *C*3). cc, corpus callosum. (Scale bar, 50 μm.)

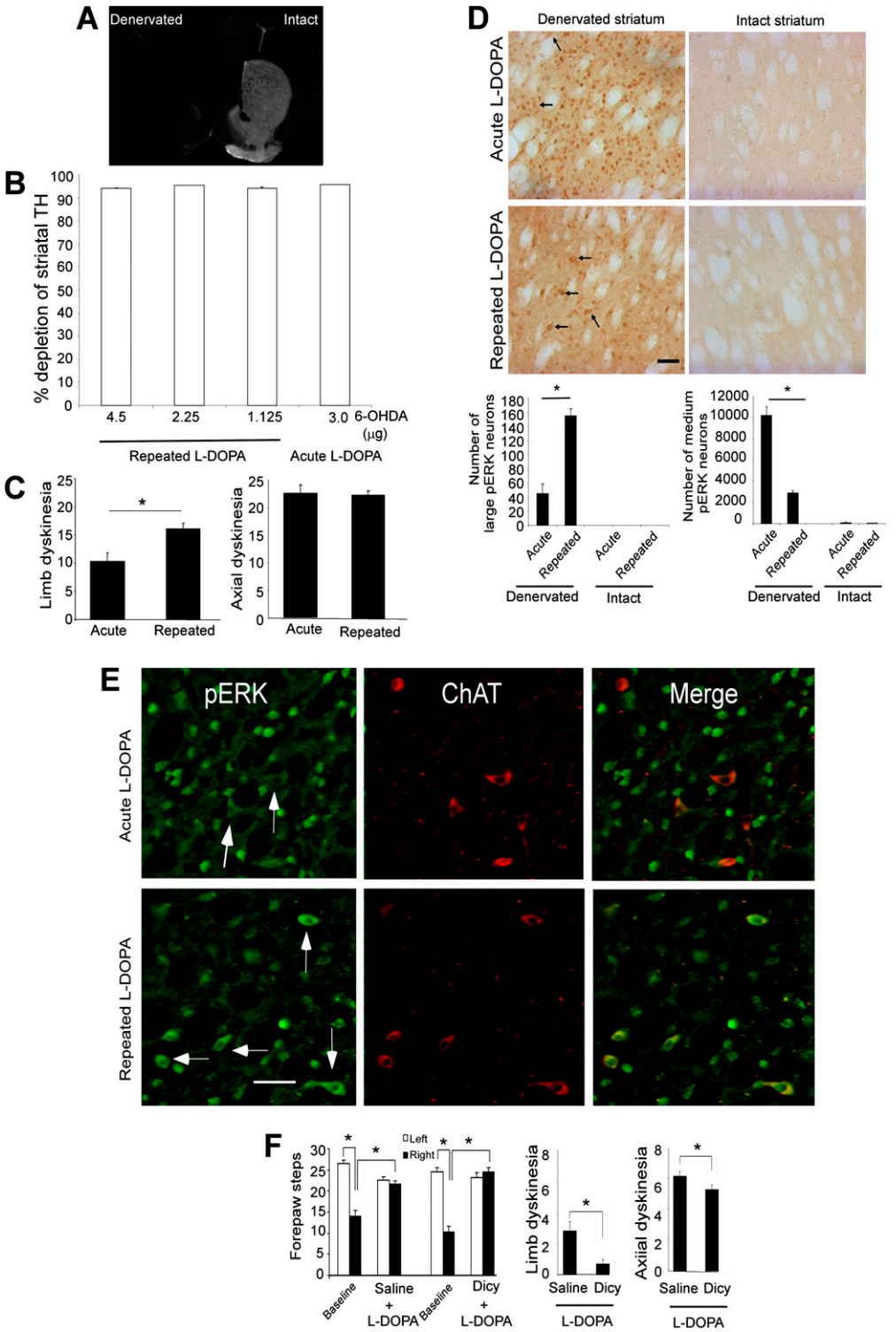


**Fig. S5.** Repeated L-DOPA–induced dyskinesia in unilateral Parkinson disease (PD) mice is associated with elevated cholinergic tone which is inhibited by muscarinic antagonism. (*A*) Unilateral 6-OHDA lesion of the medial forebrain bundle significantly depletes striatal tyrosine hydroxylase (TH) immunostaining on the lesioned side. (*B*) Mice used for L-DOPA–induced dyskinesias (LID) in this study received a single injection of 1.125, 2.25, or 4.5 μg (repeated L -DOPA group) or 3.0 μg (acute L-DOPA group) of 6-OHDA into the medial forebrain bundle, all of which produced a similar extent of striatal TH depletion (see *SI Materials and Methods* for details). (*C*) Repeated L-DOPA administration to unilateral 6-OHDA–lesioned mice for 5 wk resulted in a significant increase in limb dyskinesia, but had no effect on axial dyskinesia, compared with the first exposure of these animals to L-DOPA ($n = 26$; $^*P < 0.05$, *t* test). Dyskinetic behaviors were scored using a previously described rating scale (1). (*D*) Lesioned mice received either repeated saline (4 wk) or L-DOPA (1 mg/kg, twice a day, i.p., 10 wk) and were killed 20 min after a challenge injection of L-DOPA (3 mg/kg) and processed for immunohistochemical staining for pERK. Overall, many more pERK-labeled cells were present in denervated striatum after acute compared with repeated L-DOPA administration (*Upper*). Arrows indicate presumed positively labeled large cholinergic neurons (Scale bar, 50 μm.) (*Lower*) Quantitation of the number of medium- and large-sized pERK immunoperoxidase stained cells in

Legend continued on following page

denervated striatum showed that there were fewer labeled medium-sized neurons and a greater number of stained large-sized neurons after repeated, compared with acute L-DOPA treatment ($n$ = 4 mice acute group/$n$ = 26 mice repeated group; *$P < 0.05$, $t$ test). (*E*) Merged images (*Right*) showing colocalization of pERK (arrow, *Left*) and ChAT (*Center*) in the denervated striatum of unilateral 6-OHDA–lesioned mice challenged with acute (*Upper*) or repeated (*Lower*) administration of L-DOPA (3 mg/kg). (Scale bar, 40 μm.) (*F*) Lesioned mice repeatedly treated with L-DOPA (1 mg/kg, twice a day, i.p.) for 8 to 10 wk were injected with saline or the muscarinic receptor antagonist, dicyclomine (Dicy) (45 mg/kg, intraperitonally), 20 min after a challenge dose of L-DOPA (3 mg/kg). Behavioral testing was conducted 40 min after L-DOPA challenge. (*Left*) Forepaw stepping with the contralateral limb (right paw) was significantly improved by L-DOPA and the antiparkinsonian effect of L-DOPA was unaffected by dicyclomine ($n$ = 13 per group; *$P < 0.05$, two-way ANOVA followed by Tukey post hoc test). Dicyclomine treatment reduced L-DOPA–induced limb dyskinesia (*Center*) ($n$ = 21 per group; *$P < 0.05$, Mann-Whitney rank sum test) and axial dyskinesia (*Right*) ($n$ = 21 mice per group; *$P < 0.05$, $t$ test). The data for *B–D* and *F* represent the mean ± SEM.

1. Winkler C, Kirik D, Björklund A, Cenci MA (2002) L-DOPA–induced dyskinesia in the intrastriatal 6-hydroxydopamine model of Parkinson's disease: Relation to motor and cellular parameters of nigrostriatal function. *Neurobiol Dis* 10:165–186.

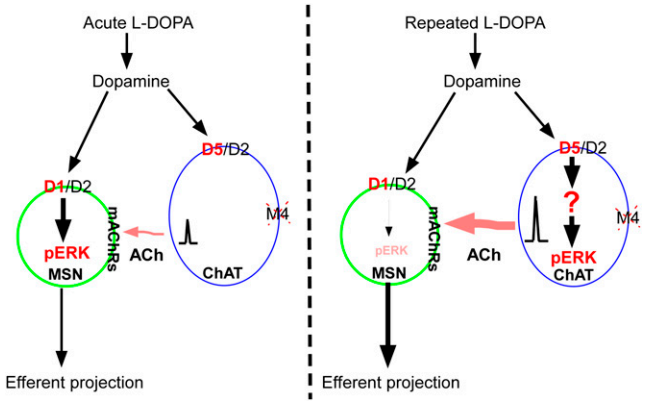


**Fig. S6.** Schematic model illustrating the relative shift of ERK activation from striatal medium spiny projection neurons (MSN) induced by acute L-DOPA exposure to cholinergic interneurons (ChAT) following repeated L-DOPA administration. In the DA-denervated striatum, acute L-DOPA administration activates pERK in MSN through stimulation of D1 DA receptors (1, 2). Repeated L-DOPA exposure leads to decreased activation of pERK in MSN and enhanced pERK in ChAT. The functional significance of these changes in producing dyskinesia has been demonstrated in this study by reversal of behavioral and electrophysiological changes upon inhibition of pERK activation or treatment with a muscarinic receptor antagonist. The mechanisms underlying the relative shift of ERK activation are currently not known. However, repeated L-DOPA treatment may result in sensitization of D5 receptors to DA and increased coupling to effector signaling pathways, as has been reported for the D1 receptor on MSN (3, 4). Because the cholinergic neurons do not express DARPP-32 or FosB (Fig. S1), L-DOPA induction of pERK probably occurs via a protein kinase A-independent mechanism, such as one involving phospholipase C (PLC). The activation of ERK leads to an increase in neuronal excitability, resulting in further augmentation of the increase in acetylcholine (ACh) release occurring in the DA-denervated striatum because of muscarinic (M4) receptor malfunction (5). The increased cholinergic transmission acting on postsynaptic muscarinic receptors (mAChRs) on MSNs along with concurrent stimulation of D1/D2 receptors by DA results in synergistic activation of these neurons.

1. Darmopil S, Martín AB, De Diego IR, Ares S, Moratalla R (2009) Genetic inactivation of dopamine D1 but not D2 receptors inhibits L-DOPA-induced dyskinesia and histone activation. *Biol Psychiatry* 66:603–613.
2. Santini E, et al. (2009) L-DOPA activates ERK signaling and phosphorylates histone H3 in the striatonigral medium spiny neurons of hemiparkinsonian mice. *J Neurochem* 108:621–633.
3. Konradi C, et al. (2004) Transcriptome analysis in a rat model of L-DOPA–induced dyskinesia. *Neurobiol Dis* 17:219–236.
4. Aubert I, et al. (2005) Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. *Ann Neurol* 57:17–26.
5. Ding J, et al. (2006) RGS4-dependent attenuation of M4 autoreceptor function in striatal cholinergic interneurons following dopamine depletion. *Nat Neurosci* 9:832–842.