Cell-specific pallidal intervention induces long-lasting motor recovery in dopamine-depleted mice

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The identification of distinct cell types in the basal ganglia has been critical to our understanding of basal ganglia function and the treatment of neurological disorders. The external globus pallidus (GPe) is a key contributor to motor suppressing pathways in the basal ganglia, yet its neuronal heterogeneity has remained an untapped resource for therapeutic interventions. Here we demonstrate that optogenetic interventions that dissociate the activity of two neuronal populations in the GPe, elevating the activity of parvalbumin (PV)-expressing GPe neurons over that of Lim homeobox 6 (Lhx6)-expressing GPe neurons, restores movement in dopamine-depleted mice and attenuates pathological activity of basal ganglia output neurons for hours beyond stimulation. These results establish the utility of cell-specific interventions in the GPe to target functionally distinct pathways, with the potential to induce long-lasting recovery of movement despite the continued absence of dopamine.

Cell types in neural circuits provide a functional diversity that can be harnessed to treat neurological disorders. The identification of distinct cell types in the basal ganglia has been critical to our understanding of basal ganglia function and the treatment of neurological disorders, particularly Parkinson's disease. However, a major limitation of Parkinson's disease treatments is that they provide only transient relief of symptoms, which rapidly return if a drug dose is missed or deep brain stimulation (DBS) is discontinued.

The external globus pallidus (GPe) is a key contributor to motor-suppressing pathways in the basal ganglia, yet its neuronal heterogeneity has remained an untapped resource for therapeutic interventions. It extends projections to all nuclei within the basal ganglia, as well as to the thalamus, amygdala, brainstem and cortex1–3, and has been implicated as a critical node in the generation and amplification of pathological activity in the dopamine-depleted (DD) state4–6. Recently, molecular and genetic strategies have been developed to subdivide GPe neurons into different subpopulations that vary in physiological and anatomical projections1–3,7–10. Two main subdivisions are 'prototypical' and 'arkypallidal' neurons8,10,11. Approximately 75–80% of GPe neurons are prototypical, meaning they have high, regular firing rates in vivo and project strongly to downstream basal ganglia nuclei7,8. Within the prototypical population, neurons can be further subdivided on the basis of expression of parvalbumin (PV-GPe neurons) and Lim homeobox 6 (Lhx6-GPe neurons)2,12. Although expression of these markers is partially overlapping7–9,12, as a whole, PV and Lhx6 populations differ in their intrinsic physiology and projection densities to downstream nuclei2. However, the behavioral significance of these neuronal subdivisions has not been directly demonstrated.

Here we demonstrate that, in DD mice, transiently dissociating the activity of PV-GPe and Lhx6-GPe subpopulations induces long-lasting recovery of movement and reversal of pathological activity in the basal ganglia circuit that persists for hours beyond stimulation. These prokinetic effects are engaged only by restricting manipulations to particular neuronal subsets and not by manipulations that modulate all GPe neurons simultaneously. These results establish the behavioral relevance of functionally distinct neuronal subpopulations in the GPe and suggest their potential as therapeutic nodes for the long-term restoration of movement in Parkinson’s disease.

RESULTS
Global GPe stimulation does not restore movement in DD mice
A prediction of the classic model of basal ganglia function under DD conditions is that increasing firing rates of GPe neurons should improve movement13,14. To test this hypothesis, we expressed channelrhodopsin-2 (ChR2) in all GPe neurons under control of the human synapsin-1 (SYN1) gene promoter (hSyn-ChR2) (Fig. 1a) and measured the efficacy of global GPe stimulation to reduce immobility and bradykinesia in bilaterally DD mice (Online Methods and Supplementary Figs. 1a,b and 2a–c).

Even though GPe neurons exhibited the expected pathophysiology (Supplementary Fig. 1c–e), hSyn-ChR2 did not rescue movement (Fig. 1b,c), nor did it reduce bradykinesia (defined as a decrease in movement velocity normalized to that of dopamine-intact controls) (Fig. 1d).

This inability to rescue movement with global GPe stimulation could challenge predictions of the classic basal ganglia model13,14, but at least two other interpretations are possible. The first is that bilaterally DD...
mice are so impaired that they are no longer capable of robust movement. To address this possibility, we increased direct pathway activity by driving ChR2 expression in spiny projection neurons expressing D1 dopamine receptors (D1-ChR2) (Fig. 1e and Supplementary Fig. 2d,e). Consistent with previous results, D1-ChR2 relieved immobility in a predominantly light-locked manner (Fig. 1fg). As compared to the 10-min epoch before the first light pulse (Pre: 82 ± 24%; values reported throughout main text as mean ± s.d.), some movement persisted in the 10-min epoch after the tenth light pulse; the difference was not significant (Post10min: 63 ± 21%, n = 4, P = 0.15, paired t-test). When movement bouts did occur, velocities were significantly greater than before stimulation (normalized to dopamine-intact controls: Pre: 0.25 ± 0.22 versus Post10min: 0.91 ± 0.46, n = 4, P = 0.02, paired t-test) (Fig. 1h). These results demonstrate that bilaterally DD mice are still capable of robust movement, so this cannot account for the inability of global GPe stimulation to rescue movement.

A second possibility is that our optogenetic stimulation does not effectively drive firing of GPe neurons. To test this, we recorded the responses of GPe neurons in vivo during optical stimulation with hSyn-ChR2 (Online Methods and Fig. 1i). Due to the large numbers of neurons responding, single-unit activity could not be well isolated during stimulation, so these data reflect multiunit activity. On average, all units (n = 68 of 68 units across 3 animals) significantly increased in firing rate within 100 ms (z-score100ms = 17.6 ± 1.2; t(67) = 1.6679, P = 0.0495, one-tailed t-test) (Fig. 1j,k). In a subset of recordings, we verified that neural responses in the GPe were stable across each of the ten repeated stimulations (n = 34 units across 3 animals) (Fig. 1l). These results confirmed that hSyn-ChR2 was effective at increasing firing rates of GPe neurons, yet this did not rescue movement in DD mice.

Selective activation of PV-GPe neurons restores movement persistently in DD mice

The finding that movement can be rescued during D1-ChR2 stimulation, but not by global GPe stimulation, challenges the classic rate-based basal ganglia model and motivated a more in-depth analysis of GPe circuitry. The GPe contains a heterogeneous population of neurons. Since these populations have different anatomical and physiological properties, we reasoned that they might make different contributions to behavior. As such, cell-specific interventions might be more effective than global ones. To test this hypothesis, we first restricted ChR2 expression to PV-containing GPe neurons (Fig. 2a and Supplementary Fig. 3a,b).
Ten days to 2 weeks after viral injections, mice were bilaterally depleted. The prokinetic effects of PV-ChR2 stimulation were measured 3–5 d later. In contrast to global GPe stimulation, selective stimulation of PV-GPe neurons provided robust relief of immobility and bradykinesia (Fig. 2b–d and Supplementary Videos 1 and 2). In mice expressing a control fluorescent construct (DIO-EYFP), immobility was not reduced (Fig. 2b). Initial responses to PV-ChR2 were highly light-locked, but as stimulation progressed, continuous movement gradually accumulated between light pulses (Fig. 2bc). By the tenth light pulse, PV-ChR2 stimulation had reduced immobility to the same degree as D1-ChR2 stimulation (PV-ChR2: 21 ± 20%, n = 10, versus D1-ChR2: 17 ± 23%, n = 4; P = 0.995, Sidak’s post hoc test). While the effects of D1-ChR2 decayed shortly after stimulation, the effects of PV-ChR2 persisted significantly after the tenth stimulation (Pre: 81 ± 21% versus Post10min: 28 ± 16%, n = 10, P < 0.0001, paired t-test). Movement velocities were also significantly increased (normalized to dopamine-intact controls: Pre: 0.26 ± 0.10 versus Post10min: 1.04 ± 0.67, n = 10, P < 0.0001, paired t-test) (Fig. 2d).

To test the duration of the prokinetic effects of PV-ChR2, a subset of mice were left in the open field for 3 h after stimulation (n = 7) (Fig. 2e,f); four of seven mice remained highly mobile for the duration of the test (Fig. 2f), and immobility (averaged across all mice, n = 7) was significantly reduced for the entire 3 h (Fig. 2e). When mice were returned to their home cage after stimulation, locomotion ramped down within minutes. However, when mice were placed at one end of a 30 × 20 cm cage with food and water positioned at the other end, eight of ten PV-ChR2 mice reached the food within 5 min (range: 0.08–3.9 min) whereas only one of five PV-EYFP mice reached the food (latency = 0.58 min) (P = 0.03, chi-squared test) (Fig. 2g). Combined, these results demonstrate that the prokinetic effects of PV-ChR2 stimulation are long lasting and persist for hours after stimulation.

To characterize the behavioral patterns expressed by PV-ChR2 mice, open field behaviors were manually scored during the 10-min post-stimulation period. Overall, behavioral patterns were remarkably similar between PV-ChR2 mice and dopamine-intact controls (Fig. 2h). Control (n = 7) and PV-ChR2 (n = 10) mice spent similar percentages of time walking (control: 48 ± 6% versus PV: 52 ± 25%; P = 0.6, Mann–Whitney U), grooming (control: 6 ± 4% versus PV: 5 ± 4%; P = 0.734, Mann–Whitney U) and performing fine movements (control: 10 ± 4% versus PV: 8 ± 7%; P = 0.270, Mann–Whitney U). On average, walking bouts were longer in PV-ChR2 mice (6.8 ± 4.9 s) compared to controls (3.1 ± 0.48 s, P = 0.043, Mann–Whitney U). Rearing was not rescued by stimulation (control: 27 ± 9% versus PV: 0.6 ± 1%; P = 0.0001, Mann–Whitney U), and PV-ChR2 mice spent more time immobile compared to controls (control: 9 ± 4% versus PV: 33 ± 19%; P = 0.003, Mann–Whitney U). Furthermore, PV-ChR2 mice retained a hunched posture and irregular gait (Supplementary Video 2), suggesting that stimulation is more effective at alleviating bradykinesia and immobility than postural or gait symptoms.

Because the severity of motor symptoms induced by bilateral DD restricted our experimental time window to 3–5 d after depletion, we performed several control experiments to ensure that behavioral rescue was specific for symptoms related to dopamine loss and not symptoms induced by other factors, such as acute inflammation. First, to control for the effects of inflammation, dopamine-intact mice were injected with lipopolysaccharide, an inflammatory agent, in the medial forebrain bundle17. Five days after injections of lipopolysaccharide,
mice exhibited no locomotor deficits in the open field and PV-ChR2 stimulation had no effects on immobility (Supplementary Fig. 4a).

Second, we observed that the persistent component of behavioral rescue could not be induced in mice with partial dopamine depletions (mice with >20% striatal tyrosine hydroxylase left on either side) (Supplementary Fig. 4b-f), suggesting the long-lasting prokinetic effects of PV-ChR2 are specific to the DD state of the mouse and not other factors associated with our depletion protocol. Third, consistent with data from partially DD mice, PV-ChR2 stimulation in unilaterally DD mice (14 of 21) ChR2 + neurons were inhibited during optical pulses (data not shown). A subset of recordings that were stable enough to track inhibited units held across all ten 30-s pulses (n = 11 across 3 animals). Shaded bars (green) indicate periods of stimulation and each point represents 30-s bins immediately before, during and after the stimulation. Error bars, s.e.m. (f) Schematic of GPe network during Lhx6-Arch stimulation. (g) Light-evoked responses of ten single units during onset of a 30-s pulse. (h) Average z-score of inhibited (blue, n = 27 of 42 across 3 animals) and unchanged (gray, n = 15 of 42) neurons during a 30-s optical pulse. Shaded area, s.e.m. (i) Firing rates of individual neurons before, during (time 0) and in 10-s bins after a 30-s light pulse (excited: t(13) = 4.789, *P < 0.0001, paired t-test). Population averages are shown as thick, colored lines. (e) Average firing rates of units across all ten 30-s pulses for excited (top, n = 5 across 3 animals) and inhibited units (bottom, n = 4 across 2 animals). Shaded bars (blue) indicate periods of stimulation and each point represents 30-s bins immediately before, during and after the stimulation. Error bars, s.e.m. (k) Summary of average firing rates before, during and after 30-s stimulations of PV-ChR2 and Lhx6-Arch.

Neuronal responses in the GPe during PV-ChR2 stimulation

Our behavioral results demonstrate that movement is restored when PV-GPe neurons are stimulated selectively, but not when they are stimulated with all other GPe neurons (hSyn-ChR2). To investigate how PV-ChR2 stimulation differs at the network level from hSyn-ChR2 stimulation, we recorded responses of neurons in vivo during PV-ChR2 stimulation (Fig. 3a). To identify putative PV-GPe neurons, we first classified neurons as ChR2 + or ChR2 − on the basis of their short-latency responses to brief (5 ms) optical pulses (Online Methods and Supplementary Fig. 5a). Characteristic firing patterns and waveform distributions of ChR2 + and ChR2 − neurons are summarized in Supplementary Figure 5b,c. Because the average firing rate of our putative PV-GPe population (24 ± 4 Hz, n = 18 across 3 animals) was lower than what has been reported by other groups8,18, we validated our optical identification strategy in dopamine-intact mice (Supplementary Fig. 5d). In dopamine-intact controls, the average firing rate of the PV-GPe population was 46 ± 2 Hz, consistent with results from previous studies: 48 ± 3 Hz (ref. 8) and 47 ± 6 Hz (ref. 18). These results suggest that the lower firing rates of putative PV-GPe neurons in our study are due to changes in population activity induced by dopamine loss and not by errors in neuronal classification.

In DD mice, all 18 ChR2 + (putative PV-GPe) neurons tested responded to 30-s optical pulses with sustained increases in firing rates (Fig. 3b,c), averaging 24 ± 4 Hz before stimulation and 52 ± 8 Hz during stimulation (P < 0.0002, paired t-test) (Fig. 3d). A subset of recordings that were stable enough to track single units across a ten-pulse stimulation series (n = 7 across 3 animals) revealed that responses were consistent across all ten pulses (Fig. 3e). In contrast, most (14 of 21) ChR2 − neurons were inhibited during optical pulses (Fig. 3b,c), averaging 30 ± 3 Hz before stimulation and 18 ± 2 Hz during stimulation (P < 0.0004, paired t-test) (Fig. 3d). Firing rates of the remaining (7 of 21) ChR2 − neurons were not significantly modulated (data not shown). A subset of recordings that were stable enough to track inhibited units across a ten-pulse stimulation series (n = 4 across 2 animals) revealed that inhibitory responses were consistent across all ten pulses (Fig. 3e).
These data reveal a key difference between the effects of PV-ChR2 and hSyn-ChR2 stimulation on population dynamics in the GPe. PV-ChR2 stimulation produces a bidirectional response that transiently elevates the firing of PV-GPe neurons but suppresses the firing of other GPe neurons, a dissociation that is occluded during global stimulation with hSyn-ChR2. To test whether this dissociation is critical for the induction of behavioral rescue, we sought to mimic this effect by inhibiting a subset of neurons directly with archaerhodopsin (Arch). Because the firing rates and waveforms of ChR2− neurons were highly overlapping with those of ChR2+ neurons (Supplementary Fig. 5b,c), we reasoned that both subtypes are part of the prototypical population. PV-GPe neurons constitute a major fraction of this population, but neurons expressing Lhx6 represent a second, partially non-overlapping fraction (Supplementary Fig. 6a,b).

To measure the impact of inhibiting Lhx6-GPe neurons on population activity in the GPe, we recorded in vivo in Lhx6-Cre mice 2 weeks after virally mediated expression of Arch (Fig. 3f). Neurons were classified as Arch+ or Arch− based on their response to 1-s optical pulses of green light (Online Methods and Supplementary Fig. 5e). Characteristic firing patterns and waveform distributions of Arch+ and Arch− neurons were highly overlapping and are summarized in Supplementary Figure 5f.g.

In response to 30-s optical pulses, all 27 Arch+ neurons tested responded with sustained decreases in firing rates (Fig. 3g,h), averaging 29 ± 3 Hz before stimulation and 6 ± 2 Hz during stimulation (P = 2.68 × 10−10, paired t-test) (Fig. 3i). A subset of recordings that were stable enough to track single units across a ten-pulse stimulation series (n = 11 across 3 animals) revealed that responses were consistent across all ten pulses (Fig. 3j). All 15 remaining neurons were Arch− and showed no net change in firing rate during optical stimulation (Fig. 3g−i). Although some neurons exhibited sharp firing rate increases at the onset of a light pulse, this effect was transient, seldom persisting for >100 ms (Fig. 3h). These results demonstrate that Lhx6-Arch, like PV-ChR2, transiently dissociates population activity in the GPe, but with different effects on absolute firing rate (Fig. 3k).

**Selective inhibition of Lhx6-GPe neurons restores movement persistently in DD mice**

To test whether the transient dissociation of GPe activity produced by Lhx6-Arch was also sufficient to induce behavioral rescue, we assessed its effects on immobility and bradykinnesia of DD mice in the open field (Fig. 4a−d and Supplementary Fig. 3e). Initially, mice were highly immobile (Pre: 86 ± 11%, n = 9), but over the course of ten stimulations, Lhx6-Arch reduced immobility to a similar degree as PV-ChR2 (tenth pulse: Lhx6-Arch: 28 ± 38%, n = 9 versus PV-ChR2: 21 ± 20%, n = 10, P = 0.80, Tukey’s post hoc test) (Fig. 4b,f). Control (n = 6) and Lhx6-EYFP (n = 7) mice remained highly mobile for the duration of the test (Fig. 4g). These results demonstrate that Lhx6-Arch, like PV-ChR2, transiently dissociates population activity in the GPe, but with different effects on absolute firing rate (Fig. 3k).

Intriguingly, Lhx6-Arch induced the gradual, persistent component of behavioral recovery but not the early, light-locked component present in PV-ChR2 mice. Bradykinnesia was also greatly reduced (Pre: 0.22 ± 0.08 versus Post10min: 0.85 ± 0.45, n = 9, P = 0.002, paired t-test) (Fig. 4d). Ten minutes after the last stimulation, Lhx6-Arch mice remained highly mobile, spending only 34 ± 21% (versus Pre: 86 ± 11%, n = 9, P = 0.012, Tukey’s post hoc test) of their time in the immobile state. As with the long-lasting effects of PV-ChR2, immobility in Lhx6-Arch mice remained significantly reduced for hours after stimulation (Post3h: Lhx6-Arch: 45 ± 11%, n = 5 versus PV-ChR2: 41 ± 36%, n = 7, P = 0.990, Tukey’s post hoc test) (Fig. 4f,g). Contrary to three of five Lhx6-Arch mice remained highly mobile for the duration of the test (Fig. 4g).

Behavioral patterns in Lhx6-Arch mice were similar to those of dopamine-intact controls (Fig. 4e). Control (n = 6) and Lhx6-Arch mice...
(n = 7) spent similar percentages of time walking (control: 42 ± 12% versus Lhx6: 44 ± 28%; P = 0.886, Mann–Whitney U), grooming (control: 6.9 ± 7.7% versus Lhx6: 11.2 ± 9.2%, P = 0.568, Mann–Whitney U) and performing fine movements (control: 21 ± 9% versus Lhx6: 18 ± 9%, P = 0.445, Mann–Whitney U), but rearing behavior did not recover (control: 23 ± 10% versus Lhx6: 0.3 ± 0.3%, P = 0.001, Mann–Whitney U). Lhx6-ChR2 mice spent a wide range (7–50%) of time in the immobile state (control: 7.6 ± 2.0% versus Lhx6: 26.5 ± 18.3%, P = 0.101, Mann–Whitney U). Walking bouts in Lhx6-ChR2 mice were significantly longer than in control (control: 2.7 ± 0.8 ms versus Lhx6: 5.6 ± 2.4 ms, P = 0.02, Mann–Whitney U), and they walked with a hunched posture and shuffling gait (Supplementary Video 4). Combined, our behavioral results suggest that transiently dissociating the activity of GPe neurons with Lhx6-ChR2 induces a gradual, long-lasting recovery of movement that is qualitatively and quantitatively similar to that induced by PV-ChR2.

**Persistent behavioral rescue depends on the ratio of Lhx6 and PV activity**

Thus far, our results have shown that movement can be persistently rescued by manipulations that dissociate the activity of PV-GPe neurons above that of Lhx6-GPe neurons. But because these subpopulations are partially overlapping at the molecular level (Supplementary Fig. 6a,b), we wanted to determine whether their effects were truly segregated at the behavioral level. To test this, we assessed the prokinetic effects of inverse manipulations: Lhx6-ChR2 and PV-Arch. In mice stimulated with Lhx6-ChR2 (Fig. 5a and Supplementary Fig. 3e), some relief from immobility was observed in three of five mice during the stimulation period (Pre: 91 ± 13% versus tenth stimulation: 47 ± 46%, n = 5, P = 0.067, Tukey’s post hoc test), but this effect did not persist beyond 30 min after stimulation (Pre: 91 ± 13% versus Post30min: 72 ± 37%, n = 5, P = 0.684, Tukey’s post hoc test) (Fig. 5b,c).

In mice stimulated with PV-Arch (Fig. 5d and Supplementary Fig. 3f), optogenetic suppression of PV-GPe neurons did not rescue movement (Fig. 5e). Immobility in PV-Arch mice was 94 ± 4% before the first stimulation and 92 ± 8% during the tenth stimulation (n = 5, P = 0.448, paired t-test), and no persistent effects were observed (Fig. 5e). Taken together, these results confirm that the induction of long-lasting behavioral recovery is cell-type specific and is induced by interventions that dissociate the firing rates of PV-GPe neurons above that of Lhx6-GPe neurons (Fig. 5f).

**PV-ChR2 and Lhx6-Arch reverse pathological burst firing in SNr**

How do transient imbalances between the firing rates of two subpopulations of GPe neurons produce a long-lasting effect on movement? To study the impact of GPe interventions on the basal ganglia circuit, we recorded neural activity in the substantia nigra reticulata (SNr), the main basal ganglia output nucleus in rodents.

A pathological hallmark of SNr dysfunction following dopamine depletion is an increase in burst firing and the percentage of bursting neurons19–21. Consistent with these findings, we observed a rightward shift in the proportion of bursting neurons in DD mice compared to dopamine-intact controls (P = 0.005, Kolmogorov–Smirnov (K-S) two-sample test) (Fig. 6a,b). To test whether optogenetic stimuli that rescue movement would reduce the proportion of bursting neurons, we recorded from the SNr before, during and after stimulation (Fig. 6c,d). PV-ChR2 induced a leftward shift in the distribution of bursting neurons in the SNr (P = 0.005, K-S two-sample test) (Fig. 6e). This effect was most pronounced for highly ‘bursty’ units, as identified by burst frequencies that exceeded 1 median absolute deviation above the median (Fig. 6f). The fraction of highly bursting neurons was reduced from 27% before stimulation to 10% after (Pre: n = 22 of 81 versus Post: n = 6 of 58 across 3 animals, P = 0.044, chi-squared test) (Fig. 6g). In contrast, hSyn-ChR2 did not shift the distribution of bursting neurons (P = 0.188, K-S two-sample test) (Fig. 6h). 31% of units were classified as highly bursty before stimulation compared to 20% after (Pre: n = 17 of 55 versus Post: n = 14 of 69 across 3 animals, P = 0.295, chi-squared test) (Fig. 6i,j). In recordings stable enough
to track the activity of single units across all ten light pulses, a reduction in the number of bursts was apparent at the level of individual neurons recorded in PV-ChR2 mice (Fig. 6k), but not in hSyn-ChR2 mice (Fig. 6l). Notably, bursts diminished gradually over the first two to four stimuli, mirroring the kinetics of behavioral rescue in the open field.

To test whether Lhx6-Arch works through a similar mechanism, we repeated experiments in Lhx6-Arch mice. Consistent with the effects of PV-ChR2, Lhx6-Arch produced a leftward shift in the distribution of bursting neurons in the SNr (Fig. 6m), and the fraction of highly bursty neurons was reduced from 31% before stimulation to 3% after (Pre: n = 9 of 29 versus Post: n = 2 of 69 across 3 animals, P = 0.0006, chi-squared test) (Fig. 6n,o).

To determine how long burst attenuation persists after stimulation, we separated the population activity into 1-h-time bins and calculated the fraction of bursty neurons over time. Consistent with the persistent effects on behavior, the fraction of bursty neurons across the population remained persistently reduced for hours after stimulation (Pre versus Post1–3h: PV-ChR2: P = 0.007, Lhx6-Arch: P = 0.003, Dunnett’s multiple-comparisons test) and only began to drift back to pre-stimulation levels by ~3 h after stimulation (Pre versus Post2h: PV-ChR2: P = 0.007, Lhx6-Arch: P = 0.001, Dunnett’s multiple comparisons test) (Fig. 6p). These data suggest a mechanism through which PV-ChR2 and Lhx6-Arch persistently rescue movement through a long-lasting normalization of basal ganglia output pattern. We also considered effects of stimulation on SNr firing rates, and although burst firing decreased, there was no change in the firing rate before and after PV-ChR2 stimulation (Fig. 6p).

Taken together, these results suggest that transient cell-specific interventions in the GPe induce...
long-lasting reductions in the pathological activity of basal ganglia output neurons in the SNr that persist for hours beyond stimulation.

**DISCUSSION**

Here, we demonstrate that cell-specific interventions in the GPe induce robust, long-lasting recovery of motor function in DD mice. Manipulations that transiently elevate the firing of PV-GPe neurons above that of Lhx6-Arch neurons reverse pathological burst firing in the SNr and ameliorate symptoms of immobility and bradykinesia for hours beyond stimulation. These results establish the behavioral significance of cell type heterogeneity in the GPe, with potential implications for the treatment of Parkinson's disease.

Nearly 30 years ago, the discovery that striatal neurons can be molecularly divided into D1 and D2 dopamine receptor-expressing subpopulations provided a cellular basis for the direct/indirect pathway model of the basal ganglia that has long dominated our conceptual framework. However, with the exception of neuronal diversity in the striatum, the classical direct/indirect pathway model treats all other downstream nuclei as relay structures, an oversimplification whose limits have become increasingly apparent as techniques for studying circuit function become more sophisticated. The GPe contains different cell populations, but this knowledge has been slow to translate into therapeutic strategies. Here, leveraging tools to optogenetically target subpopulations of GPe neurons, we were able to induce long-term recovery of motor function in DD mice.

Strategies that persistently restored movement shared a common mechanism of elevating the firing rates of PV-GPe neurons relative to that of Lhx6-GPe neurons (PV-ChR2, Lhx6-Arch). Interventions that activated or suppressed all GPe neurons (hSyn-ChR2, CAG-Arch) were not effective, nor were interventions that elevated the firing rates of Lhx6-GPe neurons relative to PV-GPe neurons (Lhx6-ChR2, PV-Arch). Thus, despite some overlap between the Lhx6 and PV subpopulations at the molecular level, the behavioral effects produced by manipulating these populations were well segregated.

The GPe’s impact on movement is thought to be mediated by its influence over basal ganglia output nuclei, predominantly the SNr in rodents. Under DD conditions, neurons in the basal ganglia become more rhythmic and bursty, impairing basal ganglia output. Attenuation of pathological activity is well correlated with the therapeutic effects of DBS on bradykinesia and rigidity. The dissociation of pallidal subpopulations—elevating the activity of PV-GPe above that of Lhx6-GPe neurons—and the subsequent remediation of pathological activity in the SNr may be a possible mechanism for DBS. Although the 6-hydroxydopamine model does not replicate all the features of a complex human disorder such as Parkinson’s disease, it provides key insights into the function of neural circuits under conditions of low dopamine. The cardinal motor symptoms of Parkinson’s disease, such as immobility and bradykinesia, do not arise until dopamine levels have decreased by ~70%, highlighting the need to discover strategies to restore motor function, even at advanced stages of dopamine loss.

It has long been assumed that the prokinetic effects of DBS are limited to the stimulation period, with symptoms (and pathological activity) rapidly returning within minutes after stimulation. However, a modified DBS protocol, called coordinated reset, has been shown to provide some prokinetic benefits that persist for hours or even days after stimulation in both 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate models and Parkinson’s disease patients. The ability to destabilize the network by shifting the balance of neuronal subpopulations may be sufficient to rescue motor function.

Although we do not know whether GPe stimulation engages similar mechanisms, the GPe is a central node for the amplification and propagation of pathological network oscillations in Parkinson’s disease. Our data identify PV and Lhx6-GPe neurons as critical nodes in the basal ganglia circuit for the induction of long-lasting attenuation of pathological activity in the SNr. The convergent effects of PV and Lhx6-GPe neurons on the SNr could be mediated via their direct projections to the nucleus or via their indirect projections by way of the subthalamic nucleus, a nucleus that is differentially innervated by Lhx6 versus PV subpopulations. Elucidating the circuit and synaptic mechanisms that give rise to pathological rhythmicity in disease and discovering interventions to best counteract this rhythmicity represent important areas of research.

**Conclusion**

In conclusion, our results demonstrate that cell-specific, but not global, interventions in the GPe induce long-lasting behavioral rescue and physiological restoration of basal ganglia output in DD mice. These results establish important functional distinctions between subpopulations of GPe neurons, delineated in part by their expression of Lhx6 and PV. These results reconcile some conflicting reports in the literature: that successful DBS can either increase or decrease firing rates of GPe neurons, that DBS directly in the GPe can reduce bradykinesia but restoring autonomous firing after DD has no effect. Finally, because the proportion of PV-GPe neurons in humans is similar to that in mice, our results suggest that interventions that preferentially increase their activity relative to other GPe neurons might provide more persistent prokinetic benefits than current treatments.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

K.J.M., K.T.Z., and K.H.L. performed behavioral experiments, including the histological verification, and with A.H.G. analyzed the data. K.J.M. and A.M.W. were responsible for the collection and analysis of the in vivo experiments. All authors discussed results and interpretations. K.J.M. and A.H.G. designed the experiments and wrote the manuscript.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. Experimental procedures were approved by the Carnegie Mellon University Committee for the Use and Care of Animals and in accordance to the guidelines set forth by the National Institute of Health and Society for Neuroscience Use of Animals in Neuroscience Research. Male and female heterozygous mice 8–15 weeks old on a C57BL/6J background were used for all experiments. D1-SPNs were targeted using the D1-cre mouse line44. PV-GPe neurons were targeted using the Lhx6-iCre mouse line46. To ensure health of the animals undergoing the dopamine depletion, weights were closely monitored and every animal weighed greater than 20 g before initial surgery. Animals were group housed (2–8 per group) in a 12-h/12-h light dark cycle until the time of second surgery (as noted below) and all experiments were completed during the light cycle.

Viral transfection. Injections of purified double-floxed AAV2-DIO-EYFP (controls), AAV2-DIO-ChR2-EYFP (cell-specific activation), AAV2-DIO-Arch-T-tomato (cell-specific inhibition), AAV2-hsyn-hChR2(H132R)-tdTomato (non-specific activation) or AAV2-CAG-Arch-T-tomato (nonspecific inhibition) produced at the University of North Carolina (Vector Core Facility) were made in D1-cre, PV-Cre or Lhx6-iCre transgenic mice 8–12 weeks old. Littersmates were randomly assigned to either the rhodopsin-positive or control groups. Injections into the dorsomedial striatum12 or GPe2 were completed in accordance to methods previously described. Briefly, anesthesia was induced using ketamine (100 mg/kg) and xylazine (30 mg/kg) and maintained throughout surgery using 1.5% isoflurane. Mice were placed in a stereotaxic frame (Kopf Instruments), where the scalp was opened and bilateral holes were drilled in the skull (striatum: 0.5 mm anterior, 1.5 mm lateral, GPe: 0.27–0.30 mm anterior, 2.1–2.2 mm lateral from bregma). Virus (200–250 nL) was injected with a Nanoject (Drummond Scientific) through a pulled glass pipet (tip diameter ~30 µm) whose tip was positioned below the top of the skull (striatum: 2.80 mm, GPe: 3.65 mm). To prevent backflow of virus, the pipet was left in the brain for 5 min after completion of the injection. All experiments were performed at least 2 weeks after injection to allow time for full viral expression. At that point, mice underwent a second surgery for either behavioral optogenetics or physiology, during which experimenters were blind to the experimental condition. Sample sizes for each experiment are in line with previous published studies15,47.

Optogenetic behavioral implantation and dopamine depletion. For behavioral optogenetic experiments in freely moving mice, a second surgery was performed at least 10 d after viral injections to deplete dopamine and insert optical fibers. For the second surgery, mice were anesthetized and placed on the stereotaxic frame, and holes were redrilled from the previous viral injection. In addition, bilateral holes were drilled over the medial forebrain bundle (MFB: ~0.45 mm posterior, ±1.15 mm lateral from bregma) for 6-hydroxydopamine (6-OHDA) injections. A 33-gauge cannula (Plastics One, Roanoke, VA, USA) attached to a syringe pump was slowly lowered into place (MFB: 4.95 mm from the skull) and allowed to settle for 5 min. At this point, 1 µL of 6-OHDA (5 µg/µL in 0.9% NaCl), saline (0.9% NaCl) for unilateral depletions or lipopolysaccharide (Sigma-Aldrich) for bilateral depletions were injected using the Omniplex system (Plexon Inc) and stored for offline analysis. After the bilateral injections, a custom-made plastic button containing two polished ferrules was placed over the holes previously used for viral injections. The fibers were slowly advanced to the top of the viral expression (striatum: 2.60 mm, GPe: 3.45 mm). Dental cement was used to secure the button to the top of the skull. After all dopamine depletions, mice were individually housed and placed in a recovery station. The station consisted of a new cage, soft food, trail mix and water dishes, and half of the cage was placed on a heating pad. In a subset of conditions, mice were observed for another 3 h to examine the persistence of behavioral intervention.

For the food-retrieval task, mice were placed in a new cage (30 × 20 cm) following the completion of the behavioral testing. Mice were placed in the opposite end of the cage (~30 cm away) from two small dishes of food and water. Mice were tracked overhead using Noldus EthoVision. Successful trials were defined as when mice reached the food or water dishes within 5 min.

Implantation of head-fixation system. A subset of virally injected mice were used for head-fixed in vivo physiology. Two weeks after viral expression, mice followed the same dopamine depletion protocol described above. After the bilateral injection of 6-OHDA, bilateral craniotomies were created over the GPe (~0.45 to 0.45 mm anterior, 1.75 to 2.35 mm lateral to bregma) or SNr (~2.75 to 3.25 mm anterior, 1.15 to 2.00 mm lateral to bregma). For SNr recordings, holes were drilled over the site of viral injections into the GPe and fibers (output transmission = 1 mW, measured at the end of the fiber) were implanted for direct optical stimulation during recordings. After this, all animals were implanted with a copper headpost fixed to the posterior portion of the skull (approximately ~3.5 mm posterior to bregma) using a combination of glue and dental cement. The dental cement fixation was extended to surround the entirety of both craniotomies. The subsequent well that was formed was filled with a silicone elastomer (Kwik-sil, WP1) that prevented infection and damage to a brain tissue. During recording, this well was filled with saline and used as a ground reference.

Head fixation training and recording. Mice were placed atop a running wheel and allowed to run freely for 60 min the day before recording. Movement was tracked for the full period of recordings using an inverted optical mouse and custom MATLAB script. Craniotomies were cleaned and prepared for recordings the following day, and silicone elastomer was replaced.

GPe recordings. On the day of recording, mice were fixed to the top of the wheel and allowed 15 min to acclimate to the head-fixed position. After removal of the silicone elastomer and clearing of the craniotomy, a linear 16-channel silicon optrode with sites spaced 50 µm apart (Neuronexus) and a 100-µm fiber terminating 50 µm above the uppermost site were attached to the micromanipulator and centered on bregma. Transmittance through the optical fiber was measured before recording to ensure ~0.5 mW in both the blue and green light conditions. The probe was slowly advanced (~5–7 µm/s) until the top of the GPe (~3.20 mm from top of the skull) was found. GPe activity was distinguished based on a combination of physiological features: presence of high-firing neurons; presence of low firing, irregular neurons; lack of spindle-like activity (thalamic) and responsiveness to light activation or inhibition. Post-mortem tissue analysis for viral injection and craniotomy placements were further evidence for proper targeting.

The probe was left in place for approximately 15 min before neuronal activity was measured. During this time, a drop of saline was placed in the well that surrounds the craniotomy and a reference ground electrode was placed in contact with the saline. Extracellular recordings and local field potentials were acquired using the Omnispot system (Plexon Inc) and stored for offline analysis.

Optical tagging method. In the cell-type manipulations, single units could be isolated. An optical tagging strategy was employed to distinguish a neuron’s activity as positive or negative for rhodopsin expression. For ChR2 conditions, brief pulses (pulse width = 5 ms, 10 Hz, 120 pulses) were administered at the start and end of each recording session before advancing the probe to the next location. For Arch conditions, a set of longer pulses (pulse width = 1 s, 0.5 Hz, 10–20 pulses) was administered to clearly denote neurons that were directly inhibited by the light. After the optical tag, activity of the neurons was measured in response to a 30-s period of light. In a subset of neurons, stability of recording was great enough to allow the application of the full optogenetic protocol (pulse width = 30 s, interpulse interval = 3 min, 10 pulses). After the recording period, mice were sacrificed and tissue was used to verify placement of the craniotomy and documentation of probe entrance and placement.

For analysis, we used a previously published identification tool to classify neurons based on responsiveness to brief pulses (5 ms)48. Briefly, baseline activity
Shortly after the behavioral or electrophysiology experiments, data were filtered at 150–8,000 Hz for spiking activity during gradual dopamine depletion with 6-hydroxydopamine in mice. Spike detection was completed using the Plexon offline sorter, where principal component analysis was used to delineate single units and multitunits. To be classified as a single unit, a unit had to meet the following criteria: (i) PCA clusters were significantly different (P < 0.001); (ii) J3-statistic was greater than 1; (iii) percentage of ISI violations (<2 ms) was less than 0.15%; (iv) Davies–Bouldin test statistic was less than 0.5; (v) under manual verification, optical stimulation did not occlude the ability to delineate single units from noise.

Following spike sorting, data were processed in Neuroexplorer and with custom scripts in Matlab. Rest period analysis: Periods of rest were analyzed to identify the firing rates (FR) and coefficient of variation of the interspike intervals (CV_ISI) in the dopamine-intact versus DD state (Supplementary Fig. 1c–e). Burst analysis: Using the surprise algorithm (surprise = 2), bursts were identified in the single-unit SNr activity (FR > 5 Hz) and calculated across a 2 min period; this was referred to as number of bursts (Fig. 6). For Figure 6k1, the number of bursts were calculated for 30-s time bins. Each unit was then normalized to baseline (Pre) and then averaged across all stably recorded units. Fractional analysis: The fraction of highly bursty neurons was identified by calculating the number of units that exceeded median absolute deviation from the median in the distribution across all conditions, before optogenetic manipulation (bursty threshold: 70).

Statistics. Statistical analyses were completed using SPSS software or GraphPad Prism 7 (GraphPad Software). All data were tested for normality and equal variance. Behavioral data were analyzed using two-sided, paired or unpaired Student’s t-tests for normally distributed data. In all other cases, a Mann–Whitney U or a chi-squared test was applied. For the analysis of behavioral persistence, behavioral conditions were tested using two-way, repeated-measures ANOVA. As noted in the main text, comparisons made within or across conditions used Tukey’s or Sidak’s post hoc test, respectively. Results are reported in the text as mean ± s.d.

Physiology data were first tested for normality and equal variance. Then the appropriate parametric (two-sided, paired or unpaired Student’s t-test) or non-parametric (Mann–Whitney U or Kruskal–Wallis H) tests were applied. Cumulative distributions were compared using the Kolmogorov–Smirnov (K-S) two-sample test. Fractional burst analysis applied a chi-squared statistic to measure whether there was a change in the population burst firing before and after optogenetic intervention. Physiology results were reported as mean ± s.e.m. A Supplementary Methods Checklist is available.

Behavioral analysis. Videos collected during the behavioral testing were analyzed using Noldus EthoVision. Immobility was quantified as the period of time where there was less than 1% change in pixels corresponding to the body segment. Center-point detection was used to calculate movement velocities during movement bouts (velocity > 1 cm/s for at least 1 s). For quantification of behavioral patterns, the behavior of a subset of randomly selected animals in the dopamine-intact control, PV-ChR2 and Lhx6-Arch conditions was manually scored using Observer software to denote start and stop times of periods during which mice engaged in walking, rearing, grooming, and fine movements (scratching, sniffing, looking around). Periods of time not engaged in any of these movements were classified as ‘immobile’ in behavioral pattern analyses.

Electrophysiology analysis. Data were filtered at 150–8,000 Hz for spiking activity and 0.7–300 Hz for local field potentials. Spike detection was completed using the Plexon offline sorter, where principal component analysis was used to delineate single units and multitunits. To be classified as a single unit, a unit had to meet the following criteria: (i) PCA clusters were significantly different (P < 0.001); (ii) J3-statistic was greater than 1; (iii) percentage of ISI violations (<2 ms) was less than 0.15%; (iv) Davies–Bouldin test statistic was less than 0.5; (v) under manual verification, optical stimulation did not occlude the ability to delineate single units from noise.

Following spike sorting, data were processed in Neuroexplorer and with custom scripts in Matlab. Rest period analysis: Periods of rest were analyzed to identify the firing rates (FR) and coefficient of variation of the interspike intervals (CV_ISI) in the dopamine-intact versus DD state (Supplementary Fig. 1c–e). Burst analysis: Using the surprise algorithm (surprise = 2), bursts were identified in the single-unit SNr activity (FR > 5 Hz) and calculated across a 2 min period; this was referred to as number of bursts (Fig. 6). For Figure 6k1, the number of bursts were calculated for 30-s time bins. Each unit was then normalized to baseline (Pre) and then averaged across all stably recorded units. Fractional analysis: The fraction of highly bursty neurons was identified by calculating the number of units that exceeded 1 median absolute deviation from the median in the distribution across all conditions, before optogenetic manipulation (bursty threshold: 70).

Tyrosine hydroxylase quantification. Quantification of tyrosine hydroxylase staining was used as a measure of dopamine lesion on both hemispheres. As described previously49, slices containing the dorsal striatum were imaged using an epifluorescence microscope at 10x magnification. To analyze the fluorescence intensity, we used the pixel intensity-measuring tool in ImageJ. A 100 × 100 μm square from each hemisphere was measured and normalized to the pixel intensities of a healthy control tissue that was processed and imaged in parallel. Unless noted, all mice had <20% TH remaining on both sides for all behavioral and electrophysiology experiments to limit behavioral variability.

Behavioral analysis. Videos collected during the behavioral testing were analyzed using Noldus EthoVision. Immobility was quantified as the period of time where there was less than 1.2% change in pixels corresponding to the body segment. Center-point detection was used to calculate movement velocities during movement bouts (velocity > 1 cm/s for at least 1 s). For quantification of behavioral patterns, the behavior of a subset of randomly selected animals in the dopamine-intact control, PV-ChR2 and Lhx6-Arch conditions was manually scored using Observer software to denote start and stop times of periods during which mice engaged in walking, rearing, grooming, and fine movements (scratching, sniffing, looking around). Periods of time not engaged in any of these movements were classified as ‘immobile’ in behavioral pattern analyses.