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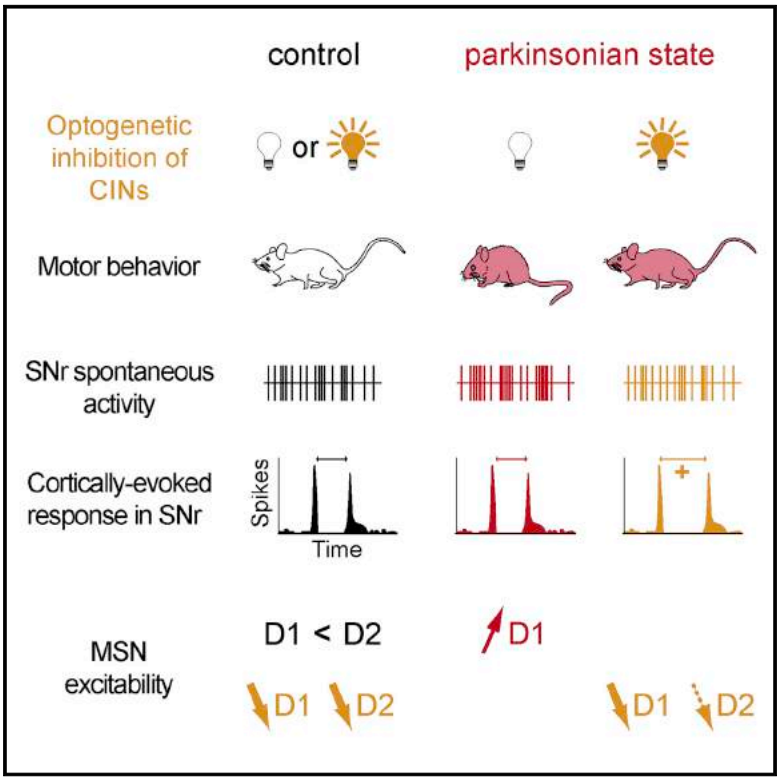
Striatal Cholinergic Interneurons Control Motor Behavior and Basal Ganglia Function in Experimental Parkinsonism

Graphical Abstract



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In Brief

By combining optogenetics with behavioral and electrophysiological approaches in mice, Maurice et al. provide evidence for a causal role of striatal cholinergic interneurons in parkinsonian symptomatology and identify underlying neural substrates in the basal ganglia network.

Highlights

- CIN activity impacts motor function and basal ganglia output in parkinsonian state
- CIN inhibition alleviates parkinsonian symptoms, while activation has no effect
- CIN inhibition corrects burst firing and enhances cortically evoked inhibition in SNr
- CIN's control of D2-, but not D1-MSN, excitability is reduced in parkinsonian state

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Striatal Cholinergic Interneurons Control Motor Behavior and Basal Ganglia Function in Experimental Parkinsonism

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SUMMARY

Despite evidence showing that anticholinergic drugs are of clinical relevance in Parkinson's disease (PD), the causal role of striatal cholinergic interneurons (CINs) in PD pathophysiology remains elusive. Here, we show that optogenetic inhibition of CINs alleviates motor deficits in PD mouse models, providing direct demonstration for their implication in parkinsonian motor dysfunctions. As neural correlates, CIN inhibition in parkinsonian mice differentially impacts the excitability of striatal D1 and D2 medium spiny neurons, normalizes pathological bursting activity in the main basal ganglia output structure, and increases the functional weight of the direct striatonigral pathway in cortical information processing. By contrast, CIN inhibition in nonlesioned mice does not affect locomotor activity, equally modulates medium spiny neuron excitability, and does not modify spontaneous or cortically driven activity in the basal ganglia output, suggesting that the role of these interneurons in motor function is highly dependent on dopamine tone.

INTRODUCTION

Parkinson's disease (PD) is a debilitating neurodegenerative movement disorder resulting from the loss of nigral dopaminergic (DA) neurons that project massively to the striatum, the main basal ganglia input structure. The hypokinetic parkinsonian syndrome is thought to be the consequence of opposite changes in the activity of the two populations of GABAergic striatal projection neurons, also called medium spiny neurons (MSNs), which control the basal ganglia output nuclei, mainly the substantia nigra pars reticulata (SNr) in rodent. Dopamine D1 receptor-expressing MSNs (D1 or direct MSNs), giving rise to a monosynaptic inhibitory projection (direct pathway) onto SNr, become hypoactive, whereas dopamine D2 receptor-expressing MSNs (D2 or indirect MSNs), at the origin of a polysynaptic projection with excitatory influence onto SNr (indirect pathway), become hyperactive (Albin et al., 1989; Mallet et al., 2006). This imbalance leads to pathological activation of SNr, reinforcing its inhibitory tone onto the thalamocortical circuit and, hence, on motor cortical outflow. The pathological changes in the activity of the two striatal pathways, whose functional relationship with movement control is still debated (Calabresi et al., 2014; Cui et al., 2013), may involve profound reorganizations within the striatal circuitry.

Striatal cholinergic interneurons (CINs), which correspond to the tonically active neurons recorded in vivo, constitute 1%–3% of all striatal neurons. Despite being few in numbers, they are the main source of acetylcholine (ACh) within the striatum, and their dense terminal fields are primarily directed to MSNs (Phelps et al., 1985). The improvement of parkinsonian tremor by both DA agonists and anticholinergic drugs led to the DA-ACh balance hypothesis, where DA and ACh are believed to play opposite roles in the striatum (Barbeau, 1962). This clinical observation particularly underlines the functional impact of ACh as DA levels fall. There is indeed compelling evidence showing that DA depletion triggers complex alterations in striatal cholinergic signaling and activity (Aosaki et al., 1994; Ding et al., 2006), leading, among other things, to morphofunctional alterations of striatal output neurons (Pisani et al., 2007; Shen et al., 2007). However, whether and how this cholinergic-dependent disruption of striatal properties contributes to motor symptoms in PD and affects basal ganglia circuitry remain an open question.

The diversity of cholinergic receptors expressed in the striatum, located both at the presynaptic and postsynaptic levels (Goldberg et al., 2012), suggests that CINs exert complex and powerful influence on striatal functioning and, hence, on basal ganglia outflow. An additional level of complexity in understanding cholinergic regulation of striatal function comes from recent studies showing the following: (1) CINs co-release glutamate able to evoke fast glutamatergic responses in MSNs (Higley

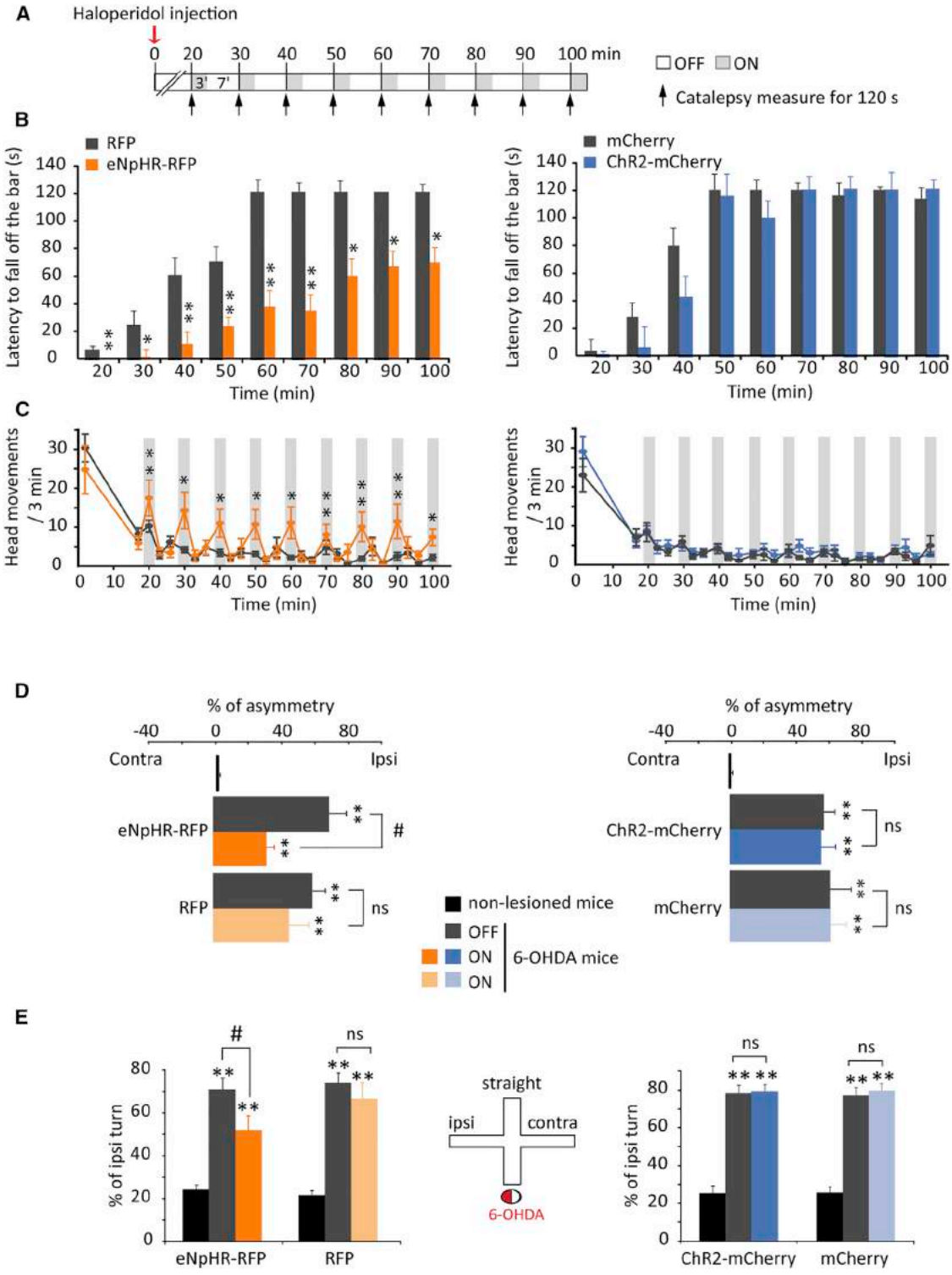

Figure 1. Photoinhibition of CINs rReduces Haloperidol-Induced Catalepsy and Relieves Parkinsonian-like Motor Deficits

(A) Experimental design. Mice received haloperidol (0.25 mg/kg) and the latency to step down the bar was measured 20 min later, then every 10 min, with a 120-s cutoff. Light was turned on (blue light: 10 Hz, 25-ms pulse width; yellow light: continuous illumination) for 3 min when placing the mice on the bar and was turned off afterward for 7 min, until next measure.

(B) Yellow light reduced haloperidol-induced catalepsy in eNpHR-RFP mice versus RFP mice (*$p < 0.05$, **$p < 0.01$, U-Mann-Whitney after significant Kruskal-Wallis test), whereas blue light illumination in ChR2-mCherry mice had no effect versus mCherry mice ($p = 0.3$).

(legend continued on next page)

et al., 2011), (2) CIN activation can drive GABA release from dopaminergic terminals (Nelson et al., 2014; Tritsch et al., 2014) and neuroptide Y-expressing interneurons (English et al., 2011), and (3) their synchronous activation triggers striatal DA release (Threlfell et al., 2012). While we do not know yet how these different actions are coordinated in vivo, these results suggest that DA/ACh interactions are more complex than the traditional antagonistic model would predict. Therefore, the contribution of CINs to basal ganglia function cannot be fully understood unless an approach mimicking the diversity of their actions is used. Optogenetics that allows precise control of circuit function, was, for example, used successfully to demonstrate the role of CINs in the nucleus accumbens during cocaine conditioning (Witten et al., 2010).

Here, using a combination of optogenetic, behavioral, and electrophysiological approaches, we demonstrate a major role of CINs in PD pathophysiology. We show that selective inhibition of CINs alleviates motor deficits and corrects dysfunctions at the main input and output stages of the basal ganglia network in PD mouse models, with a preferential action on the direct striatonigral pathway.

RESULTS

Inhibition of Striatal Cholinergic Interneurons Reduces Parkinsonian-like Motor Deficits

We expressed ChR2-mCherry or eNpHR-RFP in choline acetyltransferase (ChAT)-expressing neurons by injecting a Cre-dependent adeno-associated virus (AAV) carrying the opsins or their reporter genes into the dorsal striatum of $ChAT^{cre/cre}$ mice (subsequently referred to as ChR2-mCherry and eNpHR-RFP mice). Quantifying the proportion of neurons expressing opsins that were also ChAT positive and vice versa demonstrated the specificity and efficiency of the targeting strategy (Figure S1). Recordings of optogenetically identified CINs in striatal slices and anesthetized mice showed that opsins were functional (Figure S2).

CIN photoinhibition or photoactivation had no significant effect on locomotor activity of non-lesioned RFP, eNpHR-RFP, mCherry, and ChR2-mCherry mice in an open field (Figure S3A). To determine whether CINs affect parkinsonian akinesia, the effects of CIN modulation were examined in the haloperidol-induced catalepsy model (Figure 1A). The eNpHR-RFP haloperidol-treated mice removed their forepaws from the bar significantly faster under yellow illumination than did the RFP-treated mice (Figure 1B). Consistently, yellow light restored head movements in a light-locked manner in haloperidol-injected eNpHR-RFP mice, demonstrating a robust anti-akinetic action of CIN inhibition (Figure 1C). In contrast, ChR2-mCherry mice under blue illumination exhibited a long-lasting cataleptic state, similar to mCherry mice (Figures 1B and 1C).

We next tested whether CIN inhibition could alleviate motor dysfunction in the 6-hydroxydopamine (6-OHDA) lesional model of PD. Unilateral 6-OHDA injection into the substantia nigra pars compacta (SNc) resulted in a near-total loss of DA cells after 2 weeks. In the cylinder test (Figure 1D), RFP and mCherry non-lesioned mice used their two forepaws indifferently during exploratory rearing (asymmetry score close to zero). The 6-OHDA lesions produced a significant shift toward ipsilateral forepaw use due to contralateral forelimb akinesia. Photoinhibition of CINs ipsilateral to the lesioned side in eNpHR-RFP mice induced a significant reduction in the asymmetry score. In contrast, CIN photoactivation in ChR2-mCherry 6-OHDA mice did not affect asymmetry (Figure 1D). In the cross maze test (Figure 1E), the 6-OHDA-induced bias toward ipsilateral turns, which reflects sensorimotor neglect, was significantly reduced by CIN inhibition in eNpHR-RFP mice, while CIN activation had no effect in ChR2-mCherry mice. The non-selective muscarinic receptor antagonist scopolamine (1 mg/kg) also reduced the asymmetry in the cylinder and cross maze tests in 6-OHDA mice (Figures S3B and S3C). Finally, we examined the effect of CIN photoinhibition on L-DOPA-induced dyskinesia, a main side effect of long-term DA treatment. CIN inhibition in eNpHR-RFP mice failed to affect the severity of dyskinesia once expressed (Figure S4). Taken together, these data show that CIN inhibition has no effect on spontaneous locomotion and L-DOPA-induced dyskinesia in our lesion and treatment conditions, but significantly alleviates parkinsonian-like motor deficits.

Inhibition of Striatal CINs Regulates the Basal Ganglia Output Structure in Parkinsonian Condition Only

A leading hypothesis on the origin of motor dysfunction in PD is that DA loss induces abnormal bursting activity in basal ganglia output nuclei, leading to pathological inhibitory tone onto the thalamocortical circuit that disrupts motor planning and execution. We tested whether striatal CIN inhibition modifies the activity of SNr neurons recorded in vivo in anesthetized transgenic eNpHR mice. CIN photoinhibition in non-lesioned animals did not affect spontaneous activity of SNr neurons (Table S1). The 6-OHDA mice showed altered burst firing, with significant increases in burst duration and number of spikes per burst (Figure 2A). CIN photoinhibition normalized these changes, without

(C) Light partially restored head movements in eNpHR-RFP mice (ON versus OFF preceding light: *$p < 0.05$, **$p < 0.01$, paired Student's t test after significant two-way ANOVA), but not in ChR2-mCherry mice or in control RFP and mCherry mice (n = 10–13 per group).
(D) In the cylinder test, 6-OHDA mice showed marked forelimb asymmetry compared to non-lesioned mice (**$p < 0.01$, Fisher's least significant difference [LSD] test after significant ANOVA). (Left) This asymmetry was significantly improved by yellow light in eNpHR-RFP mice (ON versus OFF: #$p < 0.05$, Student's t test). Light did not affect asymmetry in RFP control mice (n = 7–8 per group). (Right) The significant forelimb asymmetry measured in 6-OHDA versus non-lesioned mice (**$p < 0.01$, Fisher's LSD test after significant ANOVA) was not affected by blue light illumination in ChR2-mCherry (ON versus OFF: ns, Student's t test) and mCherry (ON versus OFF: ns, Student's t test) mice (n = 7–8 per group).
(E) (Left) Ipsilateral turn bias induced by 6-OHDA lesion in the cross maze (6-OHDA versus non-lesioned: **$p < 0.01$, one-way ANOVA) was partially corrected by photoinhibition in eNpHR-RFP mice (ON versus OFF: #$p < 0.05$, paired Student's t test), but not in RFP control mice (n = 14 per group). (Right) Photoactivation did not affect the ipsilateral turn bias induced by 6-OHDA lesion (6-OHDA versus non-lesioned: **$p < 0.01$, one-way ANOVA) in ChR2-mCherry mice nor in mCherry mice (ON versus OFF: ns, Student's t test, n = 12 per group). In (D) and (E), continuous illumination was for 5 min with yellow light and 25-ms pulse width was at 10 Hz for blue light. Circle under the cross maze symbolizes a mouse with the 6-OHDA-injected side in red. Errors bars, SEM.

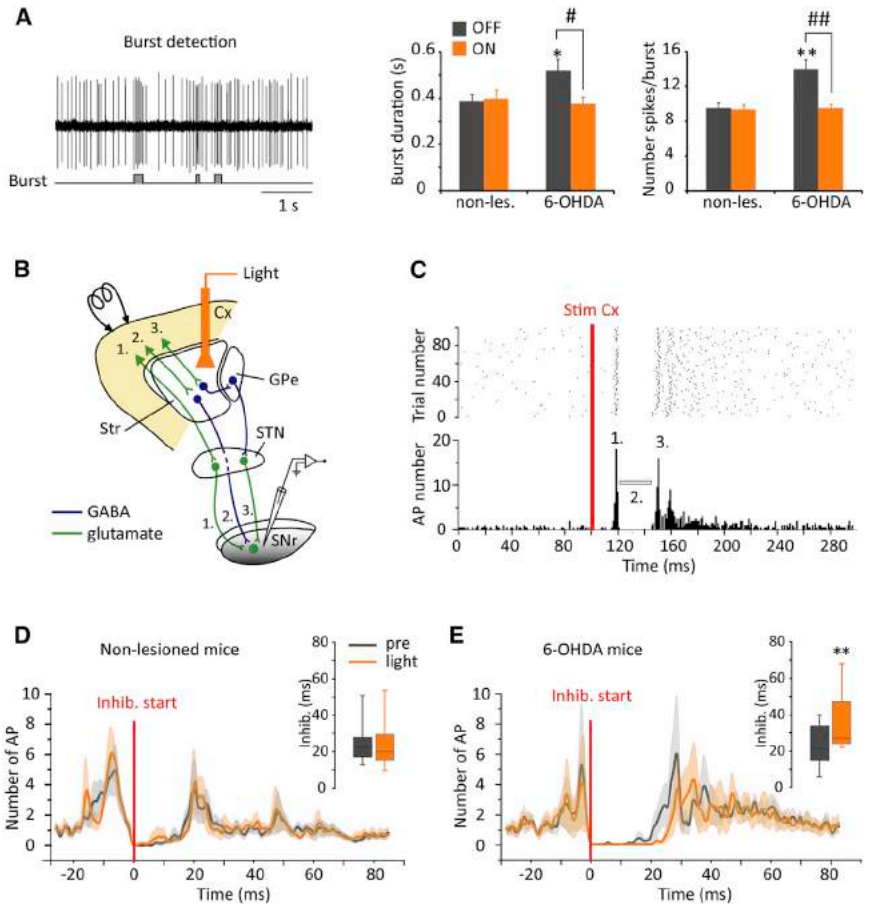

Figure 2. Photoinhibition of CINs Normalizes SNr Burst Firing and Strengthens the Inhibitory Influence of the Striatonigral Direct Pathway in 6-OHDA Mice

(A) Detection of bursts according to Poisson Surprise analysis in a spontaneously firing SNr neuron recorded in 6-OHDA transgenic eNpHR mice. The histograms show that the 6-OHDA induced increases in burst duration and number of spikes per burst (6-OHDA versus non-lesioned: *$p < 0.05$, **$p < 0.01$, Student's t test) were normalized by CIN photoinhibition (light was delivered for 1 min; 6-OHDA ON versus OFF: #$p < 0.05$, ##$p < 0.01$, Holm-Sidak test after significant one-way ANOVA). Non-lesioned mice, n = 18 cells from six mice; 6-OHDA mice, n = 13 cells from six mice.

(B) Schematic representation shows pathways activated by cortical stimulation that project to SNr neurons. Cx, cortex; Str, striatum; GPe, external globus pallidus; STN, subthalamic nucleus.

(C) Raster plot and peristimulus time histogram (PSTH) show the typical triphasic response evoked by motor cortex stimulation in one SNr neuron recorded from a 6-OHDA transgenic eNpHR mouse.

(D and E) Population PSTHs of the cortically evoked responses recorded in SNr neurons in non-lesioned (D, n = 17 cells from six mice) and 6-OHDA (E, n = 7 cells from five mice) mice. The same neuron was recorded before (pre, gray line) and during (light, orange line) CIN photoinhibition. Light was delivered for 1 s at the beginning of each trial. PSTHs are aligned on the beginning of the inhibitory component (red lines) and the light-shaded colors represent SEM. CIN photoinhibition induced a significant increase of the inhibitory component duration only in 6-OHDA mice, as illustrated by the box plots (insets) (**$p < 0.01$, Holm-Sidak test after significant one-way RM ANOVA). Errors bars, SEM.

modifying the parameters unaltered by the lesion (firing frequency and burst recurrence) (Table S1).

Next, to examine whether CINs influence the processing of cortical information through the trans-striatal pathways, we recorded the responses of individual SNr neurons to cortical stimulation (Figures 2B and 2C). As previously reported, such stimulation triggers a complex response composed, in most cases, of an early excitation followed by an inhibition and a late excitation, which has been attributed to the respective activation of the hyperdirect corticosubthalamic, the direct and indirect striatonigral pathways (Maurice et al., 1999; Ryan and Sanders, 1994; Sano et al., 2013; Tachibana et al., 2008). Because the characteristics of the evoked responses greatly vary among cells depending on the stimulation (e.g., location and number of stimulated fibers) but remain stable in a given cell over time, comparisons were made for a same cell under successive light conditions and not between cells from 6-OHDA versus non-lesioned mice. CIN photoinhibition had no effect on cortical information transfer in non-lesioned mice, whereas, in 6-OHDA mice, it increased the duration of the inhibitory component of the triphasic response without significantly affecting the excitatory components (Figures 2D and 2E; Table S2). Since parkinsonian akinesia is classically associated with overactive indirect pathway and hypoactive direct pathway, our results suggest that CIN inhibition might partially restore balance in striatal outputs by increasing the functional impact of the direct pathway. In contrast, CIN photoinhibition had no effect on SNr spontaneous activity and cortical information transfer in non-lesioned transgenic eNpHR mice.

Dopamine Depletion Affects the Intrinsic Excitability of D1 MSNs

We first determined whether CINs themselves were affected by 6-OHDA lesion, as there is no consensus on whether CIN activity or striatal ACh release increases in PD models. Optogenetically identified CINs recorded either in slices or in anesthetized mice exhibited similar firing frequency in non-lesioned and 6-OHDA conditions (Figure 3), showing that they are not hyperactive in our experimental conditions. However, CINs were more excitable in slices from 6-OHDA mice, as illustrated by a lower rheobase current (non-lesioned: 137.7 ± 16.7 pA, n = 17; 6-OHDA: 87.1 ± 16.5 pA, n = 17; $p < 0.05$, Mann-Whitney test).

We then tested whether CIN modulation of striatal functions could be altered in PD state, by examining its impact on identified D1 and D2 MSNs that form the direct and indirect striatal projection systems, 2–3 weeks after 6-OHDA lesion. The

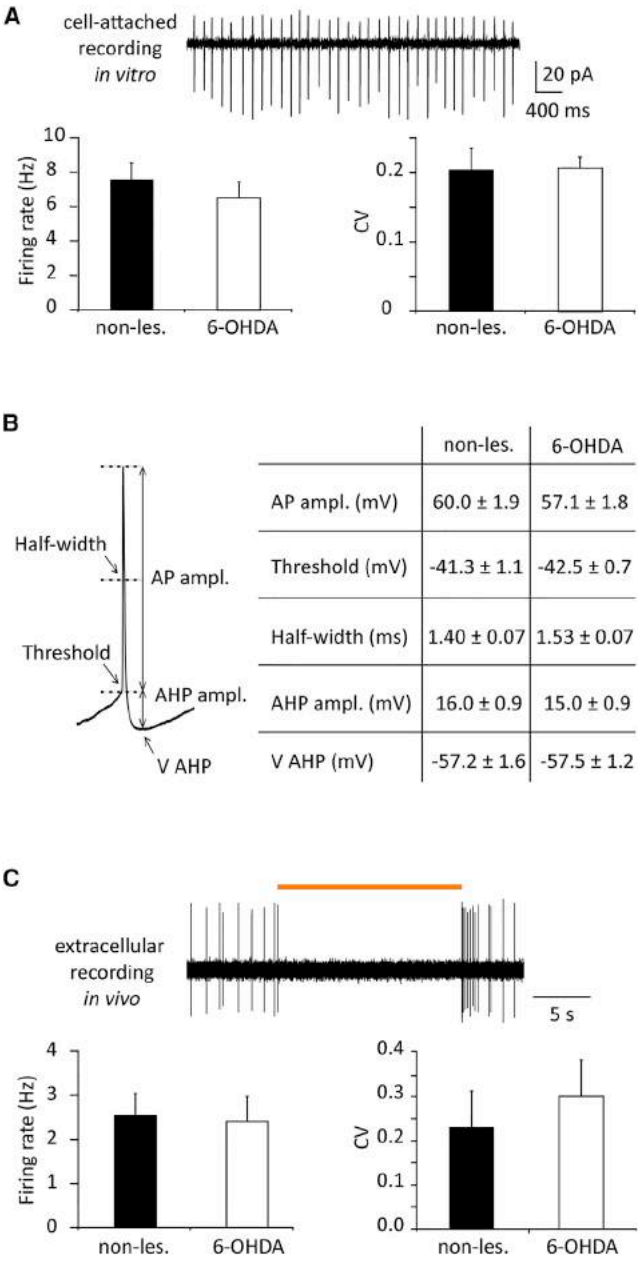

Figure 3. Impact of 6-OHDA Lesion on CIN Electrophysiological Properties

(A) Spontaneous activity from a CIN recorded in cell-attached configuration. Histograms show the mean firing rate and coefficients of variation (CV = firing rate SD/firing rate mean) in non-lesioned (n = 12 cells) and 6-OHDA (n = 20 cells) transgenic eNpHR mice (ns, Mann-Whitney test).

(B) Action potential characteristics of CINs recorded in non-lesioned (n = 13 cells) and 6-OHDA (n = 22 cells) mice. No significant differences were observed between the two groups (ns, Student's t test).

(C) Spontaneous activity and optogenetic identification of a CIN recorded in vivo. Histograms show the mean frequency rate and CVs (firing rate SD/firing rate mean) in non-lesioned (n = 8 cells) and 6-OHDA (n = 7 cells) transgenic eNpHR mice (ns, Mann-Whitney test). Errors bars, SEM.

resting membrane potentials of D1 and D2 MSNs were similar in non-lesioned and 6-OHDA mice (data not shown). However, in non-lesioned mice, D2 MSNs were more excitable than D1 MSNs, in agreement with previous reports (Gertler et al., 2008; Kreitzer and Malenka, 2007; Figure 4A). After DA depletion, D2 MSN excitability was not changed, while that of D1 MSNs greatly increased. D1 MSNs fired at higher frequency at each depolarizing current step, and the rheobase current was significantly lower in 6-OHDA versus non-lesioned mice (Figure 4). These results show that DA depletion reduces the dichotomy between D1 and D2 MSN excitability by increasing D1 MSN excitability.

Striatal CINs Differentially Modulate the Excitability of D1 and D2 MSNs in Parkinsonian Condition

Changes in intrinsic membrane excitability can occur either independently or in concert with changes in synaptic inputs. As cortical inputs strongly drive MSN activity and are modulated by presynaptic muscarinic receptors (Hernández-Echeagaray et al., 1998; Malenka and Kocsis, 1988; Pakhotin and Bracci, 2007), we investigated whether CIN inhibition affects corticostriatal transmission. Recordings were made 2–3 weeks after 6-OHDA lesion in transgenic eNpHR/D1 mice expressing both eNpHR in CINs and the fluorescent reporter tdTomato in DA D1 receptor-containing neurons (Figure S5A). In non-lesioned mice, excitatory postsynaptic currents (EPSCs) were not significantly altered by CIN inhibition, both in D1 and D2 MSNs (D1 MSNs, light versus pre-light: 325.99 ± 57.92 pA versus 311.16 ± 53.47 pA, not significant [ns], paired Student's t test, n = 14 cells; D2 MSNs, light versus pre-light: 214.98 ± 30.07 pA versus 214.59 ± 26.44 pA, ns, paired Student's t test, n = 11 cells) (Figure S5B). In contrast, CIN inhibition in 6-OHDA mice significantly potentiated EPSCs in both D1 and D2 MSNs (D1 MSNs, light versus pre-light: 262.22 ± 24.93 pA versus 240.76 ± 23.84 pA, $p < 0.05$, Wilcoxon test, n = 15 cells; D2 MSNs, light versus pre-light: 231.06 ± 34.00 pA versus 198.06 ± 24.07 pA, $p < 0.05$, Wilcoxon test, n = 9 cells) (Figure S5B). This potentiation was blocked by scopolamine (10 μM) (data pooled for D1 and D2 MSNs: light versus pre-light: 181.24 ± 35.92 pA versus 178.31 ± 33.35 pA, ns, paired Student's t test, n = 8 cells, data not shown). These results show that CIN inhibition potentiates corticostriatal transmission onto both D1 and D2 MSNs in parkinsonian condition. Therefore, corticostriatal transmission is unlikely to contribute to a differential impact of CIN inhibition on D1 and D2 MSNs.

In the healthy striatum, cholinergic modulation facilitates the firing of MSNs through M1 receptors (Galarraga et al., 1999; Goldberg et al., 2012; Pisani et al., 2007). What happens after chronic DA depletion is still unclear. We therefore examined whether and how CIN photoinhibition impacts MSN excitability in non-lesioned and 6-OHDA mice. D1 and D2 MSNs fired significantly less action potential during light illumination in both non-lesioned and 6-OHDA mice (Figures 5A and 5B). However, while the magnitude of firing inhibition was similar in D2 and D1 MSNs in non-lesioned mice, firing inhibition was weaker in D2 MSNs compared to D1 MSNs in 6-OHDA mice (Figure 5C). This result shows that D2 MSNs are less sensitive to CIN inhibition than D1 MSNs after DA depletion.

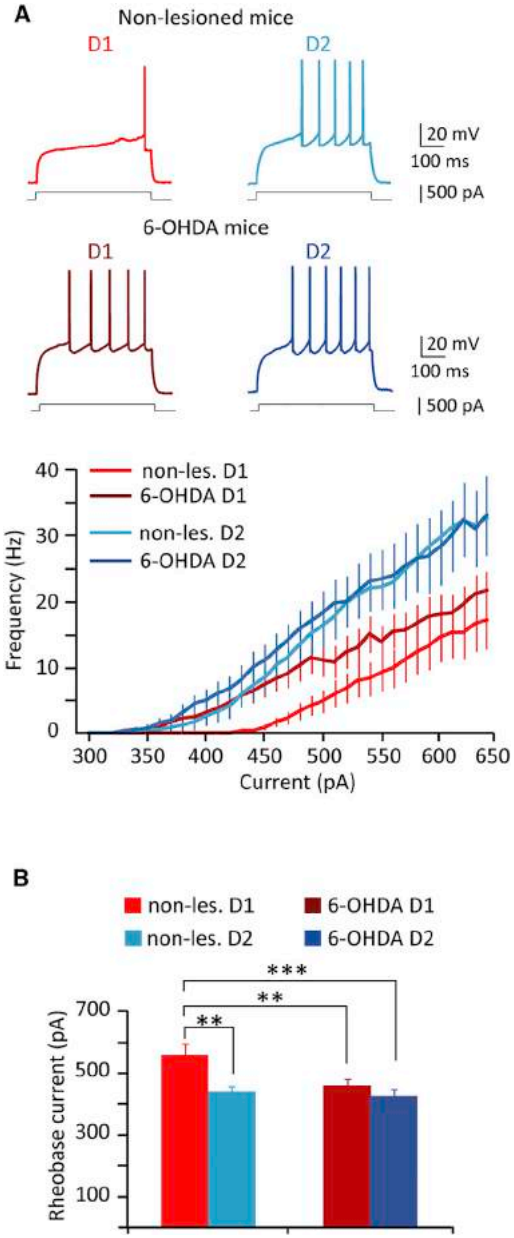

Figure 4. Impact of 6-OHDA Lesion on D1 and D2 MSN Excitability

(A) Current-clamp recordings showing the responses of D1 and D2 MSNs to depolarizing current pulses in non-lesioned (+450 pA) and 6-OHDA (+400 pA) mice. Summary graph illustrates the number of action potentials as a function of injected current in D1 MSNs (non-lesioned mice, n = 14 cells; 6-OHDA mice, n = 23 cells) and D2 MSNs (non-lesioned mice, n = 13 cells; 6-OHDA mice, n = 13 cells).

(B) Bars graph shows the mean rheobase current in D1 MSNs (non-lesioned mice, n = 14 cells; 6-OHDA mice, n = 23 cells) and D2 MSNs (non-lesioned mice, n = 13 cells; 6-OHDA mice, n = 13 cells) (**p < 0.01, ***p < 0.001, Holm-Sidak test after significant one-way ANOVA). Errors bars, SEM.

DISCUSSION

Although ACh is undeniably a critical player in striatal functioning, the role of CINs in PD pathophysiology has remained unsolved. We tackled this issue by combining optogenetics with in vitro and in vivo electrophysiology and behavioral analyses. We first showed that selective inhibition of CINs in the dorsal striatum reduces parkinsonian motor dysfunction in pharmacological and lesional PD models, attesting to their causal involvement in PD symptomatology. This provides a functional support for the long-standing hypothesis that poses PD as a striatal cholinergic disorder. Second, we identified several physiological parameters affected by CIN inhibition in the parkinsonian condition at both the input (striatum) and output (SNr) levels of the basal ganglia network. In addition, we found that CIN inhibition does not significantly impact basic motor function nor the spontaneous and cortically driven activity of SNr neurons in non-lesioned mice. This lack of effect in SNr is consistent with previous results from selective CIN ablation (Sano et al., 2013). Also, CIN inhibition does not modify the severity of dyskinesia in 6-OHDA mice. Altogether, these data suggest that CIN actions are highly dependent on DA tone.

CIN inhibition in vivo efficiently alleviates parkinsonian deficits measured after 6-OHDA lesions and neuroleptic-induced catalepsy, two different models widely used to assess motor symptoms reminiscent of parkinsonian akinesia. As neural correlates of PD symptoms' improvement by CIN inhibition, our in vivo recordings showed that it normalized the 6-OHDA-induced changes in SNr burst firing. Over the last few years, an abundant literature has clearly reported that parkinsonism is associated with increased bursting activity in the basal ganglia, including SNr (Lobb, 2014; Rivlin-Etzion et al., 2006; Wichmann et al., 1999). Even though the mechanisms leading to this abnormal activity are still elusive, its normalization by efficient antiparkinsonian treatments, such as dopatherapy or subthalamic nucleus deep brain stimulation (Brown, 2003; Degos et al., 2005; Eusebio et al., 2011), suggests that such change may contribute importantly to the development of the behavioral manifestations of the disease. Thus, it is likely that the decrease in burst activity of SNr neurons elicited by CIN inhibition contributes to the improvement of parkinsonian symptoms that we observed in 6-OHDA mice. Future studies will be required to understand fully how CINs influence SNr burst firing in PD.

The second effect of CIN inhibition revealed by our in vivo recordings in 6-OHDA mice was an increase in the inhibitory component of the cortically evoked triphasic response in SNr neurons, suggesting that CIN silencing strengthens the inhibitory influence of the direct striatonigral pathway in parkinsonian condition. Although the relationship between the activity of the direct and indirect pathways and movement generation is still under debate, it has been demonstrated that specific activation of D1 MSNs by optogenetics efficiently relieves parkinsonian deficits (Kravitz et al., 2010). In this context, it is likely that the increased duration of the cortically evoked inhibition induced by CIN inhibition contributes importantly to the improvement of parkinsonian-like motor symptoms induced by DA depletion.

What are the striatal targets modulated by CINs that could mediate their preponderant action on basal ganglia function in PD state? We showed that CIN inhibition in 6-OHDA mice potentiates corticostriatal transmission onto both types of MSNs. However, our recordings in the SNr of 6-OHDA mice clearly showed that CIN inhibition increases the cortically evoked

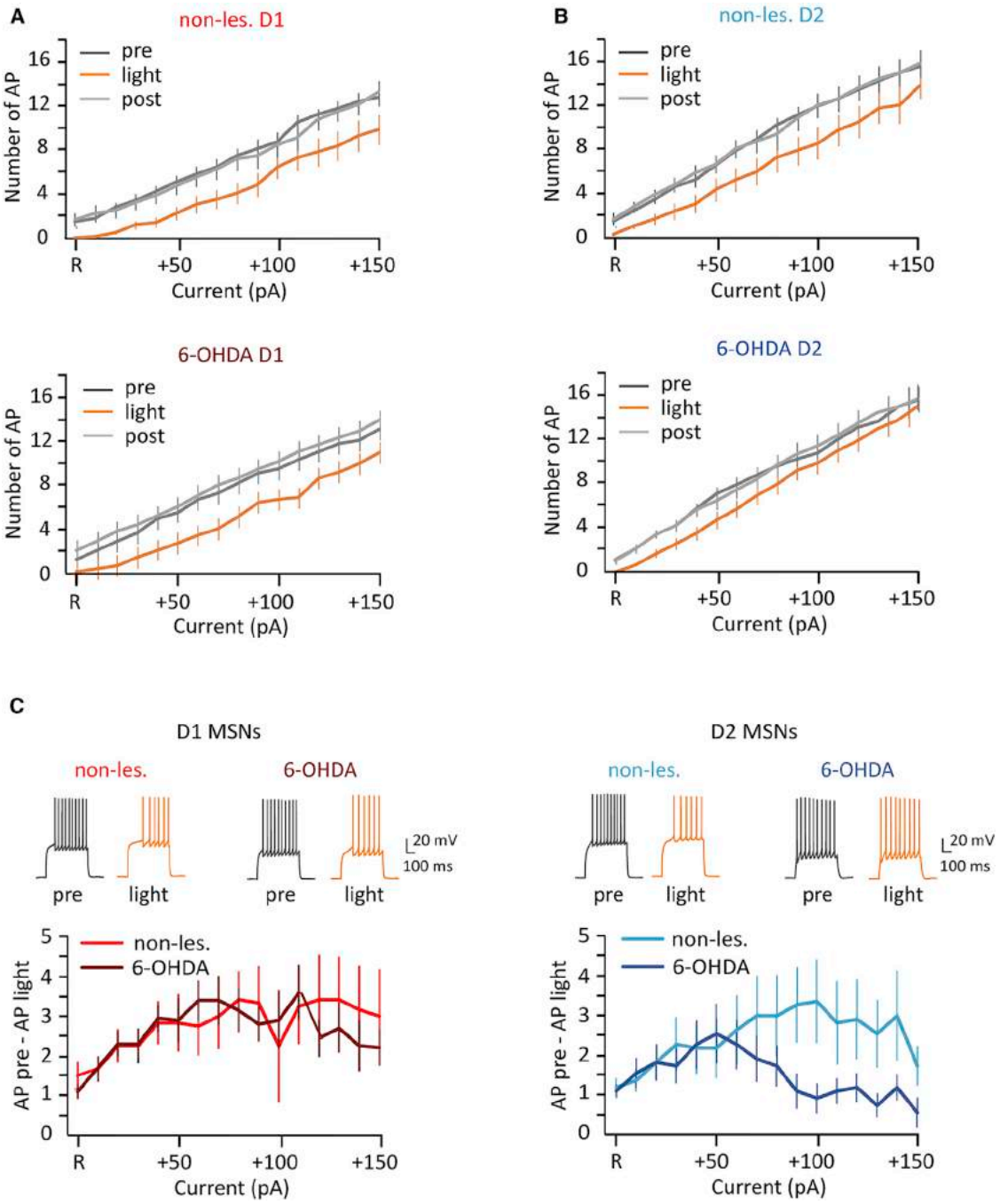

Figure 5. Photoinhibition of CINs Differentially Decreases D1 and D2 MSN Excitability in 6-OHDA Mice

(A and B) The graphs illustrate the decreased excitability of MSNs during light illumination versus pre and post conditions in non-lesioned (A, top; D1 MSNs: $F(2, 572) = 55.26$, $p < 0.0001$, $n = 12$; B, top; D2 MSNs: $F(2, 524) = 37.97$, $p < 0.0001$, $n = 11$, linear regression analysis) and 6-OHDA (A, bottom; D1 MSNs: $F(2, 953) = 209.72$, $p < 0.0001$, $n = 20$; B, bottom; D2 MSNs: $F(2, 524) = 17.09$, $p < 0.0001$, $n = 11$, linear regression analysis) mice. R is the minimal current intensity to trigger a spike in pre-light condition. From R, current intensity increased by +10-pA increment.

(C) (Top) Representative traces showing the responses to 500-ms depolarizing current steps (R+80 for D1 MSN non-les., D2 MSN non-les., and D2 MSN 6-OHDA) and R+110 (D1 MSN 6-OHDA). Light was delivered 200 ms before and continued 200 ms after the 500-ms depolarizing current step. (Bottom) The graphs show the difference in the number of action potentials evoked in pre versus light conditions in D1 MSNs (left; non-les., $n = 12$; 6-OHDA, $n = 20$) and D2 MSNs (right; non-les., $n = 11$; 6-OHDA, $n = 11$). There is a significant effect of current intensity in D1 MSNs ($F[15,450] = 3.67$, $p < 0.005$) and D2 MSNs ($F[15,300] = 2.02$, $p < 0.01$). From R+80 pA, D2 MSNs in 6-OHDA mice are less inhibited during light illumination, as shown by a significant interaction between lesion × current intensity for D2 MSNs ($F[15,300] = 2.39$, $p < 0.05$), but not for D1 MSNs. Errors bars, SEM.

inhibition linked to the activation of the direct (D1) pathway, but not the late excitation linked to the activation of the indirect (D2) pathway. The high degree of convergence of the direct pathway at the level of the SNr (48 striatal neurons converge into one SNr cell; Smith et al., 1998) may explain how a modest increase in corticostriatal transmission onto D1 MSNs impacts the cortically evoked inhibition in the SNr. In contrast, complex information processing at each stage of the polysynaptic indirect pathway might minimize the outcome of EPSC potentiation on D2 MSNs.

Another interesting result of our study is that DA depletion tends to erase the dichotomy in MSN excitability by inducing a specific increase in D1 MSN excitability. A recent study also reported increased D1 MSN excitability after DA lesion, but associated with a slight decrease in D2 MSN excitability (Fieblinger et al., 2014), whereas we failed to detect any change for D2 MSNs. This apparent discrepancy might be due to different lesion models or post-lesion delay, since opposite changes in D2 MSN excitability have been described after acute or chronic DA depletion (Day et al., 2006; Fieblinger et al., 2014; Shen et al., 2007). Elevated excitability in D1 MSNs might be considered as a homeostatic response counteracting the decreased activity triggered by the loss of dopaminergic excitatory drive on these neurons. However, if we assume that the dichotomy between D1 and D2 MSN excitability is a fundamental process for normal striatal function, making both MSNs more alike after DA depletion might be more harmful than beneficial. In favor of this hypothesis, we showed that CIN inhibition decreases the excitability more in D1 than in D2 MSNs after DA depletion, whereas it has similar impact on the two populations in control condition. Thus, restoring the dichotomy between D1 and D2 MSN excitability might be one component of the anti-parkinsonian effect of CIN inhibition. What happens following DA depletion to explain the reduced sensitivity of D2 MSNs to CIN inhibition remains an open and challenging question. It will be also interesting to investigate whether the cholinergic projection from the brainstem to the striatum (Dautan et al., 2014) cooperates with CINs to modulate striatal output.

Our results show that CINs are critical players in the host of cellular and synaptic changes induced in MSNs by DA depletion. Without excluding indirect pathway contributions, our findings point to CIN control of the direct striatonigral pathway as a critical component involved in the control of striatal output and motor dysfunction in PD state. These results should stimulate the development of therapeutic strategies targeting striatal CIN activity in PD.

EXPERIMENTAL PROCEDURES

Mice

All procedures were approved by the French National Ethical Committee (45-29102012) and were in accordance with the recommendations of the European Commission (2010/63/EU) for care and use of laboratory animals. See the Supplemental Experimental Procedures for a detailed description of the mice used.

Stereotaxic Surgery

All coordinates were adapted from the mouse stereotaxic atlas by Paxinos and Franklin (2001) with bregma and dura as references. See the Supplemental Experimental Procedures for details.

In Vitro Electrophysiology

Patch-clamp recordings on brain slices and data analysis were performed as described in the Supplemental Experimental Procedures. Excitation of opsins was achieved with a light-emitting diode source (Spectra light engine, Lumencor) connected to a 3-mm liquid core fiber. Statistical analyses were performed using paired or unpaired Student's t test. Nonparametric tests (Mann-Whitney test for unpaired data or Wilcoxon test for paired data) were used if the normality or equal variance test failed. For multiple group comparisons, one- or two-way ANOVA was used. We applied the log2 transformation to make the data normal if necessary. Linear regression analysis was used to compare the effect of CIN inhibition on MSN excitability. A significance of $p < 0.05$ was required for rejection of the null hypothesis.

Behavioral Testing

All behavioral analyses were conducted on littermates that entered the study at around 3–5 months of age. See the Supplemental Experimental Procedures for a detailed description of the behavioral tests. For open field, data were analyzed using two-way ANOVA followed by paired Student's t test, when appropriate. For haloperidol-induced catalepsy, median latencies (±semi-quartile) were compared among groups over time using the nonparametric Kruskal-Wallis test, followed by U-Mann-Whitney for pairwise comparisons at each time. Head movements were summed for the 3-min ON periods and compared to those measured 3-min OFF periods, using a two-way ANOVA. For cylinder and cross maze tests, data were analyzed using one-way ANOVA, followed by Student's t test. For L-DOPA-induced dyskinesia, data were analyzed by two-way repeated-measures (RM) ANOVA with opsins (eNpHR-RFP versus RFP) as between factor and time as within factor.

In Vivo Recordings in Anesthetized Mice

Extracellular recordings in the striatum and the SNr were performed as described in the Supplemental Experimental Procedures. The patterns of cortically evoked discharges in the same SNr neuron were analyzed before, during, and after CIN photoinhibition in the striatum. Results are given as means ± SEM of the individual responses per condition. Spontaneous activity in the three conditions was compared using one-way ANOVA followed by comparison versus pre-light condition (Holm-Sidak method). Cortically evoked responses of the same SNr neurons in the three light conditions were compared using one-way RM ANOVA, followed by comparison versus pre-light condition (Holm-Sidak method).

Histology and Immunohistochemistry

For primary antibody exposure, brain sections were incubated overnight at 4°C in rabbit anti-RFP (1/1,000, tebu-bio, 600-401-379) and goat anti-ChAT (1/100, Millipore, AB144P) for colocalization experiments and in mouse anti-tyrosine hydroxylase (1/1,000, Millipore, MAB318) to control DA lesion. Sections were then incubated, respectively, in Alexa Fluor 555 donkey anti-rabbit (1/500, Invitrogen, A31572), Alexa Fluor 488 donkey anti-goat (1/500, Invitrogen, A11055), and Alexa Fluor 555 donkey anti-mouse (1/500, Invitrogen, A31570) for 1 hr 30 min at room temperature. Rabbit anti-GFP (1/500, Invitrogen, A11122) and Alexa fluor 488 donkey anti-rabbit (1/500, Invitrogen, A21206) were used the same way to reveal eNpHR expression in transgenic mice.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.09.034.

AUTHOR CONTRIBUTIONS

N.M., M.L., M.A., L.K.-L., and C.B. designed the study. N.M., M.L., F.J., S.Z., M.H., J.C., and C.B. performed experiments. K.D. provided reagents and critical feedback. N.M., M.L., F.J., S.Z., J.C., L.K.-L., M.A., and C.B. analyzed the results. N.M., M.L., M.A., L.K.-L., and C.B. wrote the paper.

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