Learning critically drives parkinsonian motor deficits through imbalanced striatal pathway recruitment

Timothy H. C. Cheung[a,b,c,d] (iD), Yunmin Ding[a,b,c,d] (iD), Xiaoxi Zhuang[e] (iD), and Un Jung Kang[a,b,c,d,1] (iD)

Dopamine (DA) loss in Parkinson's disease (PD) causes debilitating motor deficits. However, dopamine is also widely linked to reward prediction and learning, and the contribution of dopamine-dependent learning to movements that are impaired in PD—which often do not lead to explicit rewards—is unclear. Here, we used two distinct motor tasks to dissociate dopamine's acute motoric effects vs. its long-lasting, learning-mediated effects. In dopamine-depleted mice, motor task performance gradually worsened with task exposure. Task experience was critical, as mice that remained in the home cage during the same period were relatively unimpaired when subsequently probed on the task. Repeated dopamine replacement treatments acutely rescued deficits and gradually induced long-term rescue that persisted despite treatment withdrawal. Surprisingly, both long-term rescue and parkinsonian performance decline were task specific, implicating dopamine-dependent learning. D1R activation potently induced acute rescue that gradually consolidated into long-term rescue. Conversely, reduced D2R activation potently induced parkinsonian decline. In dopamine-depleted mice, either D1R activation or D2R activation prevented parkinsonian decline, and both restored balanced activation of direct vs. indirect striatal pathways. These findings suggest that reinforcement and maintenance of movements—even movements not leading to explicit rewards—are fundamental functions of dopamine and provide potential mechanisms for the hitherto unexplained "long-duration response" by dopaminergic therapies in PD.

striatum | Parkinson's disease | motor learning | spiny projection neurons | behavior

Dopamine (DA) is critical for movement, and loss of nigrostriatal dopamine neurons in Parkinson's disease (PD) causes debilitating motor deficits (1). Dopamine is thought to promote movement initiation and vigor of immediately ensuing actions (2, 3), and loss of this dopaminergic drive is thought to underlie parkinsonian deficits. Accordingly, increasing dopamine signaling can rapidly increase motor output and rescue PD motor deficits (2, 4, 5). However, dopamine is also implicated in learning and plasticity (6–8) and is thought to act as a reward prediction error (9, 10). While the involvement of dopamine-dependent learning is well explored in Pavlovian and instrumental conditioning (11, 12), the contribution of dopamine-dependent learning to movements without explicit rewards—such as those impaired in PD—has not been systemically examined, although fragmented reports hint at learning's importance (13–15).

Uncovering the role of dopamine-dependent learning in movements is important not just for furthering our understanding of dopamine's multifaceted functions, but also for improving PD therapy. In PD patients, rescue from movement deficits—such as finger tapping—using the dopamine precursor levodopa (L-DOPA) could still be detected days to weeks after L-DOPA withdrawal, despite L-DOPA's plasma half-life of only ~90 min (4, 5, 16). This long-term rescue, termed long-duration response (LDR), is a critical component of PD therapy, accounting for a significant portion of the total motor benefits from L-DOPA (4, 5, 17, 18). Despite its importance, LDR's mechanism is still unclear, and its long-lasting effects often confound assessment of patients' disease state in therapeutic trials (16, 19). Importantly, LDR decays faster after years of chronic L-DOPA treatment (4), and this accelerating LDR decay may contribute to the development of motoric side effects from L-DOPA, such as motor fluctuation (20). The long-lasting characteristic of LDR suggests that dopamine-dependent learning can be important even for movements without explicit rewards. Therefore, we first used two distinct motor tasks (forepaw stepping and turning asymmetry while descending a pole) to demonstrate task dependency of learning and explore the striatal pathways involved.

Results

Learning Drives Both Parkinsonian Deficit and Its Long-Term Rescue by L-DOPA.
Unilateral 6-hydroxydopamine (6-OHDA) infusion into the medial forebrain bundle caused severe and stable loss of dopaminergic neurons as shown by striatal tyrosine

Significance

Dopamine (DA) is critical for movements, and its loss causes debilitating motor deficits in Parkinson's disease (PD). However, dopamine is also strongly implicated in reward learning. Here, we used two distinct motor tasks to show that, in mice, dopamine-dependent learning also contributes to PD symptoms: dopamine depletion caused motor impairments that worsened with performance, and repeated dopamine replacement induced long-term rescue that persisted despite treatment withdrawal. We propose that dopamine-dependent motor learning is an important contributor to the critical—but poorly understood—long-duration response (LDR) from dopaminergic therapies. Further understanding of LDR may improve both "motor fluctuation" treatments and our ability to accurately assess the effectiveness of disease-modifying PD therapies.

hydroxylase (TH) (dopaminergic neuronal marker) depletion (Fig. 1*A*). We trained mice with two motor tasks known to be dopamine dependent (Fig. 1*B*, *Materials and Methods*). First, in the "Step task," the mouse's weight-bearing forepaws made adjusting steps to a moving treadmill (Movie S1). Second, in the "Pole task,"

the mouse was placed on a pole and descended by itself (Movie S2). Mice were pretrained on both tasks, underwent unilateral dopamine (DA) neuron lesion with 6-OHDA, and then were split into different groups (Fig. 1*C*). One group was reintroduced to Step task only, another group to Pole task only, and the last group
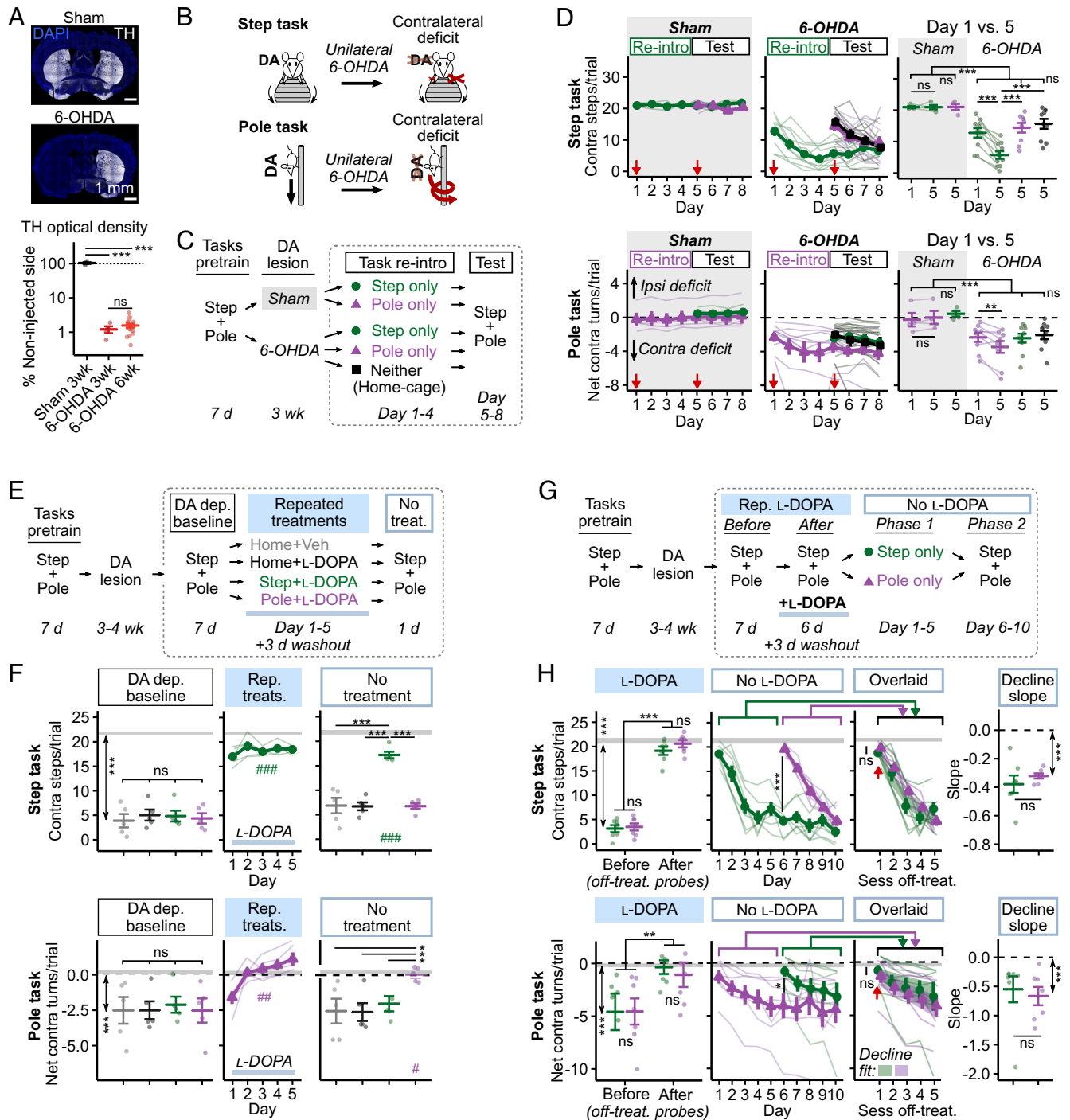

Fig. 1. Learning drives both parkinsonian motoric decline and its long-term rescue by L-DOPA. (*A*) Striatal tyrosine hydroxylase (TH) levels after unilateral 6-OHDA, spanning time points typical of present experiments. Note $\log_{10}$ *y* axis. *N* = 4, 4, 17. (*B*) Two distinct unrewarded motor tasks to test contralateral parkinsonian deficits after dopamine (DA) depletion. (*C* and *D*) Strategy to examine task-specific parkinsonian deficit (*C*), and the associated contralateral motor performance (*D*). *Right* panels in (*D*): Day 1 (first session for the group reintroduced to Step/Pole task during days 1 to 4) vs. day 5 (first session for the remaining groups). *N* = 4, 4 (sham); 10, 9, 10 (6-OHDA). (*E* and *F*) Strategy to examine acute and task-specific long-term recue by L-DOPA (*E*), and the associated contralateral motor performance (*F*). *N* = 5, 5, 5, 5. (*G* and *H*) Strategy to examine task-specific parkinsonian decline of L-DOPA-induced long-term rescue (*G*), and the associated contralateral motor performance of (*H*). "Overlaid" panels in (*H*) show parkinsonian decline during the first 5 sessions upon reintroduction to the task without L-DOPA. "Decline slope" panels show fitted decline rates of "overlaid" panels (*Materials and Methods*); negative values indicate worsening performance. *N* = 7, 7. Gray horizontal bands in (*F*) and (*H*): pre-6-OHDA performance. Data are mean ± SEM. ##*P*<0.01, ###*P*<0.001, vs. same group's DA-depleted baseline, paired *t* tests. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. ANOVAs or Holm–Bonferroni-corrected *t* tests were used for between-group comparisons, followed by Tukey's HSD post-hoc tests when needed. See Dataset S1 for full statistics. See also *SI Appendix*, Fig. S1.

remained in home cage, each for four sessions (days 1 to 4). For both Step task– and Pole task–reintroduced groups, dopamine lesion caused contralateral deficits that worsened across sessions: On the Step task, dopamine lesion reduced contralateral stepping; on the Pole task, lesion slowed the descend and increased ipsilateral turning while descending, consistent with contralateral motor deficits (Fig. 1*D* and *SI Appendix,* Fig. S1*A*). Parallel sham-lesioned groups showed no such decline. This dopamine depletion–induced worsening of motoric impairment—which we term parkinsonian decline—was experience dependent and task specific: When all groups were subsequently probed on both tasks (days 5 to 8), lesioned home-cage group showed only mild deficits initially (day 5). Similarly, Step task–reintroduced group showed mild Pole task deficit on day 5, despite its severe Step task deficit; an analogous result was found for the Pole task–reintroduced group. The experience-dependent and task-specific nature of the performance decline suggests that dopamine-dependent learning—in this case mediated by reduced dopamine signaling—is involved. In further support of task-specific learning, performance decline rates (slopes) for a given task during the first four post-6-OHDA sessions were similar across 6-OHDA-lesioned groups, despite different reintroduction histories (*SI Appendix,* Fig. S1*B*, *Materials and Methods*).

Conversely, in 6-OHDA-lesioned mice with worsened deficits on both tasks, L-DOPA not only acutely rescued deficits, repeated treatments also induced long-term rescue that persisted despite a 3-d washout (Fig. 1 *E* and *F*, *SI Appendix,* Fig. S1*C*, and Movies S3 and S4), similar to the clinically seen LDR (4, 5, 16). This long-term rescue was only seen when L-DOPA was paired with specific task performance, again consistent with dopamine-dependent learning—in this case mediated by restored dopamine signaling. Furthermore, in 6-OHDA-lesioned mice that received repeated pairings of L-DOPA with both tasks, the resultant long-term rescue declined when L-DOPA-withheld mice were reintroduced to one of the tasks (days 1 to 5; Fig. 1 *G* and *H* and *SI Appendix,* Fig. S1*D*). This decline was task specific (15), as shown by tests on both tasks on day 6. Surprisingly, the decline trajectory for the second reintroduced task was not affected by the severe decline of the first task or the prolonged L-DOPA withdrawal: Decline curves from both groups were indistinguishable when overlaid, with similar slopes (Fig. 1*H*, *Materials and Methods*). Post-6-OHDA parkinsonian motoric decline was highly replicable: It was observed in all 13 independent experiments in this paper (*SI Appendix,* Fig. S2). Altogether, these data strongly suggest that gradual motoric decline is a fundamental feature of dopamine depletion. Furthermore, the experience-dependent and task-specific nature of both parkinsonian decline and its long-term rescue highlights the importance of dopamine-dependent learning in movements without explicit rewards and in parkinsonian akinesia. Specifically, our finding suggests that dopamine-dependent learning is involved in 1) acquisition and maintenance of long-term rescue, which is task specific and depends on the presence of dopamine signaling during task experience, and 2) gradual decline of motor performance, which is also task specific and depends on the reduction/absence of dopamine signaling during task experience.

Targeting D1R/Direct-Pathway Striatal Projection Neurons Potently Induces Acute and Long-Term Rescue. Parkinsonian deficits are thought to stem from reduced dopaminergic signaling in direct-pathway and indirect-pathway striatal projection neurons (dSPNs and iSPNs), which express D1 and D2 dopamine receptors, respectively (D1Rs and D2Rs), and comprise the striatonigral vs. striatopallidal pathway (1, 21). dSPN activation promotes

movements, whereas iSPN activation inhibits movements and promotes task quitting (22, 23). Dopamine, through D1R and D2R stimulation, respectively, is hypothesized to i) acutely activate dSPNs and suppress iSPNs (21, 24), ii) promote long-term synaptic plasticity via dSPN long-term potentiation (LTP) and iSPN long-term depression (LTD) (6–8). Both dSPN-LTP and iSPN-LTD could theoretically cause long-term rescue. We thus examined the effects of D1R- vs. D2R-selective manipulations, using the Step task due to its lower between-subject variability. For simplicity, we focused on contralateral stepping, as it was much more strongly impaired by dopamine depletion than ipsilateral stepping (*SI Appendix,* Figs. S2 and S3).

We first examined the effect of blocking D1Rs vs. D2Rs on L-DOPA's acute vs. long-term rescue (Fig. 2*A*). L-DOPA+Veh acutely rescued 6-OHDA-induced deficits, as expected (Fig. 2*B*). Furthermore, treatment-free probe trials given before daily treatments (~23 h washout) showed that long-term rescue gradually increased, which persisted after a 3-d washout, consistent with learning. SCH23390 (D1R antagonist) reduced both L-DOPA's acute and long-term rescue, whereas eticlopride (D2R antagonist) did not (Fig. 2*B*), even though the doses of SCH23390 and eticlopride were chosen to induce similar acute motor impairment in dopamine-normal mice (25). These results suggest that D1R stimulation by L-DOPA potently induced acute and long-term rescue. Because L-DOPA+SCH23390 should inhibit iSPNs via D2R stimulation, we also examined the effect of selective iSPN inhibition with chemogenetics, using *Adora2a-Cre* mice to drive selective expression of the inhibitory DREADD hM4Di in iSPNs (Fig. 2 *C* and *D*). Clozapine N-oxide (CNO, hM4Di agonist) acutely rescued deficits from day 1, consistent with iSPN inhibition (26) and gradually induced long-term rescue in hM4Di-group. The rescue induced by CNO was likely not due to CNO's off-target effects, as it was not seen in control mice that did not express hM4Di. Thus, D1R/dSPN activation and iSPN inhibition each induced long-term rescue.

Long-term rescue likely reflects learning driven by acute rescue, because long-term rescue both required the experience of acute rescue (Fig. 1*E*) and built up gradually, lagging acute rescue (Fig. 2 *B* and *D*). While our data above suggest that targeting D1R/dSPNs may be more effective in inducing both acute and long-term rescue than targeting D2R/iSPNs, whether the relationship between acute and long-term rescue depends on the targeted SPN pathway is unknown. To examine this, we grouped the above manipulations into those that targeted i) D1R/dSPNs; ii) D2R/iSPNs; and iii) both pathways (Fig. 2*E*), and compared acute, on-treatment rescue (from the final on-treatment session, after acute rescue has stabilized) vs. long-term rescue (from the no-treatment session after washout). We found a significant linear relationship between acute and long-term rescue (Fig. 2*F*), consistent with clinical reports (5). Furthermore, manipulations that targeted D2R/iSPNs formed a separate cluster with both lower acute rescue and lower long-term rescue (Fig. 2 *F* and *G*). Despite this difference in rescue magnitudes, the ratio between long-term and acute rescue was similar between the pathway manipulations (Fig. 2*H*). This suggests that the underlying relationship between acute and long-term rescue did not depend on the SPN pathway targeted. Therefore, the lower long-term rescue obtained from targeting D2R/iSPNs may stem from its lower acute rescue.

To extend our findings, we also examined the effects of D1R vs. D2R agonists (*SI Appendix,* Fig. S4*A*). Consistent with D1R's importance, SKF81297 (D1R agonist) potently induced both acute and long-term rescue (*SI Appendix,* Fig. S4*B*). By contrast, acute rescue from quinpirole (D2R agonist) was not detectable until day 3, after which it induced moderate acute and long-term
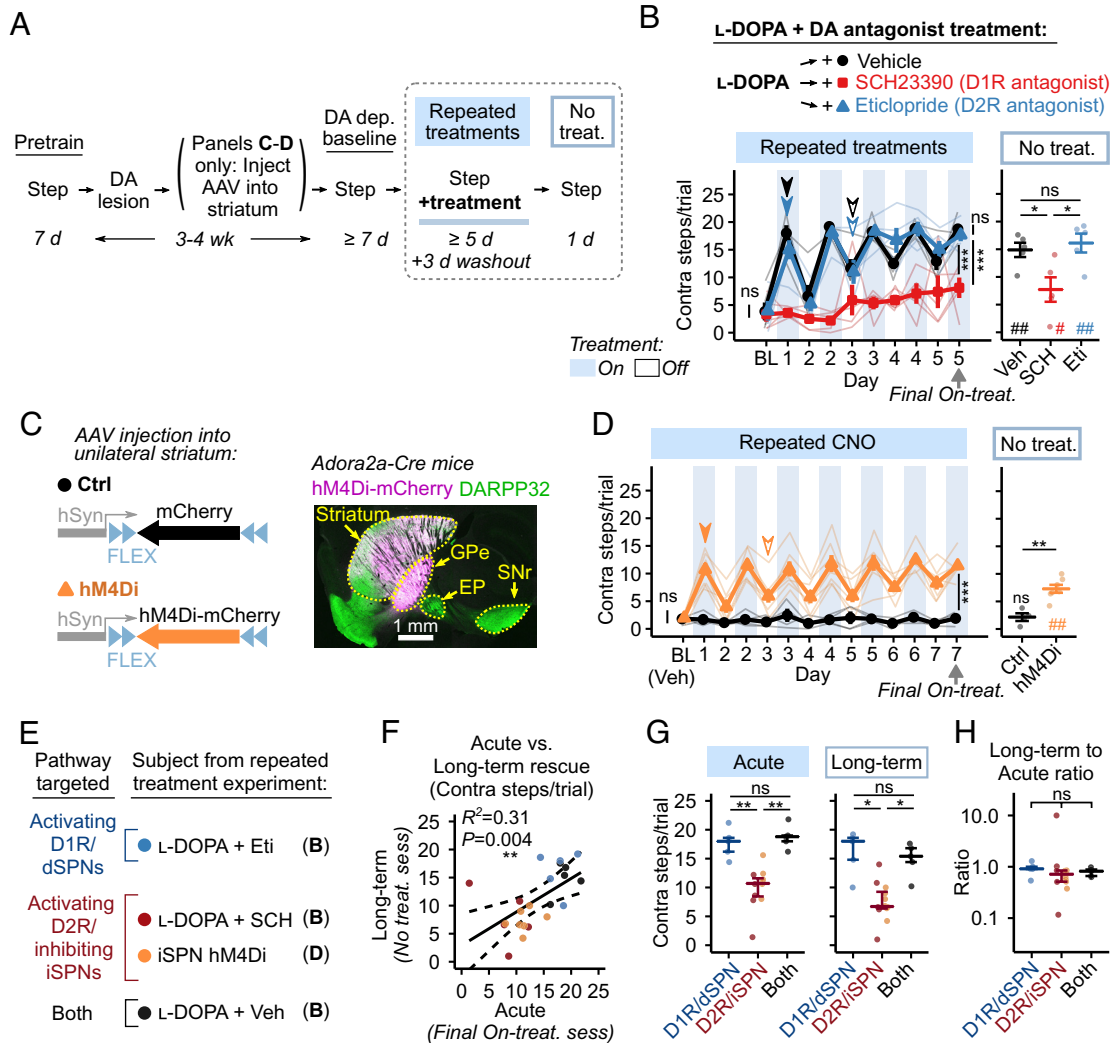
Fig. 2. D1R/dSPN activation from L-DOPA potently induces acute and long-term rescue of parkinsonian deficits. (*A*) Strategy to examine acute vs. long-term rescue induced by targeting D1R/dSPNs vs. D2R/iSPNs. (*B*) Effect of repeated L-DOPA, cotreated with D1R vs. D2R antagonist. BL: parkinsonian baseline final session. Filled arrow: first day of detectable acute rescue vs. BL. Open arrow: first day of detectable long-term rescue vs. BL (Holm–Bonferroni-corrected paired *t* tests, *Materials and Methods*). N = 5, 5, 5. (*C* and *D*) iSPN-specific expression of hM4Di or control virus (*C*); GPe: external globus pallidus; EP: entopeduncular nucleus; SNr: substantia nigra pars reticulata, and (*D*) effect of repeated iSPN inhibition with CNO (hM4Di agonist). Symbol conventions as in (*B*). N = 4, 7. (*E*) Grouping subjects from experiments in (*B*) and (*D*) according to DA receptor/SPN pathway targeted. (*F*) Acute rescue (final on-treatment session) vs. long-term rescue (postwashout session) for each subject. Solid and dotted lines: linear fit and 95% CI. (*G*) Median ± interquartile range (IQR) of acute rescue (*Left*) and long-term rescue (*Right*). (*H*) Median ± IQR of long-term rescue-to-acute rescue ratio (note $\log_{10}$ y axis). Data are mean ± SEM. unless specified. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, vs. same group's DA-depleted baseline, paired *t* tests. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. ANOVAs or Holm–Bonferroni-corrected *t*-tests were used for between-group comparisons, followed by Tukey's HSD post-hoc tests when needed. For (*G* and *H*), Kruskal–Wallis test followed by Wilcoxon rank-sum tests was used instead. See Dataset S1 for full statistics. See also *SI Appendix*, Fig. S4.

rescue, consistent with our finding above that rescue from D1R stimulation may be more effective. Despite this difference in rescue magnitudes, the ratio between long-term and acute rescue was similar between SKF81297 and quinpirole (*SI Appendix*, Fig. S4C), again suggesting that the underlying relationship between acute and long-term rescue did not depend on whether D1R or D2R was targeted.

Targeting D2R/iSPNs Potently Recapitulates Parkinsonian Decline in Dopamine-Intact Mice, While D2R Knockdown Occludes Parkinsonian Decline in Dopamine-Depleted Mice. According to classical basal ganglia model, after dopamine depletion, dSPN underactivation or iSPN overactivation can each decrease motor output (1). However, whether SPN recruitments is altered during learned parkinsonian decline (Fig. 1) is unknown. We used c-Fos (Fos) to examine SPN activation associated with parkinsonian decline, utilizing Drd2-EGFP mice to visualize

iSPNs (GFP$^+$) and putative dSPNs (p-dSPNs; GFP$^-$). On test day, we repeatedly exposed 6-OHDA-lesioned, long-term rescued but L-DOPA-withheld mice to the Step task, causing performance to decline (Fig. 3 *A* and *B* and *SI Appendix*, Fig. S5A). Another 6-OHDA-lesioned group remained in their home cage, thus performance did not decline. Two parallel sham-lesioned groups served as dopamine-intact controls. We then immediately perfused the mice for Fos immunohistochemistry (IHC; Fig. 3C). In the intact dorsal striatum (contralateral to 6-OHDA/sham-lesioned hemisphere), task performance activated both dSPNs and iSPNs (Fig. 3D), consistent with recruitment by dopamine-intact movements (27–30). In the sham/6-OHDA-lesioned striatum, 6-OHDA lesion reduced overall dSPN activation; nevertheless, the stepping-induced dSPN activation was still evident (main effects of 6-OHDA and Task; Fig. 3E). By contrast, 6-OHDA lesion caused task performance to strongly overrecruit iSPNs (significant 6-OHDA×Task interaction). Thus, parkinsonian
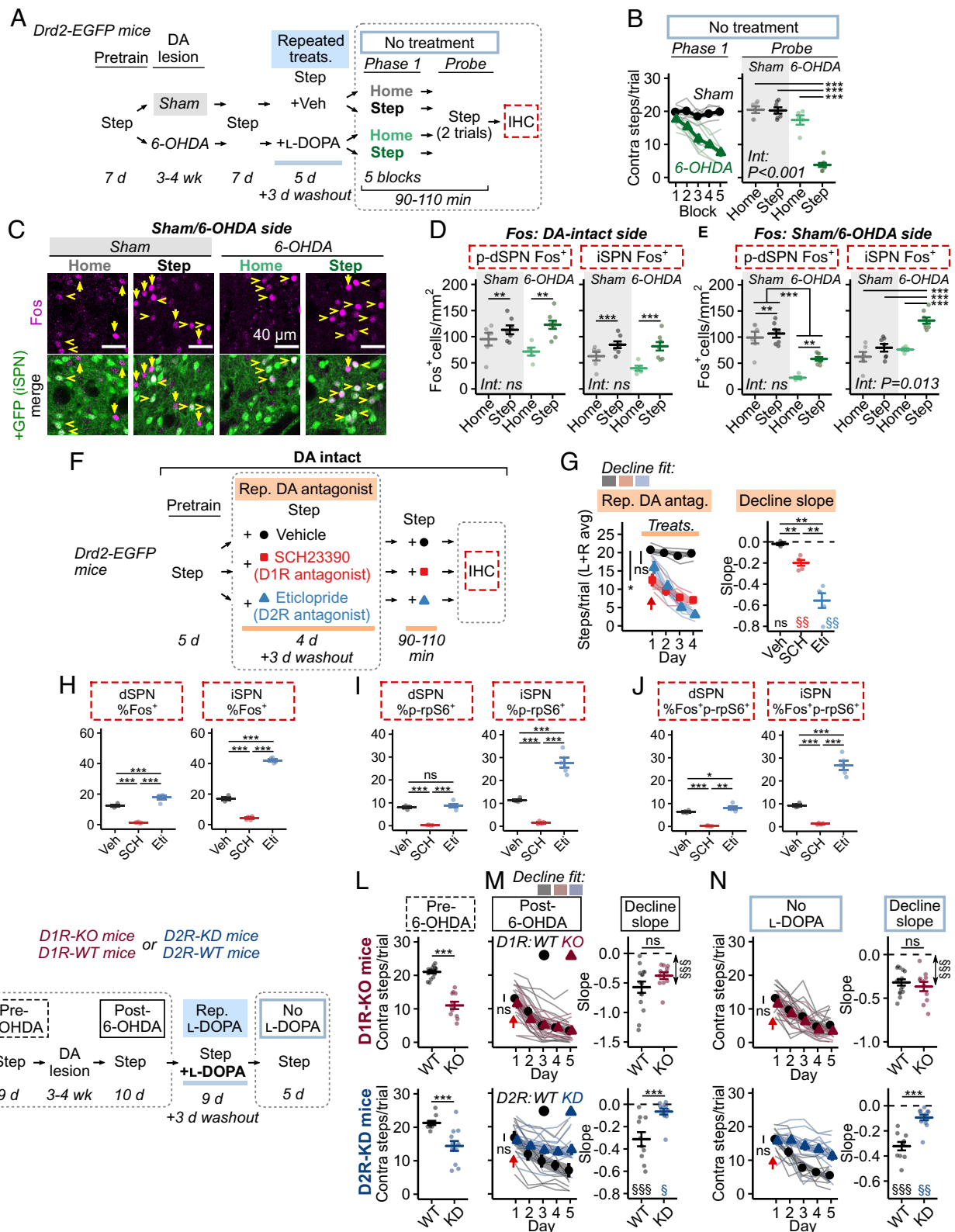
Fig. 3. Reduced D2R stimulation in dopamine-intact mice potently recapitulates parkinsonian decline, while D2R knockdown occludes it in dopamine-depleted mice. (*A–E*) Strategy to examine SPN activation associated with parkinsonian decline (*A*), contralateral performance on test day (*B*), representative IHC images (*C*), Fos density for DA-intact side (*D*) vs. sham/6-OHDA side (*E*). Vertical arrows in (*C*): Fos$^+$ putative-dSPNs (GFP$^-$); horizontal V in (*C*): Fos$^+$ iSPNs (GFP$^+$). N = 6, 7 (sham); 5, 8 (6-OHDA). (*F*) Testing D1R vs. D2R antagonist's ability to induce parkinsonian decline and perturb SPN activation in DA-intact mice. N = 4, 5, 5. (*G*) Stepping performance. Negative slope indicates decline (*Materials and Methods*). (*H–J*) Quantification of dSPN (*Left*) vs. iSPN (*Right*) activation, for Fos$^+$ (*H*), p-rpS6$^{S235/236+}$ (p-rpS6$^+$) (*I*), and Fos$^+$p-rpS6$^{S235/236+}$ double-labeled cells (*J*). (*K–N*) Strategy to examine whether D1R-knockout (D1R-KO) or D2R-knockdown (D2R-KD) occlude parkinsonian decline. (*L*) Pre-6-OHDA baseline contralateral stepping. (*M*) Post-6-OHDA parkinsonian decline. (*N*) Parkinsonian decline after L-DOPA-induced long-term rescue. Stepping declines are fitted with exponential decay, negative slope indicates decline (*Materials and Methods*). Data are mean ± SEM. $^§P < 0.05$, $^{§§}P < 0.01$, $^{§§§}P < 0.001$, vs. zero slope (no decline), Holm–Bonferroni-corrected one-sample t tests. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. ANOVAs or Holm–Bonferroni-corrected t tests were used for between-group comparisons, followed by Tukey's HSD post-hoc tests when needed. See Dataset S1 for full statistics. See also *SI Appendix*, Fig. S5.

decline was associated with both dSPN underrecruitment and iSPN overrecruitment.

To establish causal roles, we perturbed SPN recruitment by blocking D1R vs. D2R signaling in dopamine-intact mice with dopamine receptor antagonists, and then examined the resultant SPN activation using IHC. To facilitate comparison with previous studies, parkinsonian decline was assessed across sessions in this experiment, instead of within a single session as shown in Fig. 3 A–E. It is likely that similar mechanisms underlie both forms of parkinsonian decline, because their behavioral phenotypes are similar. To better identify dSPNs and iSPNs, we used Drd2-EGFP mice and the SPN marker CTIP2. Furthermore, we examined two neuronal activation markers: Fos and ribosomal protein S6 Ser235/236 phosphorylation (p-rpS6$^{S235/236}$; *SI Appendix*, Fig. S5B, *Materials and Methods*). SPN p-rpS6$^{S235/236}$ served as a proxy marker for protein kinase A (PKA) activation (31) which critically controls SPN plasticity (6–8), thereby potentially contributing to parkinsonian decline. Surprisingly, although the D1R antagonist SCH23390 potently blocked L-DOPA-induced rescues in dopamine-depleted mice (Fig. 2B), we found that in dopamine-intact mice, the same dose of SCH23390 led to day 1 deficits that declined relatively mildly on days 2 to 4 (Fig. 3 F and G). By contrast, eticlopride (D2R antagonist) potently recapitulated parkinsonian decline, even though the same dose failed to block L-DOPA-induced rescue in dopamine-depleted mice (Fig. 2B). Our results are consistent with studies showing that D2R blockade leads to more severe motoric decline than D1R blockade across several motor tasks (14, 25). As predicted by reduced D1R vs. D2R signaling, SCH23390 led to dSPN underrecruitment, whereas eticlopride led to iSPN overrecruitment, using Fos, or p-rpS6$^{S235/236}$, or double-labeled as the activity measure (Fig. 3 H–J). Surprisingly, SCH23390 also led to iSPN underrecruitment. Importantly, parkinsonian decline cannot *solely* be explained by iSPN overrecruitment, because D1R blockade caused mild decline despite also causing iSPN underrecruitment. Likewise, dSPN underrecruitment likely cannot *solely* explain parkinsonian decline, because D2R blockade caused severe decline despite not causing dSPN underrecruitment. These results suggest that, while D2R blockade potently induced parkinsonian decline, both dSPN underrecruitment and iSPN overrecruitment can each induce parkinsonian decline.

We next examined the roles of D1Rs vs. D2Rs in parkinsonian decline caused by 6-OHDA, using D1R knockout (D1R-KO) vs. D2R knockdown (D2R-KD) mice. Dopamine-intact D1R-KO mice and D2R-KD mice each had baseline stepping deficits (*SI Appendix*, Fig. S5 C and D, *Materials and Methods*). Furthermore, D1R-KO vs. D2R-KD each selectively occluded both parkinsonian decline and changes in SPN activation induced by D1R vs. D2R antagonist, respectively (*SI Appendix*, Fig. S5 E and F). These data confirm that D1R-KO and D2R-KD mice allowed contributions from D1Rs vs. D2Rs to be separately examined. We next tested the effect of 6-OHDA lesion on D1R-KO and D2R-KD mice (Fig. 3 K–N). While all groups showed detectable 6-OHDA-induced parkinsonian decline (both post-6-OHDA, and after washout from repeated L-DOPA), decline slopes were occluded (less steep) in D2R-KD but not D1R-KO mice. This suggests that in WT mice, the lack of D2R signaling after 6-OHDA may play an important role in inducing parkinsonian decline. Indeed, D2R-KD mice's post-6-OHDA stepping remained high despite extended task exposure, which occluded (delayed) detection of L-DOPA-induced rescue (*SI Appendix*, Fig. S5 G and H). By contrast, L-DOPA was less effective in rescuing D1R-KO mice both acutely and long term (*SI Appendix*, Fig. S5H), consistent with D1R's importance (Fig. 2), although

L-DOPA-induced long-term rescue eventually reached D1R-WT's level with extended training (Fig. 3N). Altogether, these data suggest that, while reduced D1R and D2R stimulation both contributed to parkinsonian decline, the decline from reduced D2R stimulation may be steeper.

iSPN Inhibition, but not Cholinergic Interneuron Ablation, Attenuates Gradual Parkinsonian Decline. In addition to iSPNs, D2Rs are also expressed in other cell types, including in striatal cholinergic interneurons (ChIs) (32), thus raising the possibility that D2R manipulations above mediated their effects through cell types other than iSPNs. If iSPN overrecruitment was important for parkinsonian decline, then selective iSPN inhibition should attenuate it. To test this, we used Adora2a-Cre mice to drive iSPN-selective expression of the inhibitory DREADD hM4Di. To avoid potential off-target effects of systemic CNO treatment, CNO was directly infused into the dopamine-depleted striatum. Chemogenetic inhibition of iSPNs with CNO acutely attenuated parkinsonian decline, as evidenced by flatter decline slope during treatments (Fig. 4 A–C). Importantly, improvements persisted after CNO washout, consistent with long-term rescue. CNO's effect was not likely off target, as it was not seen in control mice that did not express hM4Di. These data provide further evidence that iSPN overrecruitment contributes to parkinsonian decline.

Additionally, we found that the A_{2A} receptor (A_{2A}R) antagonist istradefylline—used clinically to improve motor functions in PD patients (33)—similarly attenuated parkinsonian decline of long-term rescue (*SI Appendix*, Fig. S6 A and B), consistent with iSPN-selective expression of A_{2A}Rs (32), and the ability of A_{2A}R antagonists to inhibit iSPN-LTP (6, 8). Interestingly, previous work suggests that reduced D2R signaling in iSPNs can cause iSPN-to-dSPN axon collaterals (34, 35) to excessively inhibit dSPNs, thereby causing motor deficits (36). Therefore, we explored whether A_{2A}R antagonist rescued iSPN overrecruitment and/or dSPN underrecruitment. Using Drd2-EGFP mice, we found that A_{2A}R antagonist attenuated parkinsonian decline and rescued iSPN Fos overrecruitment, but not p-dSPN Fos underrecruitment (*SI Appendix*, Fig. S6 C–E). These data suggest that parkinsonian decline could be attenuated by rescuing iSPN overrecruitment, without also rescuing dSPN underrecruitment, at least when using Fos as an activity marker. These results are consistent with recent findings that stimulating iSPNs' downstream target in the external globus pallidus (GPe) can produce long-lasting motor rescue in parkinsonian mice (37).

Striatal cholinergic interneurons (ChIs) also express D2Rs (32). D2R stimulation inhibits dorsal striatal ChIs (38), and reduced D2R-mediated ChI inhibition has been implicated in parkinsonian deficits (26, 39–41). If overactive ChI drove parkinsonian decline, then ChI ablation should occlude it, mimicking D2R-KD mice. We virally expressed Cre-dependent diphtheria toxin A (DTA) unilaterally in the striatum of ChAT-IRES-Cre mice, causing ChI ablation (Fig. 4 D–G; *Materials and Methods*). In control ChAT-IRES-Cre mice that virally expressed a Cre-dependent EYFP reporter in the striatum, 88.4 ± 1.5% (mean ± SEM) of EYFP-labeled cells also expressed the ChI marker ChAT (N = 13 mice), consistent with ChI selectivity. We found that ChI ablation did not alter baseline stepping in dopamine-intact mice, but attenuated acute stepping deficits from the D2R antagonist eticlopride on day 1 (*SI Appendix*, Fig. S7 A–E), consistent with ChI's role in mediating acute catalepsy from D2R blockade (39). However, contralateral stepping worsened at a similar rate in ChI-ablated mice, as evidenced by decline slopes being the same. Eticlopride-induced striatal Fos was also unaltered (*SI Appendix*, Fig. S7C). Furthermore, we found no evidence that ChI ablation altered the
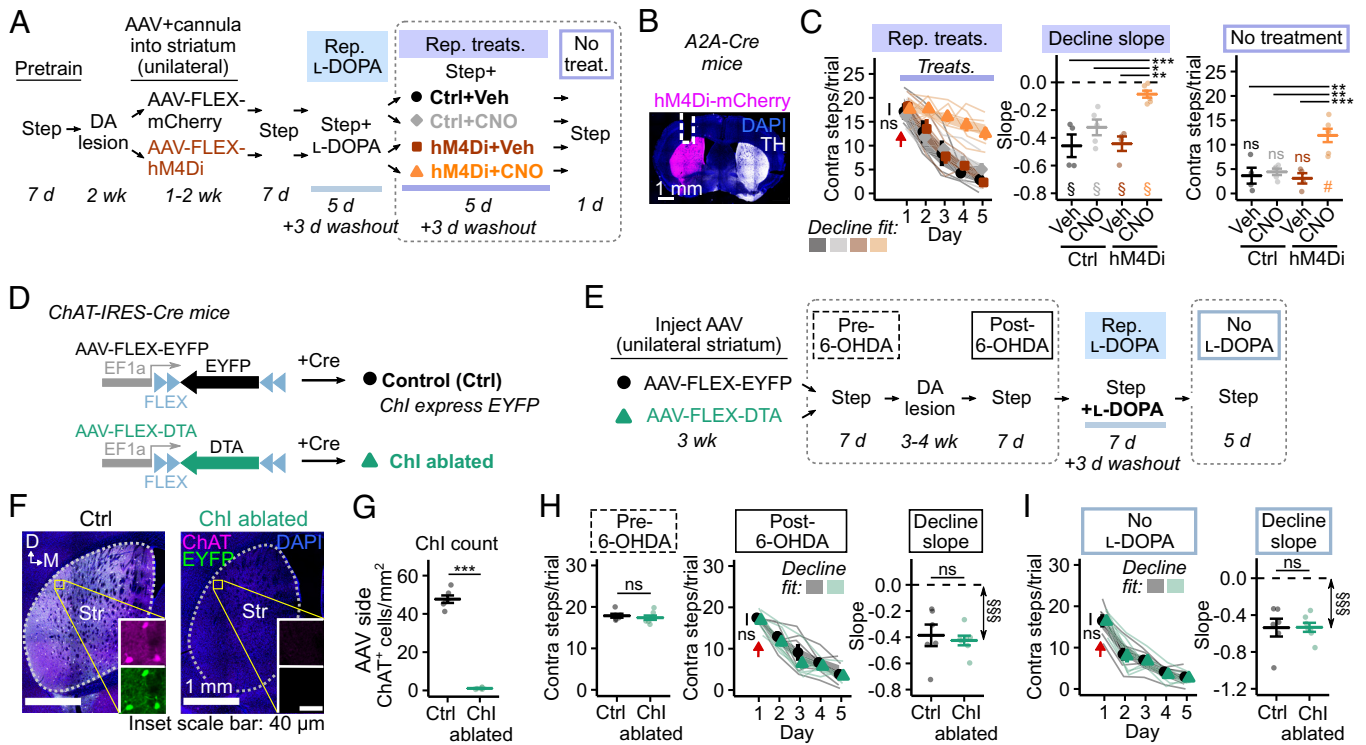
Fig. 4. iSPN-specific inhibition, but not cholinergic interneuron ablation, attenuates gradual parkinsonian decline. (*A–C*) Strategy to test whether iSPN-specific chemogenetic inhibition attenuates parkinsonian decline (*A*), striatal cannula for CNO infusion (*B*), and effect of iSPN-specific inhibition on contralateral performance (*C*). #$P < 0.05$, vs. same group's DA-depleted baseline, paired t tests. N = 4, 5, 4, 6. (*D*) Cholinergic interneuron (ChI) ablation using AAV-FLEX-DTA. (*E–I*) Strategy to examine whether ChI ablation occludes 6-OHDA-induced parkinsonian decline and rescue from L-DOPA (*E*), representative IHC images of ChI ablation (*F*), postmortem ChI quantification (*G*), contralateral stepping pre- vs. post-6-OHDA (*H*), and parkinsonian decline after L-DOPA withdrawal (*I*). N = 6, 7. Stepping declines are fitted with exponential decay, negative slope indicates decline (*Materials and Methods*). Data are mean ± SEM. §$P < 0.05$, §§§$P < 0.001$ vs. zero slope (no decline), Holm–Bonferroni-corrected one-sample t tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Independent-samples t tests were used for between-group comparisons. See Dataset S1 for full statistics. See also *SI Appendix*, Figs. S6 and S7.

rate of parkinsonian decline from 6-OHDA: ChI-ablated mice showed similar post-6-OHDA decline, had similar L-DOPA-induced acute and long-term rescue, and long-term rescue declined at a similar rate compared with controls (Fig. 4 *H* and *I* and *SI Appendix*, Fig. S7 *F* and *G*). Therefore, while ChI inhibition could attenuate acute motor deficits caused by reduced dopaminergic signaling, ChIs likely play a minor role in mediating parkinsonian motoric decline.

Balanced dSPN:iSPN Activity Predicts Prevention of Parkinsonian Decline. Experiments above suggest that reduced D2R stimulation effectively recapitulated steep parkinsonian decline in dopamine-intact mice, whereas D1R stimulation potently induced long-term rescue in severely impaired dopamine-depleted mice. Surprisingly, although the D2R agonist quinpirole induced milder acute and long-term rescue than the D1R agonist SKF81297 (*SI Appendix*, Fig. S4), we found that both SKF81297 and quinpirole were similarly effective in preventing parkinsonian decline across sessions; moreover, the rescue was long lasting (*SI Appendix*, Fig. S8 *A* and *B*). Quinpirole's effect was not blocked by ChI ablation, consistent with a role for iSPNs and not ChIs (*SI Appendix*, Fig. S8 *C* and *D*). While restoring D1R and D2R stimulation each prevented parkinsonian decline, it is unclear whether they did so by rescuing pathological recruitment of a single SPN pathway. For instance, although D1R stimulation would activate dSPNs and D2R stimulation would inhibit iSPNs, each could also theoretically alter recruitment of the opposite SPN via intrastriatal collaterals (21, 34). We used Drd2-EGFP mice and SKF81297 vs. quinpirole to block parkinsonian decline with a one-session repeated exposure protocol, then examined

SPN activation using IHC to label CTIP2 (SPN marker), GFP (D2R-expression marker), Fos, and p-rpS6$^{S235/236}$ (Fig. 5 *A–C*). While quinpirole again prevented parkinsonian decline, it had an acute suppressant effect on behavior during early trial blocks (42), possibly due to cataplexy caused by D2R stimulation in the basolateral amygdala (43). Although this one-session protocol led to quinpirole inducing a mildly positive decline slope that may be an artifact, it was consistent with quinpirole's effectiveness in preventing parkinsonian decline across sessions (*SI Appendix*, Fig. S8B). Surprisingly, although SKF81297 and quinpirole both prevented decline, they had drastically different effects on SPN activation (Fig. 5 *D–F*). SFK81297 strongly activated dSPNs, but unexpectedly further increased iSPN Fos overrecruitment relative to 6-OHDA, although this has been previously reported (29), and may reflect movement-induced iSPN recruitment. Conversely, quinpirole reversed iSPN overrecruitment but surprisingly further suppressed dSPN activation relative to 6-OHDA. These results suggest that, like the induction of parkinsonian decline in dopamine-intact mice (Fig. 3 *F–J*), the prevention of parkinsonian decline in dopamine-depleted mice cannot be explained by the activity of a single SPN pathway, but instead may be better explained by considering both dSPN and iSPN pathways together.

We examined the above proposal using modeling. If a single SPN pathway adequately explained parkinsonian decline, then its activation—measured using Fos, p-rpS6$^{S235/236}$, or Fos + p-rpS6$^{S235/236}$ double labeled—should account for the differences in parkinsonian decline slopes in different dopaminergic conditions. We first examined this using linear regression in dopamine-intact mice given D1R vs. D2R antagonists (*SI Appendix*, Fig. S9B). When we used dSPN activation measures as linear
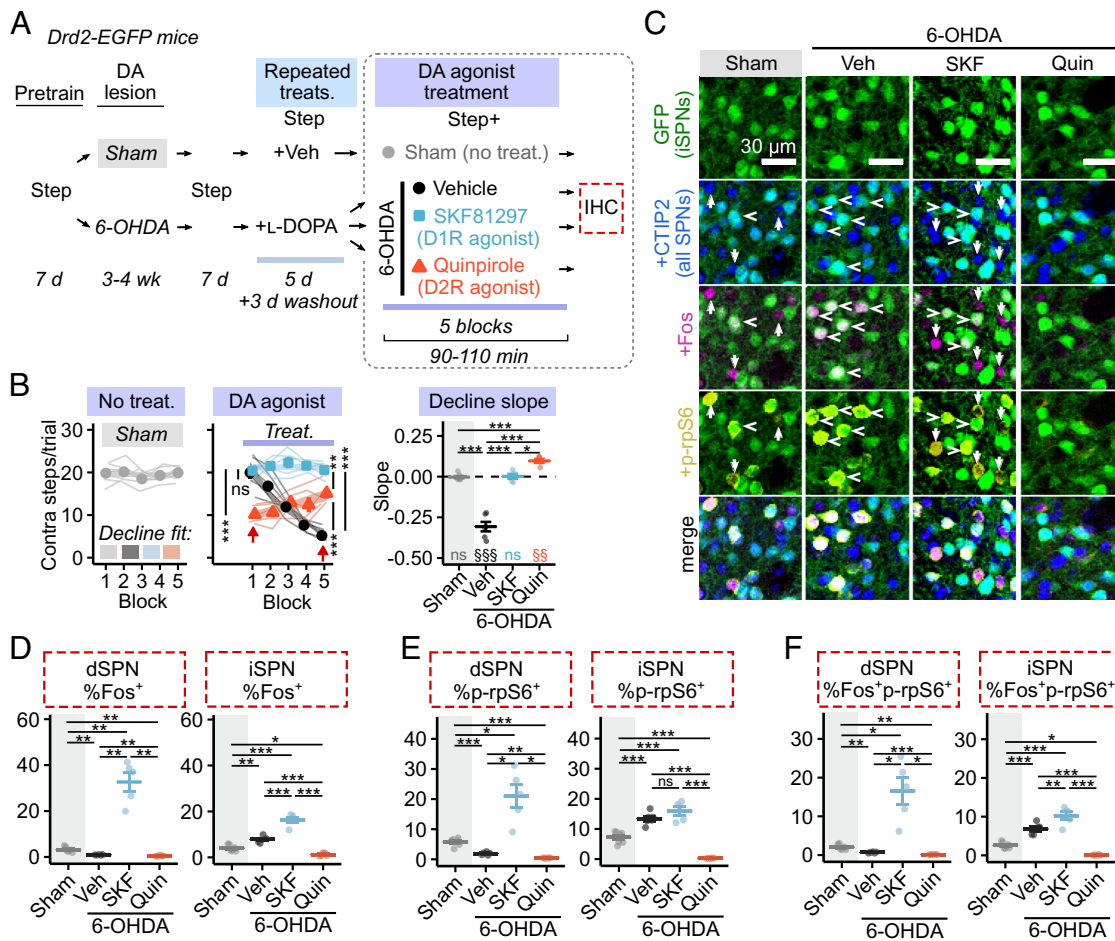
Fig. 5. D1R and D2R agonists both prevent parkinsonian decline in dopamine-depleted mice, but have drastically different effects on dSPN and iSPN activation. (*A*) Strategy to examine SPN activation associated with parkinsonian decline prevention. The sham group is taken from Fig. 3*A* "Sham+Step" group. *N* = 7 (sham); 6, 5, 5 (6-OHDA). (*B*) Contralateral performance on test day. Negative slope indicates decline (*Materials and Methods*). $^{§§}P < 0.01$, $^{§§§}P < 0.001$, vs. zero slope (no decline), Holm–Bonferroni-corrected one-sample *t* tests. (*C*) Representative IHC images. Vertical arrows: double-labeled Fos$^+$p-rpS6$^+$ dSPN (CTIP2$^+$GFP$^-$). Horizontal V: double-labeled Fos$^+$p-rpS6$^+$ iSPN (CTIP2$^+$GFP$^+$). (*D–F*) Quantification of dSPN (*Left*) vs. iSPN (*Right*) activation, for Fos$^+$ (*D*), p-rpS6$^{S235/236+}$ (p-rpS6$^+$) (*E*), and Fos$^+$p-rpS6$^{S235/236+}$ double-labeled cells (*F*). Data are mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ANOVAs or Holm–Bonferroni-corrected *t* tests were used for between-group comparisons, followed by Tukey's HSD post-hoc tests when needed. See Dataset S1 for full statistics. See also *SI Appendix*, Figs. S8–S10.

predictors of parkinsonian decline slope, none of the resultant models were significant (*SI Appendix*, Fig. S9*C*), suggesting that there was no linear relationship between dSPN activation and dopamine-antagonist-induced parkinsonian decline. By contrast, we found all the three iSPN activation measures to be negatively related to parkinsonian decline slope (*SI Appendix*, Fig. S9*D*), which suggests that increased iSPN activation is associated with more severe parkinsonian decline; this effect was largely driven by D2R antagonism (Fig. 3 *H–J*). However, this cannot explain how D1R antagonist induced moderate parkinsonian decline despite also reducing iSPN activation. We therefore combined contributions from both dSPN and iSPN together into a SPN bias index—modified from (30)—that ranges from negative (more iSPNs activated than dSPNs), to 0 (dSPNs and iSPNs equally activated), to positive (more dSPNs activated than iSPNs) (*SI Appendix*, Fig. S9*A*, *Materials and Methods*). We found that, unlike models using dSPN or iSPN activation alone, SPN bias indices correctly predicted the order of parkinsonian decline severity caused by D1R vs. D2R antagonists (*SI Appendix*, Fig. S9*E*) and provided better fits as confirmed by model comparison (*SI Appendix*, Fig. S9*G*). The superior fit with SPN bias indices suggests that parkinsonian decline occurs when more iSPNs are activated than dSPNs.

We next carried out a similar analysis in dopamine-depleted mice whose parkinsonian decline was prevented by D1R vs. D2R

agonists (*SI Appendix*, Fig. S10*A*). Again, we found that almost all linear models that used only dSPN or iSPN activation as predictors of parkinsonian decline were not statistically significant (*SI Appendix*, Fig. S10 *B* and *C*), suggesting that there was no robust linear relationship between the activation of a single SPN pathway and dopamine-agonist-induced prevention of parkinsonian decline. By contrast, SPN bias indices—especially those that incorporated p-rpS6$^{S235/236}$—provided significantly better linear fit to parkinsonian decline (*SI Appendix*, Fig. S10*D*), as confirmed by model comparison (*SI Appendix*, Fig. S10*F*). These results provide further evidence that both SPN pathways are involved, and that parkinsonian decline is prevented when balanced dSPN:iSPN activation is restored. Furthermore, the finding that p-rpS6$^{S235/236}$ activation was an important predictor is consistent with a role for PKA-dependent plasticity. Finally, we found that α (an exponentiation parameter) from SPN bias index decreased from ≈1.4 in the dopamine-intact case (*SI Appendix*, Fig. S9*F*) to ≈1 in the dopamine-depleted case (*SI Appendix*, Fig. S10*E*). Since α acts as a sensitivity threshold to SPN activation, this finding suggests that after dopamine depletion, SPNs' downstream targets may become relatively more sensitive to SPN activation (*SI Appendix*, Fig. S10*G*), as has been previously observed for both SNr and GPe (44, 45) (*Discussion*). Altogether, these results suggest that both dSPNs and iSPNs likely contribute to parkinsonian decline and its rescue by dopaminergic therapy.

Discussion

By dissociating dopamine's acute vs. long-lasting effects on motor performance, we unambiguously showed that dopamine-dependent learning is critical even for movements without explicit rewards. Deficits in these movements drastically impair everyday life in Parkinson's disease. Surprisingly, in the two tasks tested here, much of the performance deficits caused by dopamine depletion stemmed from experience-dependent, task-specific motoric decline (Fig. 1 *D* and *H*). This finding adds to previous studies that used different motor tasks to show the importance of learning in parkinsonian deficits (14, 15) and raises the possibility that the importance of learning in PD may generalize to other motor tasks not tested here. Our results suggest two roles of dopamine in movements. First, dopamine acutely activates movements, as shown by L-DOPA after 6-OHDA-induced parkinsonian decline. This is consistent with dopamine having a feedforward role in driving movement initiation (2, 3). Second, repeated task performance with dopamine increases future movement probability in a task-specific manner, such that subsequent movements can occur even without dopamine, consistent with learning. Conversely, whereas transient dopamine inhibition does not strongly affect movements that had been initiated (2), continued task performance while dopamine-depleted resulted in learning-dependent, task-specific motoric decline. This result suggests that dopamine also serves to maintain movements, and if dopamine is removed, another mechanism—triggered by task exposure—causes motoric performance to decline. Our findings thus show that dopamine acts as a teaching signal to drive learning, not only for Pavlovian and instrumental conditioning (11, 12), but also for movements without explicit rewards, such as forepaw stepping and maintaining forward locomotion while descending. Conversely, reduced dopamine signaling led to task-specific decrease in movements, analogous to extinction in reinforcement learning. These data suggest that reinforcement and maintenance of movements—even those outside of traditional conditioning tasks with rewards—may be a fundamental function of striatal dopamine. In agreement with this, a recent study showed that spontaneous open-field behavior (without explicit reward) can be clustered into discrete sub-second behavioral modules ("syllables"), and that behavioral modules with bigger striatal dopamine transients are more likely to reoccur in the future. Furthermore, pairing optogenetic stimulation of dopaminergic neurons with specific behavioral modules reinforces subsequent use of those specific modules—an effect that was detectable even during a subsequent, nonstimulated session (46). Our present results complement this study by also showing the converse: Pairing-reduced dopaminergic signaling with a specific task—akin to eliciting a specific behavioral module—reduces future performance of the same specific task. We therefore speculate that tonic dopamine signaling (11) and/or perimovement dopamine transients (3) may serve as feedback teaching signals (6–9) for movement-related set points such as balance, effort, or other movement-related metric (47, 48), to alter future probability of emitting that same movement.

Interestingly, our casual observations suggested that mice that had acquired long-term rescue on the Step or Pole task showed parkinsonian motor impairment in the home cage just before reexposure to the task (i.e., reduced locomotion—including reduced forelimb movements—and ipsilateral rotational bias). Nevertheless, performance of similar movements recovered as soon as the mice were placed on the task apparatus (Movies S3 and S4), at least before parkinsonian decline had occurred through repeated task exposure. This observation raises the intriguing possibility that the environmental context associated with the task may play

an important role in triggering the expression of long-term motoric rescue, consistent with a recent model of motor learning that emphasizes the contribution of contexts (49). While it is possible that the stress of being handled may have contributed to the long-term rescue observed in our studies (similar to paradoxical kinesia in PD patients), we think that it is unlikely: If that was the case, one might expect that reexposure to the task that had not been previously paired with repeated L-DOPA should also trigger stress-induced spontaneous motor recovery, which we did not observe (Fig. 1*F*). Similarly, the gradual motoric decline caused by repeated, off-treatment task exposure likely did not reflect declining handling stress: Even after motor performance had declined on one task, it showed long-term rescue when the animal was switched to another task, despite consistent handling stress (Fig. 1*H*). In short, the task specificity of both the induction and the decline of long-term rescue suggests an involvement of learning, instead of changes in handling stress.

The long-term rescue induced by L-DOPA and dopamine receptor agonists observed here shares several notable features with the clinically observed LDR in PD patients: They are both retained across days to weeks despite withdrawal from dopaminergic treatments, and they both decay gradually (4, 16). This raises the possibility that the long-term rescue described here can serve as a preclinical model for these important features of LDR. Although L-DOPA has been the most prescribed PD treatment for over half a century, with LDR providing most of its therapeutic benefits (4, 5), the mechanism of LDR is still unclear. The infusion of 6-OHDA into the medial forebrain bundle used in our study led to severe dopamine depletion, which better represents late-stage PD rather than early PD. This is important because L-DOPA seems to be clinically less effective at late-stage PD, largely due to a faster LDR decay and an increase in fluctuating side effects, both of which severely limit PD treatments (4, 20). The mechanism of LDR has been rarely explored, and most studies have focused on reversing the shortening of L-DOPA's pharmacokinetic duration by gene therapy, cellular transplants, and continuous infusions, but with limited success. Elucidating the mechanism of LDR is crucial to drive new hypothesis-driven approaches for therapeutic intervention (19), and here we propose a preclinical LDR model that can help to uncover the roles played by SPNs.

We found that D2R blockade potently induced severe parkinsonian decline in dopamine-intact mice that have high baseline probability of initiating movements (Fig. 3*G*), recapitulating the effect of 6-OHDA-mediated dopamine depletion; conversely, D2R knockdown occluded parkinsonian decline in dopamine-depleted mice (Fig. 3 *K–N*). These findings likely involve D2R signaling directly on iSPNs instead of on ChIs (Fig. 4). In dopamine-intact mice, iSPN activation during movements is hypothesized to inhibit competing actions (27, 28), including actions that, through experience, animals learn to be inappropriate (8, 50). Our findings suggest that when D2R signaling is globally lost (e.g., in PD), this process becomes dysregulated, resulting in experience-dependent inhibition of task-appropriate movements. By contrast, blockade of D1R signaling resulted in a somewhat milder parkinsonian decline in dopamine-intact mice, as was also observed in other motor tasks (25), even though the same SCH23390 dose potently blocked L-DOPA-mediated rescue in dopamine-depleted mice (Fig. 2*B*). These results are consistent with clinical observations that D2R antagonists may cause more severe extrapyramidal side effects than D1R antagonists—whose therapeutic uses are beginning to be explored in tics (51).

By contrast, when movement initiation probability became low after severe parkinsonian decline, D1R/dSPN activation potently

induced acute motoric rescue (Fig. 2 and *SI Appendix*, Fig. S4), consistent with dSPN's role in action initiation (22). Furthermore, the acute rescue gradually consolidated into long-term rescue, consistent with the effectiveness of L-DOPA and D1R agonist in treating motoric deficits in PD (52–54). By comparison, targeting D2R/iSPN resulted in both acute and long-term rescues that were smaller in magnitude (Fig. 2 and *SI Appendix*, Fig. S4). Because an optimal window of iSPN activation may be required for movement initiation (27, 28), the global iSPN inhibition by our manipulations could have disrupted action initiation (23) in addition to reversing 6-OHDA-induced iSPN overrecruitment, resulting in milder motoric rescues. Although targeting D2R/iSPN resulted in weaker acute and long-term rescue in our studies, the long-term-rescue to acute-rescue ratio—which may reflect the proportion of acute rescue that was "stored" as long-term rescue—was similar regardless of the pathway targeted (Fig. 2*H* and *SI Appendix*, Fig. S4C). This suggests that the underlying relationship between acute and long-term rescue likely did not depend on the dopamine receptor/SPN pathway targeted.

Although we selected the doses of SCH23390 and eticlopride based on their ability to induce similar acute motor impairment in dopamine-normal mice (25), a caveat of our studies is that we used single doses of agonists and antagonists. It is thus also possible that the smaller rescue magnitudes we observed from targeting D2R/iSPN may be caused by doses that were relatively less potent. Another caveat is that systemic drug treatments can also affect nonstriatal regions. For example, D2R stimulation outside the striatum may promote sleep-related processes (43). Drugs can also have off-target effects on nondopaminergic receptors, although we were able to use D1R-KO and D2R-KD to support our main findings that 1) D1R stimulation is important for motoric rescue in dopamine-depleted mice (*SI Appendix*, Fig. S5*H*), and 2) reduced D2R stimulation recapitulates severe parkinsonian decline in dopamine-intact mice and is occluded by D2R knockdown (Fig. 3 *M* and *N*).

Surprisingly, even though quinpirole (D2R agonist) induced milder acute and long-term rescues than SKF81297 (D1R agonist) (*SI Appendix*, Fig. S4*B*), both were similarly effective in preventing parkinsonian decline once long-term rescue had been acquired (Fig. 5*B* and *SI Appendix*, Fig. S8*B*). This was not well explained by D1R and D2R agonists rescuing a single, common SPN pathway. Instead, parkinsonian decline prevention was best explained by restoring balanced dSPN:iSPN activation (SPN bias index ≈ 0; *SI Appendix*, Fig. S10 *B–D*). Furthermore, in dopamine-intact mice, the ability of D1R and D2R antagonist to induce parkinsonian decline was also best explained by taking both dSPN and iSPN activation into account using the SPN bias index (*SI Appendix*, Fig. S9 *C–E*). These results highlight the complementary roles of dSPNs and iSPNs and suggest that balanced dSPN:iSPN activation—likely integrated by downstream regions (e.g., the SNr)—is critical for both immediate (30) and future task-specific movements. Conversely, imbalanced SPN activation that favors iSPNs leads to parkinsonian decline. Furthermore, our finding that incorporating ribosomal protein S6 Ser235/236 phosphorylation (p-rpS6$^{S235/236}$) best predicted the slope of parkinsonian decline is consistent both with dopamine's role in controlling SPNs' PKA activity (55) and with PKA's importance in driving SPN plasticity and learning (6–8).

Our modeling suggested that the sensitivity threshold α of SPN bias index decreased from 1.4 to 1 after chronic dopamine depletion (*SI Appendix*, Figs. S9*F* and S10*E*). The lower sensitivity threshold α after chronic dopamine depletion may reflect SPNs' downstream targets being relatively more sensitive to inputs from SPNs (*SI Appendix*, Fig. S10*G*), consistent with findings that chronic dopamine depletion may increase the connectivity and/or neurotransmission efficacy between SPNs and their downstream targets in SNr (44) and GPe (45). Interestingly, D2R signaling appear to play a critical role in at least one case: D2R-knockout mice have reduced connectivity of a noncanonical dSPN-to-GPe "bridging collateral" projection (56)—a projection whose connectivity is strongly increased by dopamine depletion (45). If the increase in connectivity of this projection is involved in 6-OHDA-induced parkinsonian decline, then a reduction in D2R-KD mice may explain why parkinsonian decline was occluded in D2R-KD mice but not in D1R-KO mice (Fig. 3 *L–N*). Note that the SPN bias index in *SI Appendix*, Fig. S9*A* is only one of the many possible ways of combining dSPN and iSPN activation. For instance, we have assumed the same sensitivity threshold α for dSPNs vs. iSPNs for the sake of parsimony. Future experiments may identify a model that better fits behavioral and/or neurophysiological data.

In the present study, we employed two widely used neuronal activation markers: Fos and p-rpS6$^{S235/236}$—the latter as a proxy marker for protein kinase A (PKA) activation (31) which critically controls SPN plasticity (6–8). Surprisingly, we found that the D1R antagonist SCH23390 reduced Fos and p-rpS6$^{S235/236}$ not only in dSPNs as expected, but also in iSPNs (Fig. 3 *H–J*), partly in agreement with recent findings using real-time calcium and PKA activity imaging (57). Conversely, the D1R agonist SKF81297 increased Fos in both dSPNs and iSPNs (Fig. 5 *D–F*). Similarly, the D2R agonist quinpirole reduced Fos and p-rpS6$^{S235/236}$ not only in iSPNs as expected, but also in dSPNs. These findings provide evidence of "cross talk" between D1Rs and iSPNs, and between D2Rs and dSPNs. Such cross talk, which is often not considered in the classical basal ganglia model of parallel dichotomous direct/indirect pathways, may nevertheless be important for controlling movements. The concomitant activation/inhibition of both dSPNs and iSPNs resembles their comodulation during movement initiation (27, 28, 57) and may reflect the ability of a single SPN pathway to modulate downstream structures in the cortico-striato-thalamic loop, which in turn can modulate cortico-striatal or thalamo-striatal glutamatergic inputs that can influence both types of SPNs. While the present IHC approach allowed parallel assessment of Fos and p-rpS6$^{S235/236}$ activation, these data should be complemented in the future by real-time measurements of SPN activation (29, 30, 55, 57) during similar motor tasks, because discrepancies may be informative. Indeed, in vivo electrophysiological recording found that, in 6-OHDA-treated mice, SKF81297 (D1R agonist) decreased the firing of putative iSPNs, and quinpirole (D2R agonist) increased the firing of putative dSPNs (58), in agreement with dSPNs and iSPNs laterally inhibiting each other via intrastriatal axon collaterals (35, 36), and contrary to our finding (Fig. 5 *D–F*), although a major difference from our study is their use of higher agonist doses that elicited substantial dyskinesia. Another discrepant finding involves calcium imaging studies, which suggest that movement-associated overrecruitment of iSPNs may be transient: Most obvious 1 to 2 d after 6-OHDA, but declines back to baseline levels by 14 d after 6-OHDA (29, 30). By contrast, our study suggests that movement-associated iSPN overrecruitment could still be detected by Fos and p-rpS6$^{S235/236}$ IHC >30 d after 6-OHDA (Figs. 3 *A–E* and 5). One explanation for these discrepancies is that Fos and p-rpS6$^{S235/236}$ may reflect processes other than calcium levels and neuronal firing rates, for instance processes related to long-term plasticity.

Our findings may have important implications for the treatment and management of Parkinson's disease. First, we found that long-term rescue from L-DOPA can persist for ≥9 d after

withdrawal if subjects did not perform the task during that period (Fig. 1*H*). This finding explains the difficulty in assessing PD patients' "off-treatment" baseline motor impairments in clinical trials, and how the confounding effects of LDR can occlude the efficacy of disease-modifying treatments (16). Second, we found that targeting D1R/dSPNs is important for inducing long-term rescue, but either D1R or D2R stimulation was sufficient to restore balanced dSPN:iSPN activation, which was associated with the prevention of parkinsonian decline. It remains to be determined whether L-DOPA's ability to activate both D1Rs and D2Rs concurrently underlies its greater clinical benefits compared with D2R agonist treatments alone (59). Finally, our results suggest that dopamine- and experience-dependent learning is critical in establishing long-term rescue in an animal model. Whether the development of LDR in PD patients is similarly experience dependent and task specific is only partially explored (60–62), and our results should provide the impetus for future human studies. If confirmed, one may devise procedures that facilitate LDR decay for a specific task to be assessed in the clinic—while minimally influencing PD patients' overall activities in daily living—to reveal the underlying disease states for assessing new disease-modifying therapies.

Taken together, our findings demonstrate that dopamine-dependent learning drives even nonexplicitly rewarded movements that are impaired in Parkinson's disease, and that imbalanced dSPN:iSPN activation contributes to experience-dependent parkinsonian decline. These results have important implications for optimizing treatments not only for PD, but also for other movement-related diseases including Tourette syndrome, antipsychotic-induced parkinsonism, and tardive dyskinesia.

Materials and Methods

Experimental procedures for mouse strains, surgery, behavior, and data analysis are described in detail in *SI Appendix, Materials and Methods*.

Mice. Mice of both sexes (≥3 mo old) were used. All transgenic and mutant mouse lines were backcrossed with C57BL/6J mice for ≥5 generations prior to use. Animal use followed the NIH guidelines and was approved by Institutional Animal Care and Use Committees of Columbia University and New York University Grossman School of Medicine.

Behavioral and Surgical Procedures. Mice were trained on the motor tasks, then underwent unilateral 6-OHDA lesion of the medial forebrain bundle (and adeno-associated virus (AAV) infusion if needed). After recovery, mice were reexposed to the motor tasks to establish the parkinsonian baseline. To induce long-term rescue, repeated daily dopaminergic agonist treatments (or CNO in Fig. 2*D*) were given prior to task. For a subset of mice, Step task long-term rescue during induction was probed before each day's drug treatment for two trials (overnight washout). To probe long-term rescue decay, mice were exposed to the task after 3-d treatment washout.

Immunohistochemistry. Standard immunohistochemistry techniques were used, and images were analyzed with ImageJ and custom software written in Python.

Statistics. Statistical tests were conducted using R. For comparisons involving >2 groups, ANOVAs were used. Holm–Bonferroni correction was used to control for family-wise error rate. Linear regression or nonlinear least square was used for model fitting. Likelihood ratio test or Akaike Information Criterion was used for model comparison.

Data, Materials, and Software Availability. Custom code, data have been deposited in Dataverse (63). All study data are included in the article and/or *SI Appendix*.

Author affiliations: ᵃDepartment of Neurology, Neuroscience Institute, New York University Grossman School of Medicine, New York, NY 10016; ᵇDepartment of Neuroscience and Physiology, New York University Grossman School of Medicine, New York, NY 10016; ᶜThe Marlene and Paolo Fresco Institute for Parkinson's and Movement Disorders, New York University Grossman School of Medicine, New York, NY 10016; ᵈThe Parekh Center for Interdisciplinary Neurology, Grossman School of Medicine, New York University Grossman School of Medicine, New York, NY 10016; and ᵉDepartment of Neurobiology, Neuroscience Institute, University of Chicago, Chicago, IL 60637

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