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Optogenetic Globus Pallidus Stimulation Improves Motor Deficits in 6-Hydroxydopamine-Lesioned Mouse Model of Parkinson's Disease

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1 Type of the Paper: Article

2 **Contrasting behavioral effects of optogenetic globus pallidus**
3 **modulations in normal and hemi-parkinsonian mice**

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8
9 **Abstract:** Excessive inhibition of the external globus pallidus (GPe) by striatal GABAergic neurons is consid-
10 ered as a central mechanism contributing to motor symptoms of Parkinson’s disease (PD). While electro-
11 physiological findings support this view, behavioral studies assessing beneficial effects of global GPe activa-
12 tions are scarce and the reported results are controversial. We used an optogenetic approach and the stand-
13 ard unilateral 6-hydroxydopamine nigrostriatal dopamine (DA) lesion model of PD to explore the effects of
14 GPe photostimulation on motor deficits in mice. Global optogenetic GPe inhibition was used in normal mice
15 to verify whether it reproduced the typical motor impairment induced by DA lesion. GPe activation im-
16 proved ipsilateral circling, contralateral forelimb akinesia, locomotor hypoactivity and bradykinesia in
17 hemi-parkinsonian mice at ineffective photostimulation parameters in normal mice. GPe photoinhibition
18 had no effect on locomotor activity and forelimb use in normal mice but reduced directed exploration and
19 improved working memory performances indicating that recruitment of GPe in physiological conditions
20 may depend on the behavioral task involved. Collectively, these findings suggest that GPe plays more im-
21 portant role in PD as consequence of the complex functional compensatory remodeling affecting BG circuits
22 upon DA depletion.

23 **Keywords:** external globus pallidus, motor behavior, mice, optogenetic, Parkinson’s disease.

24
25 **1. Introduction**

26 Basal ganglia (BG) are a highly organized network of subcortical nuclei that con-
27 trols various aspect of voluntary motor movements and were implicated in many neu-
28 rodegenerative disorders, including Parkinson’s disease (PD)^{1,2}. The external globus
pallidus (GPe) was traditionally considered as a simple relay nucleus within the net-
work, connecting the striatum and subthalamic nucleus (STN) in the indirect pathway^{2,3}.
However, recent anatomical and electrophysiological studies uncovered a diversity of
GABAergic neurons and anatomical connectivity suggesting that the GPe may serve a
much more complex function than just a relay station^{4–6}. GABAergic neurons of GPe
comprise distinct neuronal subpopulations that can be identified based on their electro-
physiological properties, molecular features, and projection targets. The two major clas-
ses are prototypic neurons that send widespread projections within and outside the BG
and arky pallidal neurons that project back to the striatum^{4–8}. Despite surge of interest
over the past years, our understanding of the functional importance of GPe within the
BG circuits remains incomplete. Early behavioral studies showed that pharmacological
manipulations with GABAergic and glutamatergic agents that enhance GPe activity
promote motor behaviors⁹. Unilateral GPe activations induce contralateral circling in
rats and dyskinesia in monkeys while bilateral stimulations produce locomotor hyperac-
tivity in rats^{10–16}. Consistent with pharmacological studies, unilateral optogenetic GPe
activation was also reported to induce contralateral circling and hyperkinesia in mice¹⁷.
While these findings consistently show that the net effect of global GPe activation is a
facilitation of motor behaviors, studies assessing the impact of cell-type specific
optogenetic stimulations revealed a dissociable contribution of GABAergic neuron sub-
types to motor behavior^{18–21}.

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In PD condition, the degeneration of nigrostriatal dopaminergic neurons triggers a range of functional compensatory changes in BG circuits that lead to development of motor symptoms²². Excessive inhibition of GPe neurons by striatal GABAergic neurons of the indirect pathway is considered a critical mechanism underlying the pathological overactivity of the subthalamic nucleus and BG output structures and thus the expression of the motor symptoms^{1,22,623}. While electrophysiological and neurochemical studies in patients and animal models of PD provide support for this view^{24–28}, few studies have explored behavioral effects of global GPe stimulations in the context of dopamine (DA) depletion and the reported results are controversial. Pharmacological and global chemogenetic GPe activations ameliorate motor deficits in reserpine-treated rat and in mice with bilateral medial forebrain bundle (MFB) lesion, respectively^{29–31}. By contrast, no effect was found with either global optogenetic GPe activation or inhibition in MFB-lesioned mice³². Whether the discrepancy is due to the functional heterogeneity of GABAergic projection neurons and/or the difference in neuromodulation approaches used is still an open question. To date, it remains also unclear whether GPe inhibition in physiological conditions produces motor deficits like dopaminergic lesion. While pharmacological inhibitions of GPe produce motor impairments in rats^{10,11,33,34}, recent optogenetic studies targeting striatal indirect pathway and GPe found no effects in mice^{35,36}, thus raising a question about GPe participation to locomotor behavior in physiological conditions.

In the present study we used an optogenetic approach and the standard unilateral 6-hydroxydopamine (6-OHDA) nigrostriatal dopamine (DA) lesion model in mice to reevaluate the effects of global GPe stimulation on motor deficits. The behavioral effects of optogenetic inhibition of GPe were also studied in normal mice under the same testing conditions to examine whether reduction of GPe activity reproduces the typical motor deficits of DA lesion. The findings show that global GPe photostimulation improves a range of motor deficits in hemi-parkinsonian mice. In normal mice, GPe photoinhibition had a little effect on motor behavior but promoted cognitive functions. These findings shed a new light into GPe function and show that this BG nucleus differentially contribute to modulation of motor functions in normal and PD conditions.

2. Results

2.1. GPe photostimulation improves ipsilateral circling behavior and contralateral forelimb akinesia in 6-OHDA-lesioned mice

To examine whether GPe activation improves motor impairments induced by unilateral DA lesion, the excitatory channelrhodopsin 2 (ChR2) variant, ChR2(H134R), which has a larger photocurrent than wildtype ChR2³⁷, was expressed in GPe neurons under the control of the human synapsin-1 (hSyn1) promoter using an adeno-associated virus (AAV) vector (**Figure 1A**). Only mice with successful optic fiber implantation (**Figure 1B**) and opsin expression in the GPe (**Figure 1C**) were included in the study. Immunohistochemical staining of tyrosine hydroxylase (TH) confirmed the complete loss of dopaminergic fibers in the striatum of lesioned mice (**Figure 1D**), and quantitative analysis of TH immunofluorescence in the substantia nigra pars compacta (SNc) revealed over 98% loss of TH positive cells in 6-OHDA/TAG and 6-OHDA/ChR2 mice compared to Sham/controls ($p \leq 0.005$ for both groups, Dunn's post-hoc test following a significant Kruskal-Wallis test, **Figures 1E** and **F**).

To reveal potential alterations of GPe activity, we selected an optical stimulation (532 nm, 5 Hz, 5 ms, 3 mW, 2 min application, **Figure 1G**) that was behaviorally ineffective in normal mice behavior based on pilot studies (data not shown). The illumination parameters triggered no contralateral circling (null scores) and produced no change in

spontaneous locomotor activity and forelimb use in non-lesioned control mice expressing the opsin (Sham/ChR2) or the fluorescent protein alone (Sham/TAG From **Supplementary Figure 1**). The two control groups were therefore pooled together in this study (Sham/controls, **Figure 1**). During the pre-stimulation period (first OFF, **Figure 1H**), 6-OHDA/TAG and 6-OHDA/ChR2 mice had a comparable ipsilateral circling scores that were significantly higher than those of Sham/controls ($p \leq 0.02$, non-parametric Dunn's post-hoc test). The photostimulation had no effect in 6-OHDA/TAG mice ($p < 0.0005$ for all ON periods vs Sham/controls, Dunn's post-hoc test), but it reduced ipsilateral circling in 6-OHDA/ChR2 mice ($p > 0.05$ vs Sham/controls, Dunn's post-hoc test, **Figure 1H**). The improvement immediately vanished when the optical stimulation was turned OFF. Analysis of cumulative circling scores over the OFF and ON periods revealed a significant main effect of lesion ($H(3) \leq 16.58$ for both conditions, $p < 0.0005$, Kruskal-Wallis test; **Figure 1I**) and post-hoc test confirmed a beneficial effect of the photostimulation only in 6-OHDA/ChR2 mice ($p > 0.05$ vs Sham/controls, Dunn's post-hoc test).

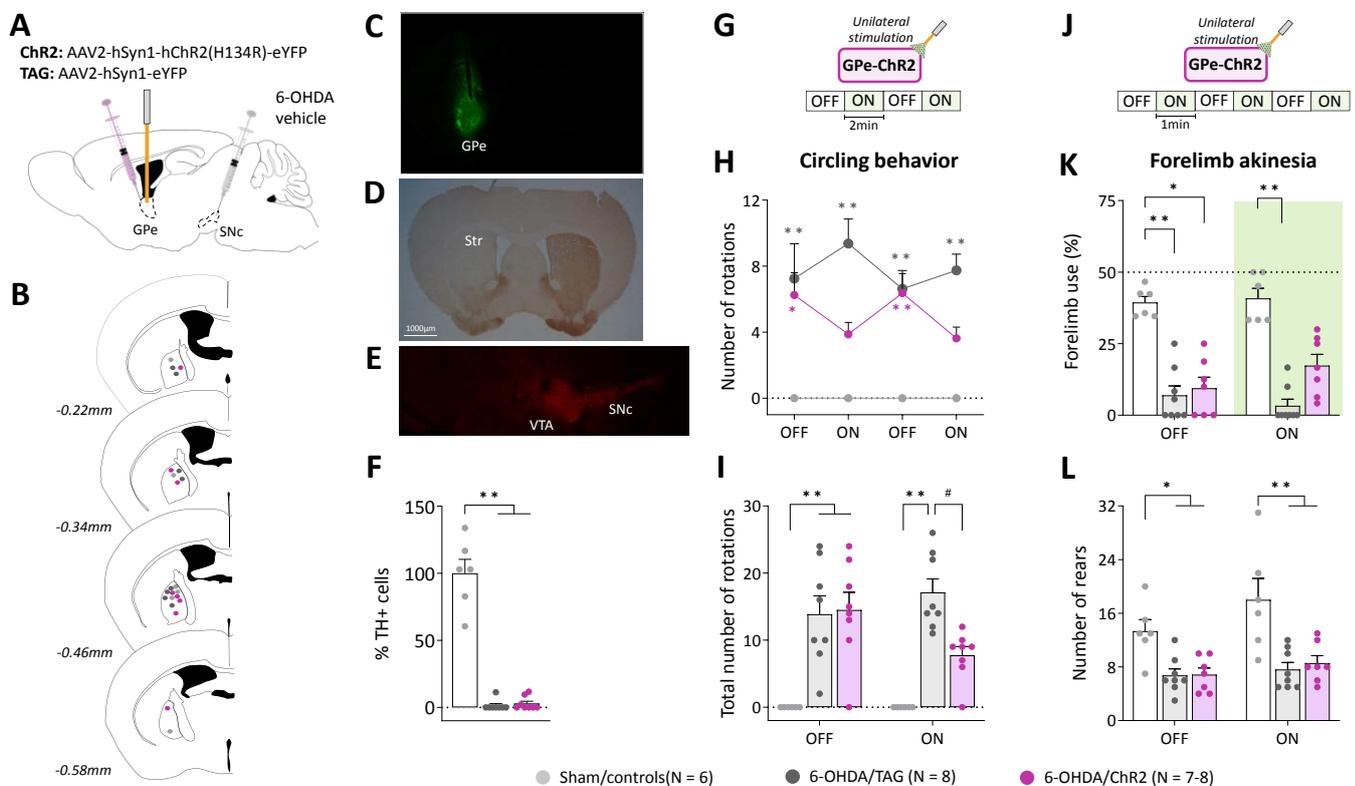


Figure 1: GPe photostimulation ameliorates circling behavior and forelimb akinesia in hemiparkinsonian mice. (A) Schematic illustration of the experimental procedure for opsin expression in GPe and nigrostriatal DA lesion. (B) Placement of individual optical fiber across the antero-posterior axis of the GPe. (C) Immunofluorescence image illustrating unilateral eYFP expression in GPe neurons. (D) DAB immunostaining for TH-positive fibers in the striatum of a lesioned mouse. (E) Immunostaining for TH+ cells in SNc of a lesioned mouse. (F) Percentage of TH+ cells loss in SNc in lesioned mice relative to Sham mice. (G) Photostimulation protocol used for the open field test. Testing was carried over 8 min with repeated sequences of light pulses stimulation (532 nm, 5 Hz, 5 ms, 3 mW) turned OFF and ON for 2 min. (H-I) Time course and total number of spontaneous ipsilateral rotations during OFF and ON periods, respectively. (J) Photostimulation protocol used for the cylinder test. Testing was carried over 6 min with repeated sequences of light pulses stimulation (532 nm, 5 Hz, 5 ms, 3 mW) turned ON and OFF for 1 min. (K) Percentage of contralateral-

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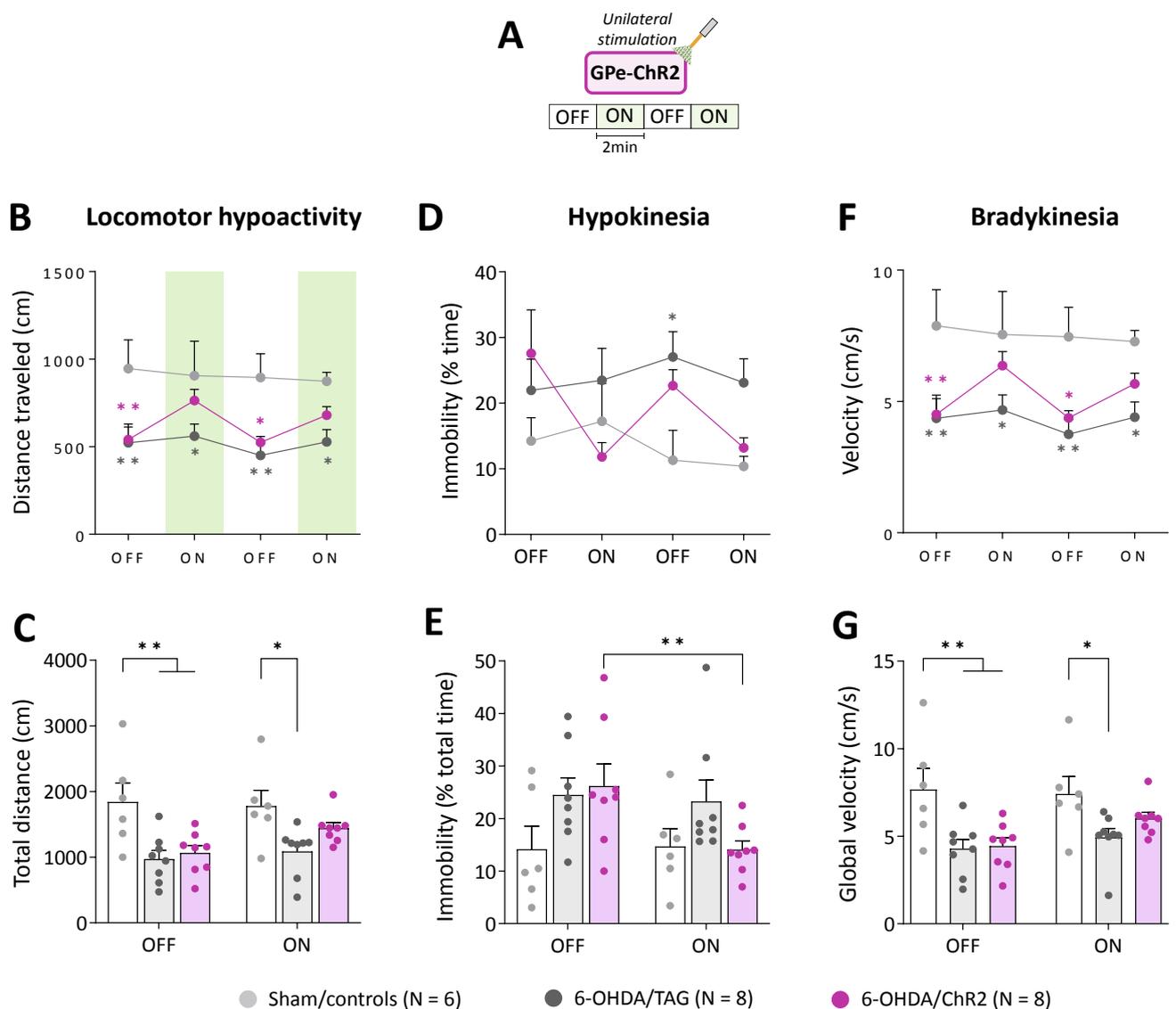
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127 teral forelimb use during OFF and ON periods relative to the total number of forepaw contacts. (L)
128 Total number of rears (forepaw contacts) during OFF and ON periods. Results are presented as
129 mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$ significantly different from Sham mice. # $p < 0.05$ significantly dif-
130 ferent from 6-OHDA/TAG mice, Dunn's test following a significant non-parametric Kruskal-Wallis
131 test for both circling behavior and forelimb use; Tukey's test following a significant two-way re-
132 peated measures ANOVA for rears.

133 We next verified whether GPe activation could restore contralateral forelimb
134 akinesia in the cylinder test. Forelimb use in the cylinder test is associated with postural
135 support when the mouse is up on its hind limbs and is a reliable index of akinesia in-
136 duced by dopaminergic depletion. **Figure 1K** illustrates the global score of contralateral
137 forelimb use in OFF and ON periods. A significant impairment was detected in all
138 lesioned groups during the OFF periods ($p \leq 0.01$ vs Sham/controls, Dunn's post-hoc test).
139 Application of the optical stimulation had no effect in 6-OHDA/TAG mice ($p < 0.001$,
140 Dunn's post-hoc test), but improved contralateral forelimb use deficit in 6-OHDA/ChR2
141 mice ($p > 0.05$, Dunn's post-hoc test). Total number of rearing was significantly reduced in
142 both lesioned groups (main effect of 6-OHDA lesion, $F_{(2, 18)} = 11.18$, $p < 0.001$, **Figure 1L**)
143 and GPe photostimulation was ineffective ($p < 0.05$ for both lesioned groups vs
144 Sham/controls, Tukey's post-hoc test, **Figure 1L**).

145 2.2. GPe photostimulation improves locomotor hypoactivity and bradykinesia in 6-OHDA- 146 lesioned mice

147 To further characterize the beneficial effects of GPe activation in lesioned mice we
148 analyzed various locomotor activity parameters. **Figure 2B** illustrates the time-course of
149 the distance traveled in the open field arena upon repeated application of the
150 photostimulation (532 nm, 5 Hz, 5 ms, 3 mW, **Figure 2A**). In the pre-stimulation period,
151 6-OHDA/TAG and 6-OHDA/ChR2 mice had a significantly lower baseline locomotor ac-
152 tivity levels than Sham/controls ($p < 0.01$ for both groups, Tukey's post-hoc test). GPe
153 photostimulation increased locomotor activity in 6-OHDA/ChR2 mice ($p > 0.05$ vs
154 Sham/controls, Tukey's post-hoc test) but not in 6-OHDA/TAG mice ($p < 0.05$ vs
155 Sham/controls, Tukey's post-hoc test). Repeated measures ANOVA performed on global
156 scores (**Figure 2C**) yielded a significant main effect of 6-OHDA lesion ($F_{(2, 19)} = 6.12$,
157 $p < 0.05$) and optical stimulation ($F_{(1, 19)} = 7.16$, $p < 0.01$). Post-hoc analysis confirmed that
158 GPe photostimulation had a beneficial effect only in 6-OHDA/ChR2 mice ($p > 0.05$ vs
159 Sham/controls, Tukey's post-hoc test).



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Figure 2: GPe photostimulation ameliorates locomotor activity measures in hemiparkinsonian mice. (A) Photostimulation protocol used for the open field test. Testing was carried over 8 min with repeated sequences of light pulses stimulation (532 nm, 5 Hz, 5 ms, 3 mW) turned OFF and ON for 2 min. (B-C) Time course and total distance traveled (cm) during OFF and ON periods, respectively. (D-E) Time course and total time spent in immobility (%) during OFF and ON periods, respectively. (F-G) Time course and global locomotor velocity during OFF and ON periods, respectively. Results are presented as mean \pm SEM. * $p < 0.05$ and ** $p < 0.01$ significantly different from Sham mice or within group, Tukey's test following a significant two-way repeated measures ANOVA.

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Hypokinesia assessed by the percentage of time spent in immobility (Figure 2D) was overall higher in lesioned groups compared to Sham/controls ($F_{(2, 19)} = 2.97$, $p < 0.05$, 6-OHDA lesion effect). Though, a significant difference between 6-OHDA/TAG mice and Sham controls was only detected at the 2nd OFF period ($p < 0.05$, Tukey's post-hoc test). The photostimulation reduced % of the time spent in immobility in 6-OHDA/ChR2 in a reversible manner ($F_{(6, 57)} = 2.44$, $p < 0.05$, 6-OHDA lesion \times photostimulation interaction, Figure 2D). Analysis of the global scores (Figure 2E) yielded a significant main effect of optical stimulation ($F_{(1, 19)} = 5.06$, $p < 0.05$) and a significant 6-OHDA lesion \times optical stimulation interaction ($F_{(2, 19)} = 4.54$, $p < 0.05$, Figure 2E), but post-hoc test failed to reveal a significant difference between groups ($p > 0.05$, Tukey's test). Further analysis performed

180 within groups confirmed that only 6-OHDA/ChR2 mice had a significantly lower scores
181 in the ON than the OFF period ($p < 0.05$, Student's t test, **Figure 2E**).

182 Lesioned mice also displayed a marked bradykinesia illustrated by a slower
183 locomotor compared to Sham controls ($F_{(2, 19)} = 6.12$, $p < 0.01$, 6-OHDA lesion effect, **Figure**
184 **2F**). The average velocity in the OFF periods was around 8 cm/s for Sham/controls and
185 below 5 cm/s for lesioned mice ($p \leq 0.04$, Tukey's post-hoc test). Application of the
186 photostimulation enhanced locomotor velocity in 6-OHDA/ChR2 ($F_{(3, 57)} = 2.26$, $p < 0.05$) at
187 all ON periods ($p > 0.05$ vs Sham/controls, Tukey's post-hoc test, **Figure 2F**). Analysis of
188 the global scores (**Figure 2G**) revealed a significant main effect of 6-OHDA lesion ($F_{(2, 19)} =$
189 5.27 , $p < 0.05$) and optical stimulation ($F_{(1, 19)} = 8.83$, $p < 0.01$) and post-hoc multiple compar-
190 isons confirmed an improvement of bradykinesia only in 6-OHDA/ChR2 ($p > 0.05$ vs
191 Sham/controls, Tukey's post-hoc test, **Figure 2G**).

192 2.3. GPe photoinhibition in normal mice does not mimic motor deficits of DA depletion

193 We next examined whether optogenetic inhibition of GPe in normal mice could
194 reproduce the typical motor deficits of unilateral 6-OHDA lesion. To do so, we used the
195 engineered blue-sensitive chloride-conducting channelrhodopsin, iC⁺⁺, which allows
196 more efficient optogenetic control than the hyperpolarization induced by light-activated
197 chloride pumps³⁸. iC⁺⁺ was expressed in GPe under the control of the hSyn1 promoter
198 (**Figure 3A**). Only mice with successful opsin expression (**Figure 3B**) and optic fiber
199 implantation within the GPe (**Figures 3C and D**) were included in the study. We first
200 examined whether unilateral GPe photoinhibition induces ipsilateral circling. Various
201 photoinhibition protocols were used with pulsed and continuous illuminations (**Figure**
202 **3E**) at different frequencies (20-60 Hz) and intensities (5-12 mW) based on published
203 studies^{38,39}. Only a tendency to circle toward the illumination side (few incomplete
204 ipsilateral rotations) was detected in iC⁺⁺ mice. From **Figures 3F and G**, it can be seen
205 that continuous photoinhibition with the highest illumination intensity (12 mW)
206 produces no notable circling behavior ($p > 0.05$, Mann-Whitney test). Analysis of
207 locomotor activity measures revealed no changes in distance traveled ($p > 0.05$, repeated
208 measures ANOVA, **Supplementary Figures 2B and C**), % of the time spent in
209 immobility ($p > 0.05$, repeated measures ANOVA, **Supplementary Figures 2D and E**) and
210 velocity ($p > 0.05$, repeated measures ANOVA, **Supplementary Figures 2F and G**). In the
211 cylinder test, the unilateral photoinhibition (12 mW, **Figure 3H**) produced no impair-
212 ment in contralateral forelimb use ($p > 0.05$, Student's t-test, **Figures 3I**) and rearing
213 behavior ($p > 0.05$ vs TAG mice, Student's t-test, **Figures 3J**).

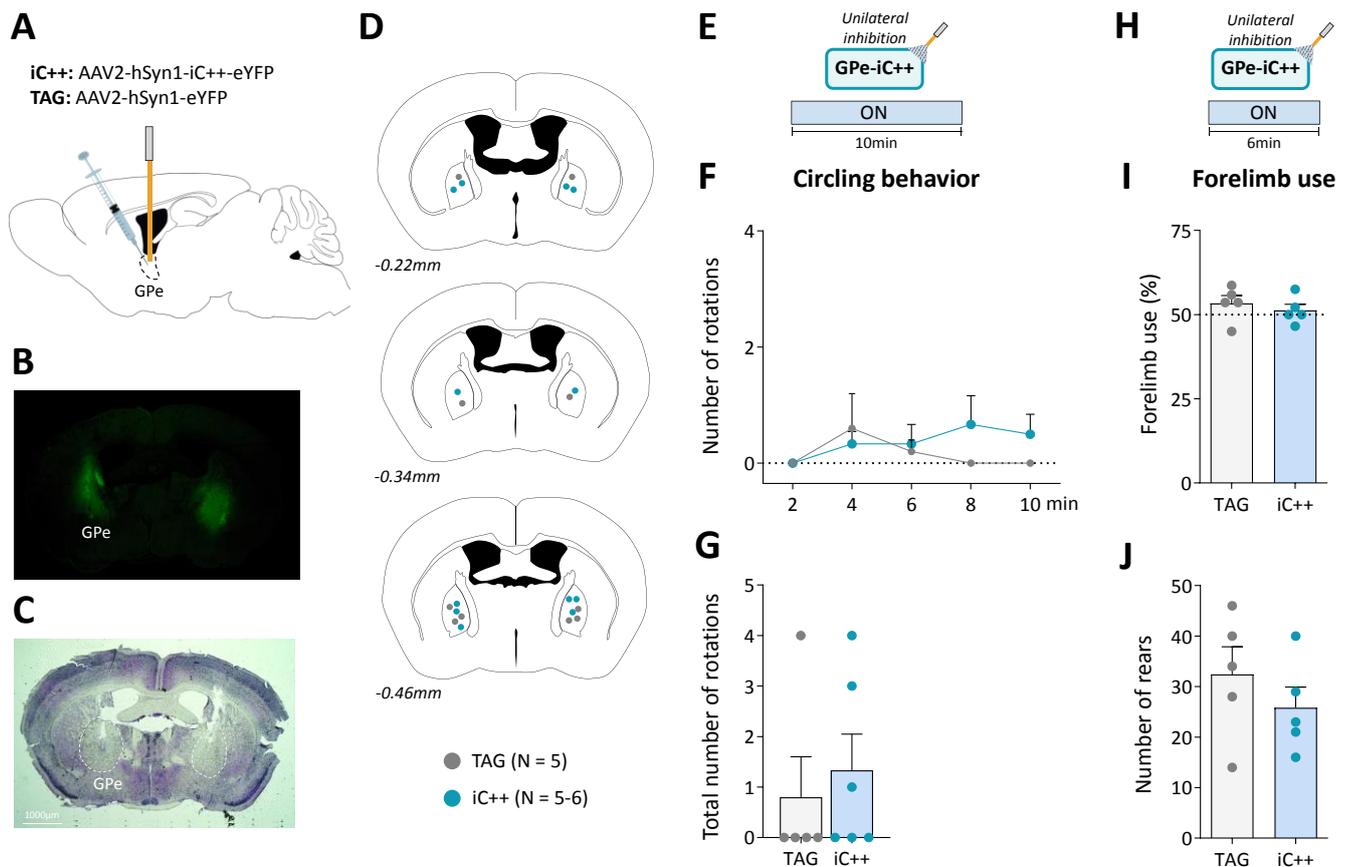


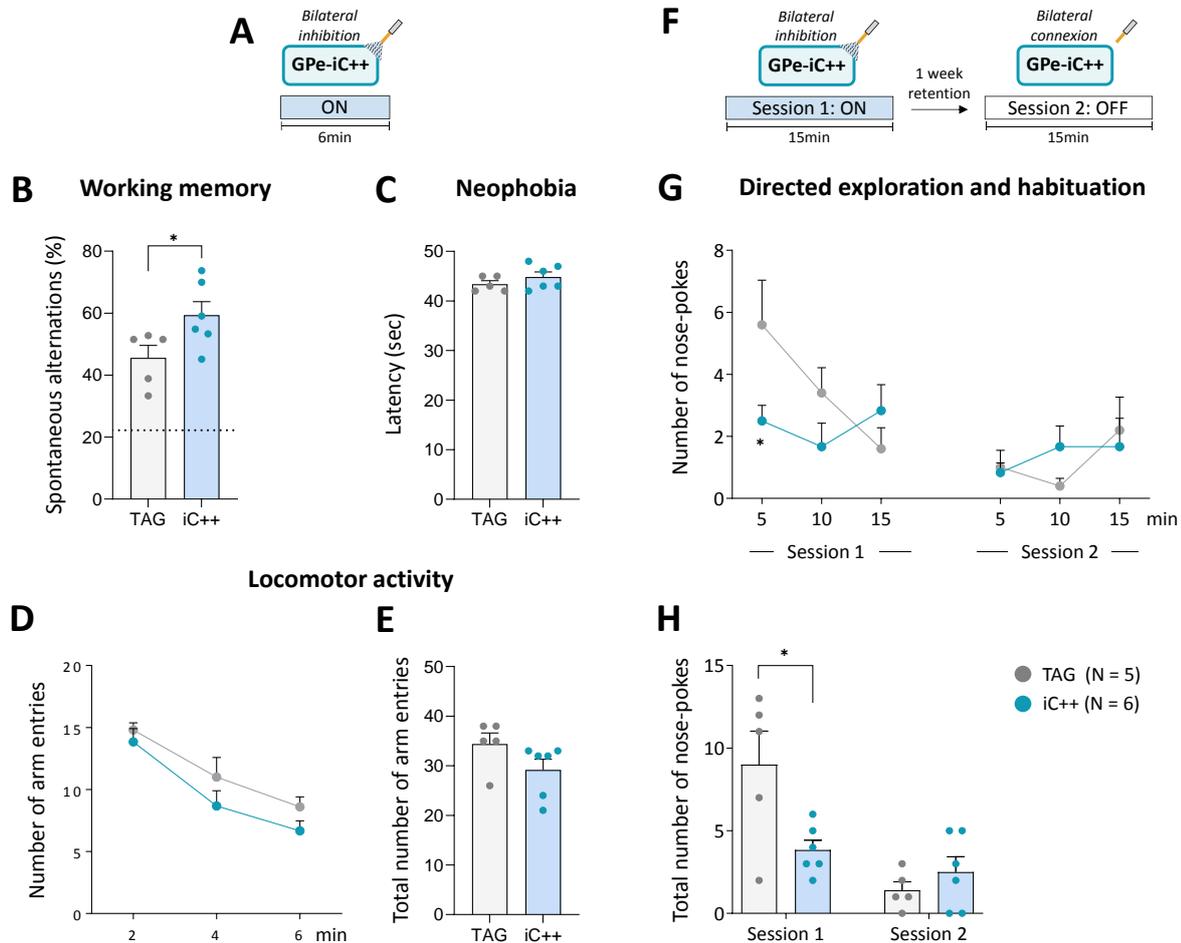
Figure 3: Unilateral GPe photoinhibition does not reproduce motor deficits. (A) Schematic illustration of the experimental procedure for opsin expression in GPe. (B) Immunofluorescence image illustrating bilateral viral expression in GPe neurons. (C) Cresyl violet staining showing bilateral fiber optic placement in the GPe. (D) Placement of individual optical fiber across antero-posterior GPe axis. (E) Photoinhibition protocol used in the open field test. Testing was carried over 10 min with continuous light illumination (450 nm, 12 mW). (F) Time course and total number of ipsilateral rotations over 10 min of testing, respectively. (G) Photoinhibition protocol used in the cylinder test. Testing was carried over 6 min with continuous light illumination (450 nm, 12 mW). (I) Percentage of contralateral forelimb use relative to the total number of forepaw contacts. (J) Total number of rears (forepaw contacts). Results are presented as mean \pm SEM. $p > 0.05$, Mann-Whitney test or unpaired t-test following a significant non-parametric Kruskal-Wallis test for circling behavior and forelimb akinesia, respectively.

2.4. GPe photoinhibition improves cognitive functions in normal mice

The above findings indicate that GPe may not contribute to modulation of spontaneous locomotor behavior in physiological conditions. GPe establishes direct anatomical connections with the cortex, thalamus, and amygdala^{19,40–43} suggesting that it may participate to modulation of motivational and cognitive aspects of motor behavior. To explore this possibility, we examined whether bilateral GPe photoinhibition (6 mW/side) could impact short-term spatial working memory performance assessed by spontaneous alternations in the Y-maze task (Figures 4A and B). The latency to exit the starting arm and the number of arm entries were used as an index of neophobia and general locomotor activity, respectively. TAG and iC++ mice, displayed good spontaneous alternation per-

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performances that were above chance level (calculated at 22.2%, $p < 0.05$, One-group test **Figure 4B**). Interestingly, iC++ mice had a better score than TAG mice ($p < 0.05$, Student's t-test) indicating that the photoinhibition promotes short-term working memory. The photoinhibition did not change the latency to exit the starting arm ($p < 0.05$ vs TAG mice, Student's t-test, **Figure 4C**) or the number of arm entries ($p < 0.05$ vs TAG mice, Student's t-test, **Figures 4D and E**).



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Figure 4: Bilateral GPe photoinhibition impacts working memory and directed exploration. (A) Photoinhibition protocol used in the Y-maze test. Testing was carried over 6 min with continuous light stimulation (450 nm, 6 mW/side). (B) Percentage of spontaneous alternations. The dashed line shows a chance level of 22.2%. (C) Latency to exit the starting arm (sec). (D-E) Time course and total number of arm entries in the Y-maze over 6 min of testing, respectively. (F) Photoinhibition protocol used in the nose-poke habituation test. Two 15-min of testing were carried one week apart, session 1 with continuous light illumination (450 nm, 6 mW/side) and session 2 with light OFF. (G-H) Time course and total number of nose-pokes per session. Results are presented as mean \pm SEM. * $p < 0.05$, unpaired t-test for Y-maze test and unpaired t-test following a significant repeated measures ANOVA for nose-poke exploration.

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The effect of the photoinhibition was also studied on the long-term habituation to spatial cues, a simple form of non-associative learning, using one week of retention delay. Testing was conducted over 15 min in operant chambers equipped with illuminated nose-poke ports placed on the front wall. We previously demonstrated that nose-poke ports can be employed as spatial cues for evaluating directed exploration and learning performances in mice^{44–46}. Mice received bilateral optical inhibition (6 mW/side) solely on the first session (**Figure 4F**). TAG mice displayed the expected exploration profile (**Fig-**

262 **ure 4G**). On session 1, they initially displayed a high number of nose-poking that de-
263 clined over the course of the session and then remained at the same low level on the se-
264 cond testing session, reflecting respectively short-term (within-session) and long-term
265 (between-session) habituation phenomenon ($F_{(9, 81)} = 5.062$, $p < 0.0001$, main effect across
266 days). iC++ mice had a remarkably low exploration level on session 1 compared to TAG
267 mice ($F_{(9, 81)} = 2.574$, $p < 0.05$, sessions \times optical inhibition interaction) and a significant dif-
268 ference between groups was detected in the first block ($p < 0.05$, Student's t-test). On ses-
269 sion 2, they displayed a normal habituation performance illustrated by a low exploration
270 level comparable to TAG mice ($p > 0.05$, Student's t-test for all blocks). Analysis of the
271 global scores confirmed that iC++ mice had a significantly lower number of nose-poking
272 than TAG mice only in session 1 ($p < 0.05$, Student's t-test, **Figure 4H**) indicating that they
273 were able to efficiently process and retain novel spatial information despite their low
274 exploration level.

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276 3. Discussion

277 The present study shows that global GPe optogenetic activation is effective at re-
278 ducing motor impairments in hemi-parkinsonian mice. As expected, unilateral SNc le-
279 sion produced the typical motor deficits in mice manifested by ipsilateral circling,
280 locomotor hypoactivity, bradykinesia, and forelimb akinesia. Global GPe optogenetic ac-
281 tivation improved the various motor abnormalities. The beneficial effects were reversible
282 and only seen in 6-OHDA-lesioned mice expressing the excitatory opsin, confirming the
283 selectivity of the optogenetic neuromodulation. The improvement of locomotor
284 hypoactivity may be due to restoration of ipsilateral circling, and thus the balance in the
285 motor circuit, which is essential to walk in a straight line and to travel over long distanc-
286 es in the arena. It may also be attributed to restoration of the exploration drive as dopa-
287 minergic lesions are well known to reduce novelty-induced exploration. The ameliora-
288 tion of hypokinesia and bradykinesia produced by GPe photostimulation is consistent
289 with this conclusion. The photostimulation was also efficient at improving akinesia as-
290 sessed by contralateral forelimb use in the cylinder test. Overall, our findings extend
291 those reported in the literature using pharmacological and chemogenetic approaches²⁹⁻
292 ³¹. However, they contrast with those of Mastro et al.³² showing that cell-specific rather
293 than global GPe optogenetic manipulations are effective in restoring motor deficits in
294 MFB-lesioned mice, a PD model of severe DA deficiency. The discrepancy between the
295 two studies may be explained by the differences in photostimulation protocols and PD
296 mouse models used. The unilateral intranigral lesion model we used produces a less se-
297 vere DA depletion and motor impairments than the bilateral MFB lesion that targets all
298 midbrain DA neurons. It is therefore possible that global GPe photostimulation may
299 more readily alleviate motor impairments induced by unilateral SNc than bilateral MFB
300 lesion. It should be stressed that in Mastro et al. study the behavioral effects of GPe
301 photostimulation were assessed 3-5 days post-lesion while in our study they were eval-
302 uated at a much later time point (5-6 weeks post-lesion). Such a difference in post-lesion
303 testing time may be another possible source of the discrepant results considering that BG
304 circuitry undergoes major functional changes several weeks after DA depletion³. An in-
305 teresting study will be to assess the beneficial effects of global GPe photostimulations in
306 hemi-parkinsonian mice at different time-points post-lesion.

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308 Numerous electrophysiological studies demonstrated a reduction of GPe neuronal
309 firings in animal models and PD patients^{3,24,26,28}. GPe hypoactivity has been attributed to
310 an abnormal elevation of extracellular GABA concentrations caused by the overactivity
311 of the striato-pallidal pathway^{23,25,47} and to local changes at the level of pallidal
312 GABAergic synapses^{48,49}. Our study provides new behavioral evidence that GPe activity
313 is altered upon DA depletion. We show that an ineffective optical stimulation in normal

314 mice is sufficient for improving many aspect of motor impairments caused by DA lesion.
315 Here, we selected low optical stimulation parameters to reveal possible functional altera-
316 tions of the GPe, and we do expect that a full restoration of the motor deficits can be
317 achieved using higher illumination frequencies and/or intensities. The greater sensitivity
318 of hemi-parkinsonian mice to behavioral effects of the photostimulation may also reflect
319 a functional hypersensitivity of GPe GABAergic neurons that develops following DA le-
320 sion. For instance, the number of GABAergic synapses and the expression of GABA_A re-
321 ceptors are increased in the STN upon DA depletion leading to a strengthening of the
322 functional connectivity between GPe and STN^{50–52}. Hence, the motor improvement seen
323 in lesioned mice may be attributed to restoration of the inhibitory drive of GPe prototyp-
324 ic neurons expressing parvalbumine (PV-neurons), which are the main source of GPe
325 inputs to STN and BG output structures (substantia nigra pars reticulata and
326 entopeduncular nucleus homologous to primates globus pallidus interna)⁴. Selective
327 optogenetic stimulations of PV-neurons was consistently shown to alleviate motor defi-
328 cits in mouse models of PD^{32,53,54}. Interestingly, we found that a behaviorally ineffective
329 optical stimulation in normal mice restores to normal level the motor deficits in lesioned
330 mice⁵⁴. It is worth noting that not all GPe neurons promote motor behaviors.
331 Optogenetic silencing of prototypic neurons expressing Lim homeobox 6 (Lhx6-neurons)
332 improves motor deficits in MFB-lesioned mice indicating that this neuronal subpopula-
333 tion plays a deleterious role in the context of DA depletion³². Optical stimulations of
334 arky pallidal neurons, namely FoxP2-expressing neurons that exclusively innervate the
335 striatum, were also found to suppress locomotion in normal mice^{21,55}. The fact that global
336 GPe photostimulation produces a beneficial effect in hemi-parkinsonian mice implies
337 that the recruitment of PV-neurons overrides deleterious influences of other GPe neu-
338 ronal populations that suppress motor behavior, an action that may be exerted through a
339 local collateral inhibition and suppression of downstream BG nuclei as recently demon-
340 strated²¹.

342 The series of experiments conducted in normal mice show that mere reduction of
343 GPe activity did not produce an impairment of spontaneous locomotor behavior and
344 forelimb use, like DA lesion did. It is unlikely that the illumination parameters used are
345 insufficient for silencing GPe neurons. We tested a range of photoinhibition protocols
346 that were previously demonstrated to be effective for silencing neurons and triggering
347 behavioral responses in normal mice^{39,56}. Our results are in a good agreement with a re-
348 cent study of Isett et al.³⁶ demonstrating a little effect of optogenetic inhibitions of GPe
349 and striatal indirect pathway on locomotor activity in mice. They also extend those re-
350 ported in rats and monkeys showing that neurotoxic lesions of GPe do not reproduce
351 changes in BG discharge patterns and motor dysfunction observed in DA-depleted
352 states^{28,57}. Collectively, these findings suggest that GPe contribution to gross motor func-
353 tion may not be critical in physiological conditions. Though, in a parkinsonian patient, a
354 lesion confined to the GPe exacerbates akinesia⁵⁸. GPe ablation was also reported to
355 worsen motor deficits in parkinsonian monkeys and to abolish the improvement in-
356 duced by the dopaminergic receptor agonist, apomorphine⁵⁹. It thus appears that in DA-
357 depleted state, GPe may play a more important role in the development of motor defi-
358 cits. In PD, the degeneration of DA neurons also leads to a hypoactivity of the direct
359 striatal pathway that promotes motor behaviors. It is therefore possible that the deleteri-
360 ous impact of reduced GPe activity on motor function may be exacerbated when direct
361 striatal pathway transmission is concomitantly reduced. Another possibility could be
362 that the inhibitory drive of some GPe GABAergic neurons that suppress motor behavior
363 is enhanced upon DA depletion. As mentioned above, optical inhibition of GPe Lhx6-
364 neurons improves motor deficits in MFB-lesioned mice³². On the other hand, striatal in-
365 puts of Npas1⁺-expressing neurons, which suppress locomotor behavior, have been
366 shown to be strengthened upon DA lesion⁵⁵. Finally, it should be noted that motor
367 symptoms of PD were linked not only to changes in local firing rates but also to an ab-

368 normal rhythmic activity in BG and cortex manifested by an enhanced oscillations in the
369 beta frequency range (13–30 Hz)^{60,61}. Pathological beta-oscillations are thought to com-
370 promise information flow and processing in the cortico-basal ganglia (CBC) network,
371 thereby leading to the impairment of motor functions. Recent preclinical studies showed
372 that GPe plays a central role in this context by promoting the expression and propaga-
373 tion of abnormal beta-oscillations throughout the CBG network^{62–66}. A such capability of
374 orchestrating changes in the pattern of activity across the CBG network may thus
375 emerge as a consequence of the compensatory alterations of GPe inhibitory drive onto
376 BG nuclei and thalamus following DA neuron loss^{19,50,63}. The contrasting effects of
377 optogenetic GPe modulations on motor functions in normal and hemi-parkinsonian
378 mice is in line with this conclusion.

380 An important finding was the improvement of cognitive performances produced by
381 GPe inactivation in normal mice. In the Y-maze task, the photoinhibition induced a spe-
382 cific improvement of short-term working memory that was manifested by an increase of
383 spontaneous alternations without any changes in locomotor exploration and emotionali-
384 ty. In the nose-poke habituation task, the promnesic effect was a less clear-cut because
385 the photoinhibition reduced nose-poking behavior. Despite the exploration deficit, iC++
386 mice displayed a normal long-term habituation performance like control mice, which al-
387 so points to a cognitive facilitation. The reduction of nose-poking behavior induced by
388 the photoinhibition contrasts with the null effects found in the open field and the Y-
389 maze tests. In both cases, higher illumination intensities (8–12 vs 6 mW) produced no
390 locomotor dysfunction in mice, supporting the view that directed exploration and
391 locomotor activity are subserved by distinct neural substrates^{44–46}. The low level of nose-
392 poke exploration displayed by iC++ mice on session 1 may also be indicative of a
393 promnesic action of the photoinhibition. An enhanced ability to process and retain spa-
394 tial information should favor a rapid familiarization of mice with surrounding salient
395 spatial cues and thus lead to a reduced investigatory activity. A such cognitive facilita-
396 tion can explain the good working memory performance of iC++ mice revealed in the Y-
397 maze task, which also requires spatial processing. The precise mechanisms underlying
398 the inhibitory influence of GPe on cognitive processing remains to be delineated. GPe
399 contains a diversity of GABAergic neurons that sends widespread projections within
400 and outside BG and could each influence specific aspects of motor and cognitive behav-
401 iors. In support of this idea, it was recently shown that PV-neurons projecting to the SNr
402 promote motor behavior while those innervating the parafascicular thalamus negatively
403 modulate behavioral flexibility in mice¹⁹. It is therefore possible that the promnesic ef-
404 fects produced by the photoinhibition may in part be attributed to an inhibition of GPe
405 inputs to the parafascicular thalamus. It may also be linked to a suppression of the GPe
406 inhibitory drive onto the frontal cortex⁴⁰ that subserves executive functions. Future stud-
407 ies are necessary to elucidate the specific contribution of the GPe pathways to the differ-
408 ent facets of cognitive behaviors.

410 In conclusion, our findings provide a new insight into the functional role of GPe in
411 health and disease state. They suggest that GPe modulation of gross motor function may
412 not be critical in physiological conditions as previously thought. This BG nucleus may
413 rather participate to specific aspects of motor and cognitive behaviors. In PD condition,
414 the GPe may however play a central role in the expression of a range motor signs of PD
415 because of the complex functional compensatory remodeling affecting BG circuits upon
416 DA depletion.

418 4. Materials and Methods

419 4.1. Subjects

420 Eight weeks old C57BL/6J (BL6) male mice were purchased from Charles River La-
421 boratories (Saint Germain Nuelles, France). Mice were housed in groups of 3-4 in indi-
422 vidually ventilated cages (Techniplast, Grostenquin, France) and kept in 12 h light/dark
423 cycle (light on at 07:00am, off at 07:00pm) with water and food ad lib. Behavioral testing
424 was performed during the light cycle between 07:00am and 07:00pm. All experimental
425 procedures were approved by the French national ethics committee (CE071) and con-
426 ducted in accordance with EEC (2010/63/UE) guidelines for care and use of laboratory
427 animals.

428 4.2. Viral constructions

429 For global optical stimulation of GPe neurons the excitatory channelrhodopsin 2
430 variant (ChR2(H134)) fused to a yellow fluorescent protein (eYFP) was expressed under
431 the hSYN1 promoter using an adeno-associated virus serotype 2 vector (AAV2-hSYN1-
432 ChR2(H134R)-eYFP, 5.6×10^{12} particles/ml). For optical inhibition we used the AAV2
433 vector encoding the engineered chloride channel, IC⁺⁺, fused to eYFP under the hSYN1
434 promoter (AAV2-hSYN1-IC⁺⁺-eYFP, 4.1×10^{12} particles/ml). AAV2 encoding the eYFP
435 reporter protein only (AAV2-hSyn1-eYFP, 2×10^{12} particles/ml) was used as a control.
436 All viral vectors were obtained from Vector Core (University of North Carolina, Chapel
437 Hill, USA and MTA from Karl Deisseroth at Stanford University).

438 4.3. Stereotaxic surgery

439 4.3.1. Unilateral 6-OHDA lesion

440 Mice were pre-treated subcutaneously with buprenorphine (0.05mg/kg) for analge-
441 sia, then deeply anesthetized with isoflurane (4% induction then 1-2%) and placed in a
442 stereotaxic frame (David Kopf instruments) on a heated pad and OcryGel was used to
443 protect from dry eyes. Lidocaine (6mg/kg) was injected subcutaneously before incising
444 the scalp. Mice received 1.5 μ l infusion of 6-OHDA hydrobromide (2.7 μ g/ μ l, free base,
445 Tocris) either into the left or right SNc over 5 min at the following coordinates: AP: 3
446 mm, ML \pm 1.3mm and DV -4.7 mm, according to the atlas of Paxinos and Watson, (2001).
447 The injection side was randomized between experimental groups. The microinjector was
448 slowly removed 5 min after the end of the injection to minimize backflow. Control mice
449 (Sham) received an injection of the corresponding volume of vehicle (0.02% ascorbic acid
450 in NaCl) in the same conditions.

451 4.3.2. Viral infusion and fiber optic implantation

452 This procedure was performed either immediately after 6-OHDA lesion for study-
453 ing the effects of GPe optostimulation in PD mice or in separate surgery session in nor-
454 mal mice for assessing behavioral effects of optogenetic manipulations of GPe activity in
455 a baseline condition. For GPe photostimulation in PD mice or in non-lesioned control
456 mice (Sham), 300 nl of AAV2-hSyn1-ChR2(H134R)-eYFP or AAV2-hSyn1-eYFP was uni-
457 laterally infused into the GPe over 6 min (AP: -0.3 mm, ML: \pm 2.0 mm and DV: -3.8 mm
458 from the skull surface, according to the atlas of Paxinos and Watson, 2001). The
459 microinjector was slowly removed 6 min after the end of the injection. Following viral
460 injection, an optical fiber-ferrule (optical fiber 200 μ m diameter, 0.22 N.A., Doric lenses,
461 Quebec, Canada) was unilaterally implanted 0.2 mm above the injection site and ce-
462 mented to the skull with Super-Bond C&B. For GPe photoinhibition in a normal condi-
463 tion, normal mice received bilateral infusion of 300 nl of AAV2-hSyn1-iC⁺⁺-eYFP or
464 AAV2-hSyn1-eYFP in GPe over 6 min at the same stereotaxic coordinates as above. Op-
465 tical fiber-ferrules were bilaterally implanted 0.2 mm above the injection site and ce-
466 mented to the skull with Super-Bond C&B.

467 Animals received a subcutaneous injection of carprofen (3mg/kg, diluted in NaCl
468 0.9% and glucose 5%) immediately after surgery and then for 2 consecutive post-
469 operative days. They were kept for 2-3 weeks in the post-operative room and their
470 weight monitored daily. Lesioned mice received supplemental food (diet gel, Nutella,
471 chocolate puffed cereals) and additional NaCl 0.9% injections if needed until complete
472 recovery.

473 474 4.4. Optogenetic manipulations

475 Mice were connected through an optical patch cable (Doric lenses, Quebec, Canada)
476 coupled either to a green DPSS laser (532 nm, LaserGlow Technologies, Toronto, Cana-
477 da) or a blue LD Fiber Light Source (450 nm, LDFS, Doric Lenses, Quebec, Canada) via a
478 rotary joint (Doric Lenses, Quebec, Canada). The light pulses were triggered by a pro-
479 grammable TTL Pulse Train Generators (custom made). The optical light power was cal-
480 ibrated before behavioral testing by measuring the output power at the tip of an optical
481 fiber-ferrule using a light power meter (PM100D, Thorlabs, France).

482 4.5. Behavioral Procedures

483 Behavioral testing was carried 5-6 weeks after the surgery. All experiments were
484 performed on 20-22 weeks old mice. Mice were handled and habituated to the optical
485 connection few days before behavioral testing. Pilot tests were carried in a viewing jar
486 (20 cm diameter and 30 cm height Plexiglas cylinder) to establish the adequate optical
487 stimulation parameters (light pulse frequency, duration, and intensity) before behavioral
488 testing. For optogenetic studies in 6-OHDA-lesioned mice, a green wavelength light
489 (DPSS laser) was used for photostimulation because the delivery of the blue LDFS was
490 delayed due to the Covid19 pandemic, and pilot tests carried in the viewing jar showed
491 that it is effective in normal mice (**Figure S3** for behavioral comparison conducted af-
492 terwards with green DPSS laser and blue LDFS, see also⁵⁶). For GPe photostimulation
493 study, mice were first submitted to the open field test for assessing circling behavior and
494 changes in spontaneous locomotor activity then the cylinder test for assessing forelimb
495 use impairment and postural asymmetry. For GPe photoinhibition study, mice were
496 tested in the following order: open field test, then the nose-poke habituation test session
497 1, Y-maze test, nose-poke habituation test session 2 and the cylinder test.

498 4.5.1. Cylinder test

499 Mice were placed in a small Plexiglas cylinder (10 cm diameter, 20 cm height) and
500 forepaw contacts performed against the wall during vertical exploration (rearing) were
501 manually scored using an ethological keyboard (LabWatcher, ViewPoint, France). For
502 studying the effects of GPe optostimulation in PD mice testing was conducted as follow:
503 after 30 sec of familiarization, mice received light pulses for 1 min (ON period) followed
504 by no stimulation for 1 min (OFF period), repeated three times. For GPe photoinhibition
505 in normal mice, the illumination was applied during the whole testing session (6 min).
506 Mice failing to reach 3 contacts during the 3 min OFF or ON period were excluded from
507 the analyses. One mouse from the 6-OHDA/ChR2 group and one from the iC++ group
508 were excluded. Data are expressed as a percentage of contralateral forepaw contacts re-
509 lative to the total number of contacts.

510 4.5.2. Open field test

511 Testing was carried in a white PVC arena (50 x 50 x 45 cm) and spontaneous
512 locomotor activity was recorded by a video-tracking system (ViewPoint, France). Each
513 mouse was individually placed facing a corner of the arena and allowed to freely ex-
514 plore the apparatus for 8 min. For GPe photostimulation in PD mice, light pulses were
515 delivered as follows: 2-min OFF periods (baseline locomotor activity level) and 2-min
516 ON periods, repeated twice to assess whether the behavioral effect of the
517 photostimulation was reversible. For GPe photoinhibition in normal mice, a repeated 2-
518 min and continuous illumination protocols were used. In each period, distance traveled,
519 velocity, % of time spent in immobility (movement threshold < 1cm/s) were analyzed.
520 Circling behaviors was hand-scored in parallel.

521 4.5.3. Y-maze test

522 Mice were placed in a Y-maze made of grey Plexiglas with 3 identical arms (40 x 9
523 x 16 cm) placed at 120° from each other. Each arm had walls with specific motifs allow-
524 ing to distinguish it from the others. Mice freely explored the maze for 6 min with light
525 ON. Spontaneous alternation performance was assessed by visually recording for each
526 mouse the pattern of entrance into each arm in the maze. Alternations were operational-
527 ly defined as successive entries into each of the three arms as on overlapping triplet sets.
528 Percent spontaneous alternation performance was defined as the ratio of actual (total al-
529 ternations) to possible alternations (total arm entries - 2) x 100. Total entries were scored
530 as an index of exploratory activity in the maze. Latency to exit the starting arm was used
531 to assess neophobia.

532 4.5.4. Nose-poke habituation test

533 Mice were tested in five-choice operant chambers (Coulbourn Instruments, Allen-
534 town, United State) dimly lit with a permanent house-light. The front panel was curved
535 and composed of five bays filled with metal wall panels interchangeable by nose-poke
536 modules (Model H21-10M). Each nose-poke hole was equipped with a controlled yellow
537 LED cue light at the end and infrared photobeam across the opening that detects the
538 nose-pokes. Two adjacent nose-poke units (spaced 4 cm apart) were presented in the
539 right corner and two others in the left corner of the front wall (no nose-poke unit in cen-
540 tral bay). The back wall was composed of a single bay fitted with metal panels and the
541 plexiglass side walls were completely covered by cardboard with distinguishable geo-
542 metrical motifs. The metal stainless-steel rod floor (the grid shock floor provided by the
543 manufacturer) was covered by a grey vinyl-coated paper that was used as the standard
544 flooring throughout the study. Testing consisted of 15 min long sessions with nose-poke
545 holes illuminated. The bilateral photoinhibition (continuous illumination) was applied
546 only the first day. One week retention delay was used to assess the impact of GPe inhibi-
547 tion on long-term habituation performances of mice. The number of nose poking was
548 monitored and used as an index of directed exploration and habituation capacity to sali-
549 ent spatial cues.

550 4.6. Immunofluorescence and histological analysis

551 4.6.1. Analysis of 6-OHDA-induced dopamine cell loss

552 Mice were perfused transcardially with 4% paraformaldehyde and the brains were
553 removed, postfixed, and cryoprotected. Free floating coronal sections (40 µm thick) con-
554 taining the SNc were incubated with rabbit anti-TH antibody (1/1000, Synaptic Systems,
555 213 102) overnight at 4°C followed by a 2h incubation with the Alexa Fluor 594 goat anti-
556 rabbit antibody (1/400, Jackson Laboratory, 111-585-003) at room temperature (RT). Sec-
557 tions were mounted onto SuperFrost Plus glass slides (VWR) and coverslipped with Ro-

558 ti®-Mount FluorCare mounting medium (Carl Roth). Immunofluorescence was ana-
559 lyzed by a laser confocal microscopy (Zeiss LM710, Germany) at a 20 X magnification.
560 TH positive cells were counted through the SNc region based on the stereotaxic mouse
561 atlas (Paxinos & Franklin, 2001) delineating the SNc from the ventral tegmental area by
562 the medial optic tract. Counting was done manually in five regions of interest (ROIs) per
563 hemisphere, covering the whole medio-lateral extension of the structure (-2.70 to -3.80
564 mm relative to bregma, 3 sections per mouse), using cell counter plugin of FIJI software
565 (ImageJ, National Institutes of Health).

566 DA depletion in the striatum was also examined. Coronal sections (40µm) were inc-
567 cubated overnight at 4 °C with a mouse anti-TH antibody (1/1000, Millipore, MAB318).
568 Thereafter, they were incubated with a biotinylated secondary antibody (goat anti-
569 mouse, 1/200; Jackson Immunoresearch; 115-065-003) for 1 hour and then in a solution
570 containing 0.01% DAB (3,3-diaminobenzidine) and 3% H₂O₂ diluted in PBS 1X for 3 min.
571 Sections were finally mounted on slides as described before. Images were acquired using
572 a bright field microscope (Nikon Leica DMLB) at 10X magnification.

573 4.6.2. Analysis of opsins expression

574 Free floating coronal sections (40 µm thick) containing the GPe were incubated a
575 48h with a rabbit anti-GFP antibody (1/500, Invitrogen, A-11122) at 4°C then 1h30 with
576 Alexa Fluor 488 donkey anti-rabbit antibody (1/500, Invitrogen, R37118) at RT. Sections
577 were mounted onto SuperFrost Plus glass slides (VWR) and coverslipped with Roti®-
578 Mount FluorCare mounting medium (Carl Roth). Immunofluorescence was analyzed by
579 a laser confocal microscopy (Zeiss LM710, Germany) at 10 X magnification.

580 4.6.3. Verification of optic fiber-ferrule placement:

581 For confirming the placement of the optical fiber in the GPe, coronal sections of the
582 GPe were stained with cresyl violet. Images were acquired using Leica DM LB Micro-
583 scope at 2.5 X magnification equipped with Nikon digital camera DXM1200. Mice with
584 the optical fiber-ferrule placements outside of the GPe were excluded from analysis.

585 4.7. Statistical analysis

586 All data are expressed as mean group value ± standard error of the mean (s.e.m.).
587 Analysis of forelimb akinesia and rears data in the cylinder test were carried out using a
588 non-parametric Kruskal-Wallis test followed by Dunn's post-hoc test and a two-way
589 repeated measures ANOVA followed by unpaired t-test, respectively. Analysis of cir-
590 cling behavior was performed using Kruskal-Wallis test followed by Dunn's post-hoc
591 test or using an Mann-Whitney test. Locomotor activity data in the open field test were
592 analyzed using two-way ANOVA with repeated measures (OFF and ON periods or
593 time). When relevant, post-hoc comparisons were carried using Tukey's multiple com-
594 parisons tests or t-tests, respectively. The accepted level of significance was p<0.05.

596 **Supplementary Materials:** The following supporting information can be downloaded at:
597 www.mdpi.com/xxx/s1,

598 Figure S1: Behavioral effect of GPe photostimulation in Sham controls groups

599 Figure S2: Unilateral GPe photoinhibition has no impact on locomotor activity measures in normal
600 mice;

601 Figure S3: Similar behavioral effects of GPe photostimulation with green or blue light in normal
602 mice ;

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604 A. C. and S. DBC.; formal analysis, AM. O., M. A., M. L., A. C. and S. DBC; investigation, AM. O.,
605 M. L., A. C. and S. DBC.; resources, AM. O., M. A. and F. A.; writing—review and editing, AM. O.,
606 M. A. and F. A., M. L. and S. DBC; supervision, AM. O., M. A. and M. L; project administration,
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615 ducted in accordance with EEC (2010/63/UE) guidelines for care and use of laboratory animals.

616 **Data Availability Statement:** The data reported in this paper will be shared by the lead contact
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