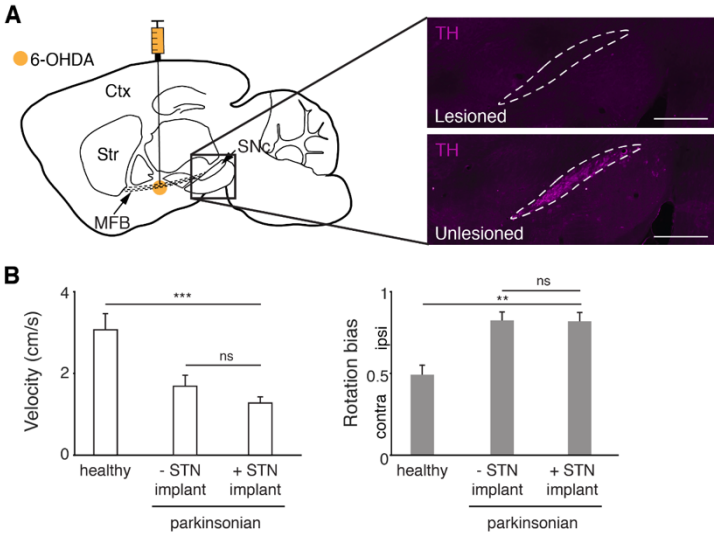
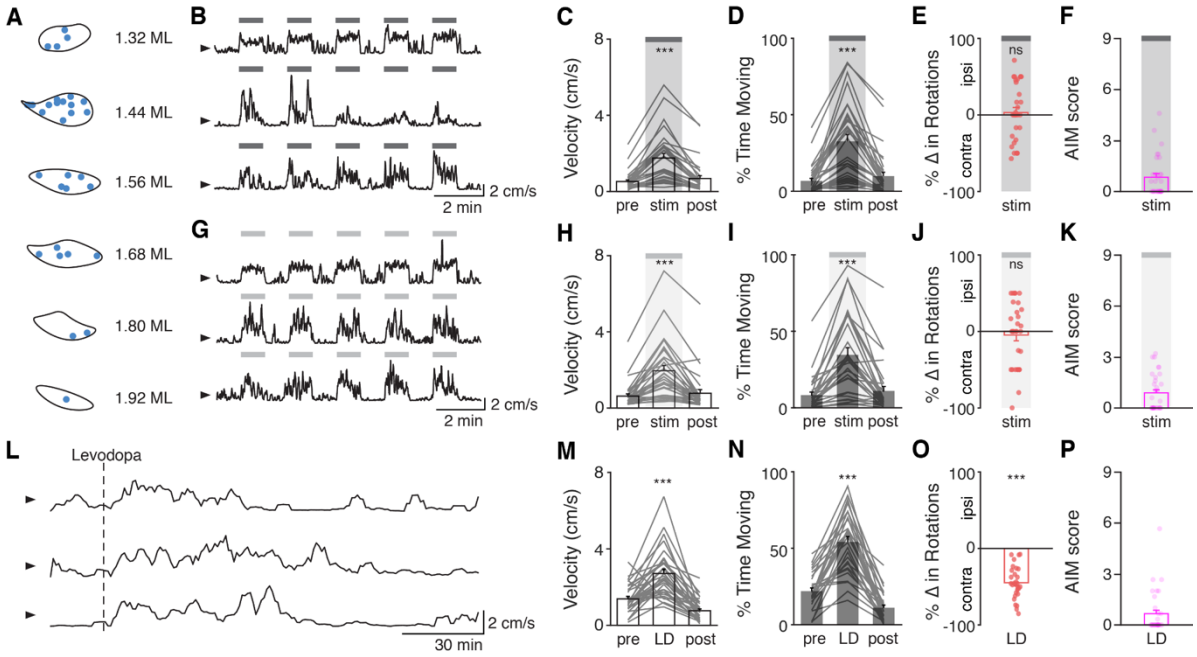
Supplementary Figures

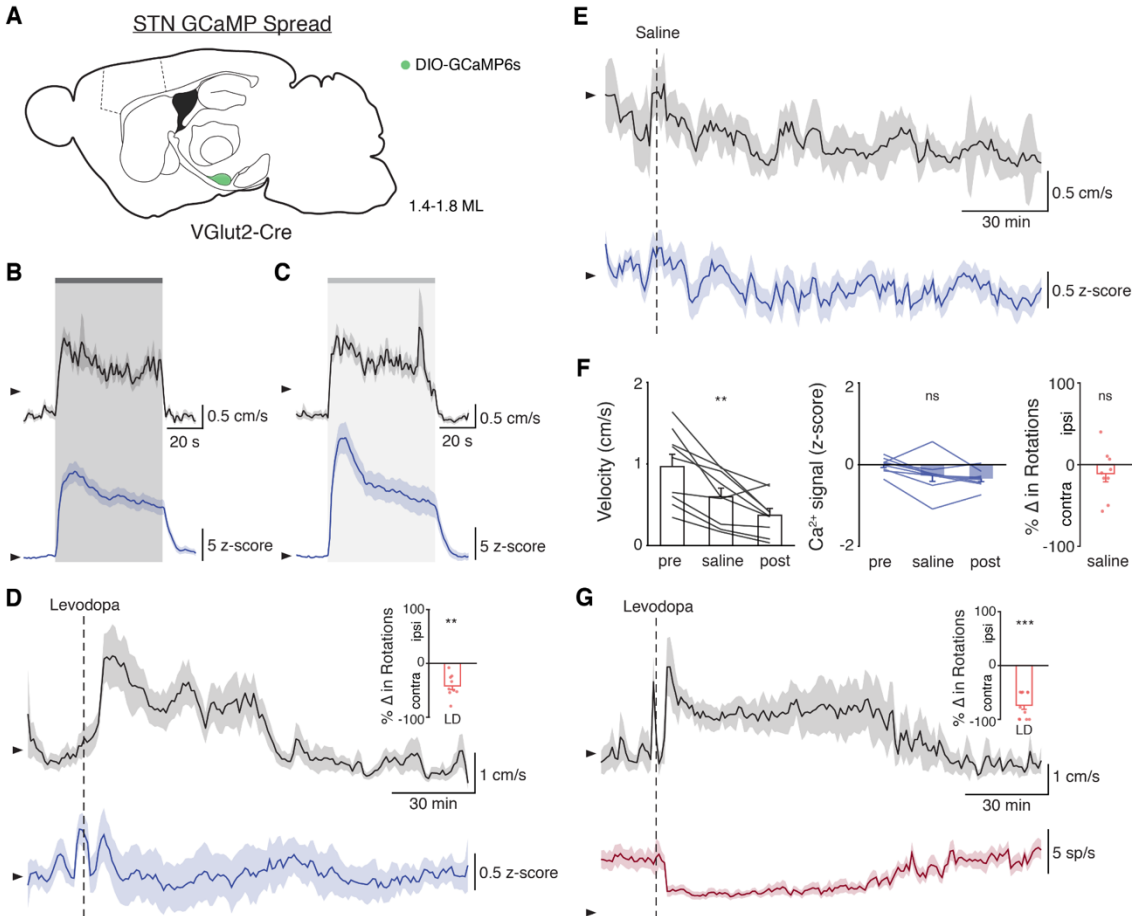


Supplementary Figure 1. Related to Figure 1. Hemiparkinsonian mice show decreased movement velocity and ipsilesional rotation bias. **(A)** Left: Sagittal schematic showing unilateral injection of 6-OHDA to deplete ipsilateral dopamine neurons. Right: Postmortem sagittal section showing depletion of TH (purple) in lesioned hemisphere (dotted lines indicate borders of substantia nigra, pars compacta; scale bar=500 µm). **(B)** Comparison of average velocity (left) and rotation bias (right) in healthy mice (N=9 mice), hemiparkinsonian mice without STN implants (N=10 mice), and hemiparkinsonian mice with STN implants (N=9 mice). Statistical significance was determined using a Wilcoxon rank-sum test; **P<.01, ***P<0.001 (see Supplementary Table 1 for detailed statistics). Bar plots show mean ± SEM.



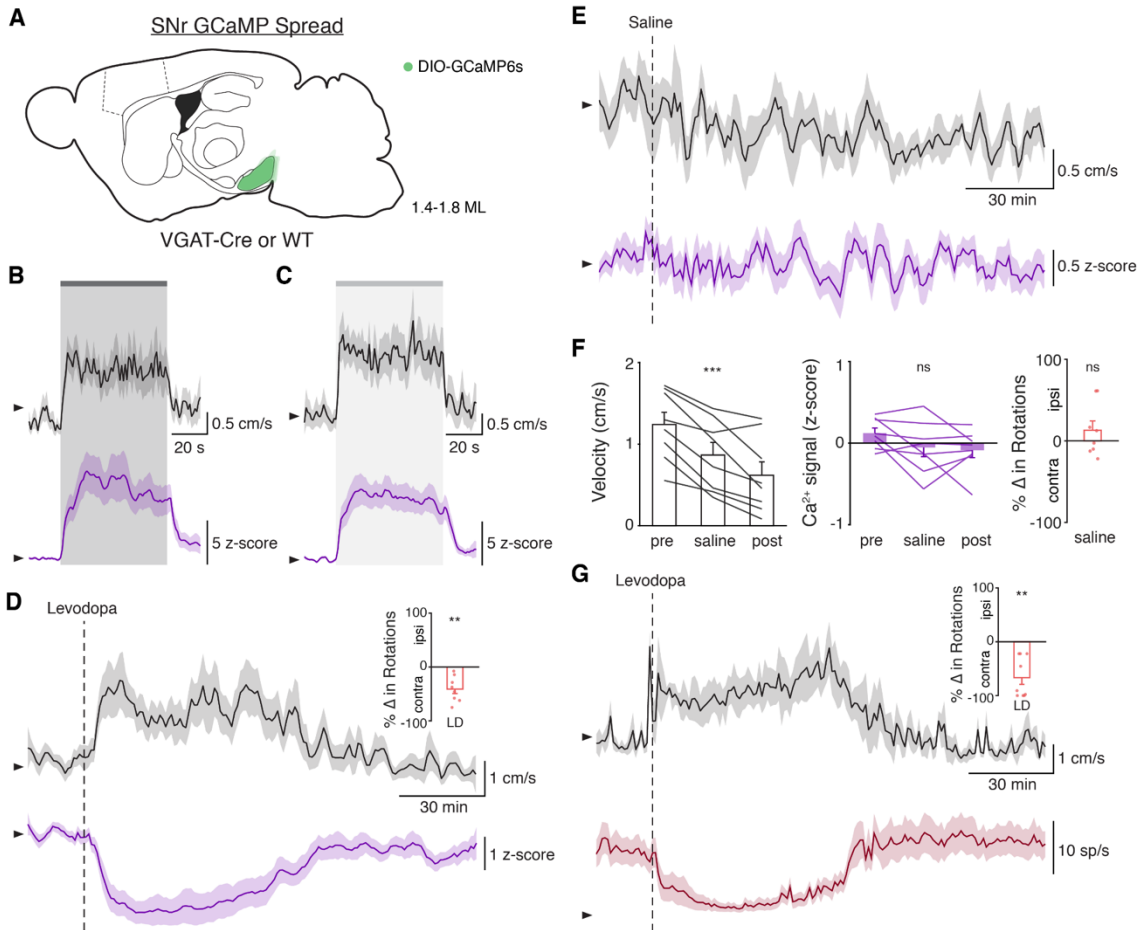
Supplementary Figure 2. Related to Figures 2-4. STN DBS and levodopa produce similar behaviors in parkinsonian mice. **(A)** Targeting of STN DBS electrodes to the STN in 32 mice. **(B)** Representative single-session velocities in response to 60 Hz STN DBS in 3 mice. **(C)** binned average velocity, **(D)** percent time moving, **(E)** change in rotational bias, and **(F)** dyskinesia in response to 60 Hz STN DBS (N=32 mice). **(G)** Representative single-session velocities in response to 100 Hz STN DBS in 3 mice. **(H)** binned average velocity, **(I)** percent time moving, **(J)** change in rotational bias, and **(K)** dyskinesia in response to 100 Hz STN DBS (N=31 mice). **(L)** Representative single-session velocities before and after levodopa injection (dotted line) in 3 mice. **(M)** binned average velocity, **(N)** percent time moving, **(O)** change in rotational bias, and **(P)** dyskinesia in response to levodopa injection (N=30 mice). Dyskinesia was quantified with as the abnormal involuntary movement (AIM) score. Statistical significance was determined using a Wilcoxon sign-rank test (E,I,O); a one-way repeated measures

ANOVA with a Tukey HSD post hoc analysis applied to correct for multiple comparisons (M-N); or a Friedman test with a Tukey HSD post hoc analysis applied to correct for multiple comparisons (C,D,H,I); ***$P<0.001$ (For ANOVA/Friedman, only comparison between pre and stim/LD shown, see Supplementary Table 1 for detailed statistics). Arrowhead in velocity traces corresponds to 1 cm/s. Bar plots show mean ± SEM.



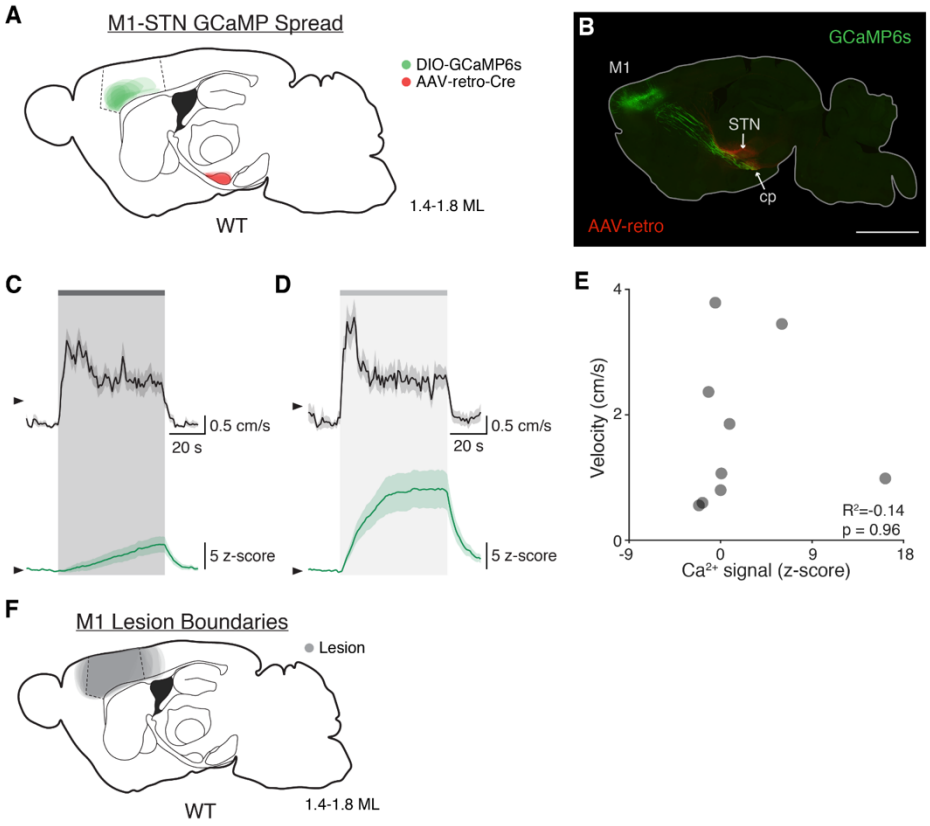
Supplementary 3. Related to Figure 2. STN stimulation increases STN activity *in vivo*. (A) Sagittal schematic showing estimated extent of viral GCaMP6s spread in the STN of VGlut2-Cre mice (N=9 mice). **(B-C)** Average movement velocity over time (black) and STN GCaMP signal (blue) following 60 Hz STN DBS (a, N=9 mice) or 100 Hz STN DBS (b, N=9 mice). **(D)** Average movement velocity over time (black) and STN GCaMP signal (blue) following administration of levodopa (N=9 mice) and average change in rotation bias during levodopa (inset). **(E)** Average movement velocity over time (black) and STN GCaMP signal (blue) following administration of saline (N=9 mice). **(F)** Average velocity (left), STN GCaMP signal (middle), and change in rotation bias before, during, and after saline injection (N=9 mice). **(G)** Average velocity over time (black) and STN

single-unit firing rate (red) following administration of levodopa (n=11 cells, N=3 mice) and average change in rotation bias during levodopa (inset). Statistical significance was determined using a Wilcoxon sign-rank test (D,F (right),G) or a one-way repeated measures ANOVA with a Tukey HSD post hoc analysis applied to correct for multiple comparisons (F (left, middle)); $^{**}P<0.01$, $^{***}P<0.001$ (For ANOVA, only comparison between pre and saline shown, see Supplementary Table 1 for detailed statistics). Arrowhead in velocity, GCaMP, and single-unit electrophysiology traces corresponds to 1 cm/s, 0 z-score, and 0 sp/s, respectively. Velocity traces, GCaMP traces, single-unit electrophysiology traces, and bar plots show mean ± SEM.



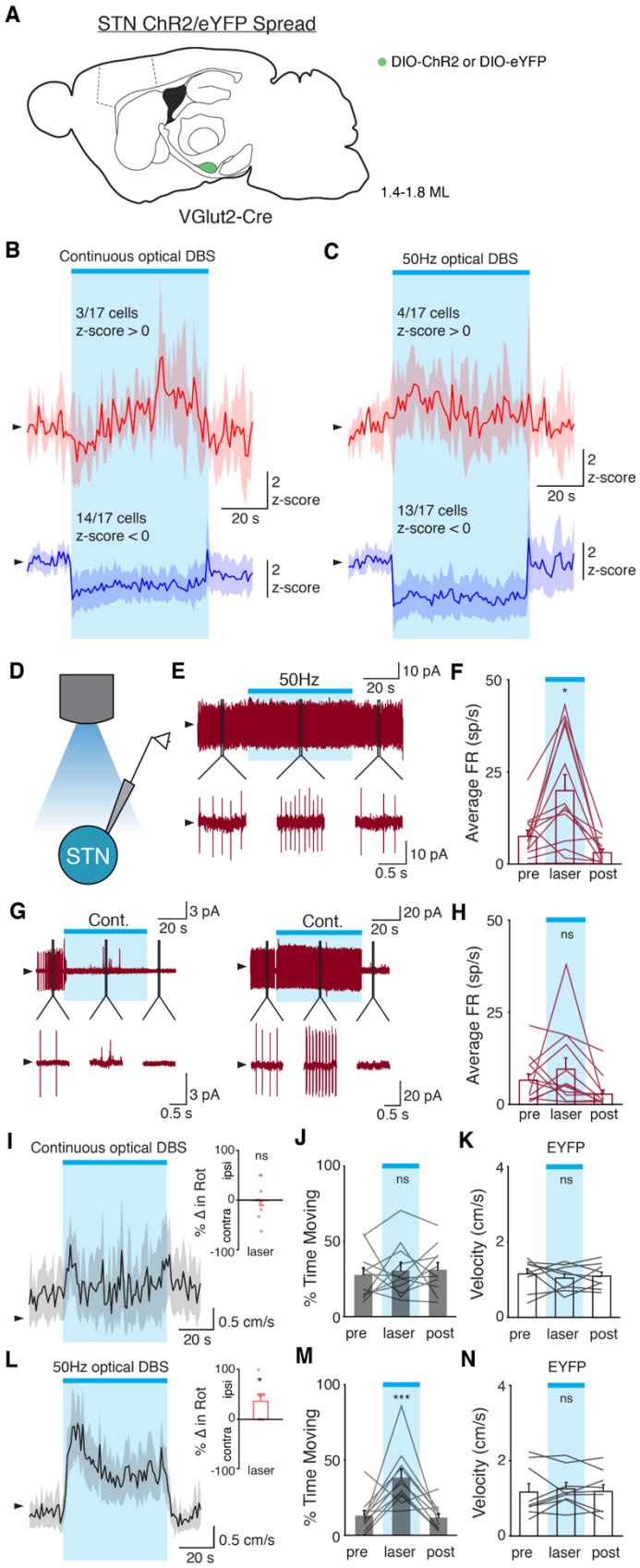
Supplementary Figure 4. Related to Figure 3. STN DBS evokes a rapid increase in SNr activity. (A) Sagittal schematic showing estimated extent of viral GCaMP6s spread in the SNr of VGAT-Cre or WT mice (N=8 mice). **(B-C)** Average velocity over time (black) and SNr GCaMP signal (purple) following 60 Hz STN DBS (a, N=7 mice) or 100 Hz STN DBS (b, N=7 mice). **(D)** Average velocity over time (black) and SNr GCaMP signal (purple) following administration of levodopa (N=8 mice) and average change in rotation bias during levodopa (inset). **(E)** Average velocity over time (black) and SNr GCaMP signal (purple) following administration of saline (N=8 mice). **(F)** Average velocity (left), SNr GCaMP signal (middle), and change in rotation bias before, during, and after saline injection (N=8 mice). **(G)** Average velocity over time (black) and SNr single-unit firing rate

(red) following administration of levodopa (n=9 cells, N=3 mice) and average change in rotation bias during levodopa (inset). Statistical significance was determined using a Wilcoxon sign-rank test (D,F (right),G) or a one-way repeated measures ANOVA with a Tukey HSD post hoc analysis applied to correct for multiple comparisons (F (left, middle)); $^{**}P<0.01$, $^{***}P<0.001$ (For ANOVA, only comparison between pre and saline shown, see Supplementary Table 1 for detailed statistics). Arrowhead in velocity, GCaMP, and single-unit electrophysiology traces corresponds to 1 cm/s, 0 z-score, and 0 sp/s, respectively. Velocity traces, GCaMP traces, single-unit electrophysiology traces, and bar plots show mean ± SEM.



Supplementary Figure 5. Related to Figure 4. STN DBS drives inconsistent and slow changes in hyperdirect M1 activity. **(A)** Sagittal schematic showing estimated extent of viral AAV-retro and GCaMP6s spread in the STN and M1, respectively, of WT mice (N=9 mice). **(B)** Postmortem sagittal section example showing AAV-retro expression in the STN (red), resulting in GCaMP6s expression in M1 neurons (green) projecting to the STN and cerebral peduncle (cp). **(C-D)** Average velocity over time (black) and M1-STN GCaMP signal (green) following 60 Hz STN DBS (a, N=9 mice) or 100 Hz STN DBS (b, N=8 mice). **(E)** Scatter plot comparing hyperdirect M1 photometry signal to movement velocity during 60 Hz STN DBS (each dot represents average for one mouse, N=9 mice). **(F)** Sagittal schematic showing estimated extent of M1 lesions in WT mice (N=11 mice). Statistical significance was determined using a one-way ANOVA (see Supplementary

Table 1 for detailed statistics). Arrowhead in velocity traces and GCaMP traces corresponds to 1 cm/s and 0 z-score, respectively. Velocity and GCaMP traces show mean ± SEM.


A
STN ChR2/eYFP Spread
DIO-ChR2 or DIO-eYFP
1.4-1.8 ML
VGlut2-Cre
B
Continuous optical DBS
3/17 cells
z-score > 0
14/17 cells
z-score < 0
2
z-score
20 s
C
50Hz optical DBS
4/17 cells
z-score > 0
13/17 cells
z-score < 0
D
STN
E
50Hz
10 pA
20 s
0.5 s
F
Average FR (sp/s)
pre laser post
G
Cont.
3 pA
20 pA
H
ns
I
Continuous optical DBS
% Δ in Rot
ipsi
contra
laser
0.5 cm/s
J
% Time Moving
K
EYFP
Velocity (cm/s)
L
50Hz optical DBS
M
***
N

Supplementary Figure 6. Related to Figure 7. *In vivo* and *ex vivo* optical STN stimulation produces distinct changes in firing rate. **(A)** Sagittal schematic showing estimated extent of viral ChR2 or eYFP spread in the STN of VGlut2-Cre mice (N=20 mice). **(B-C)** Average *in vivo* firing rate of STN single units over time in response to continuous (B) or 50 Hz (C) optical stimulation (n=17 cells, N=3 mice), subdivided into neurons in which firing rate increased to greater than 0 z-score (top traces, red) and neurons in which firing rate decreased to less than 0 z-score (bottom traces, blue). **(D)** Recording configuration for *ex vivo* recordings of STN neurons in the cell-attached configuration. **(E)** Representative STN neuron before, during, and after 50 Hz optical stimulation. 1-second portions of the sweep are shown below. **(F)** Average firing rate before, during, and after 50 Hz optical stimulation (n=13 cells, N=3 mice). **(G)** Two STN neurons before, during, and after constant optical stimulation, demonstrating the variable response of neurons. 1-second portions of the sweep are shown below. **(H)** Average firing rate before, during, and after constant optical stimulation (n=13 cells, N=3 mice). **(I)** Average movement velocity and change in rotation bias (inset) in response to constant optical stimulation in ChR2 mice (N=11 mice). **(J)** Average percent time moving before, during, and after constant optical stimulation in ChR2 mice (N=11 mice). **(K)** Average movement velocity before, during, and after constant optical stimulation in mice injected with eYFP (N=9 mice). **(L)** Average movement velocity and change in rotation bias (inset) in response to 50 Hz optical stimulation in ChR2 mice (N=11 mice). **(M)** Average percent time moving before, during, and after 50 Hz optical stimulation in ChR2 mice (N=11 mice). **(N)** Average movement velocity before, during, and after 50 Hz optical stimulation in mice

injected with eYFP (N=9 mice). Statistical significance was determined using a one-way repeated measures ANOVA with a Tukey HSD post hoc analysis applied to correct for multiple comparisons; $*P<0.05$ (only comparison between pre and laser shown, see Supplementary Table 1 for detailed statistics). Arrowhead in firing rate, cell-attached, and velocity traces corresponds to 0 z-score, 0 pA, and 1 cm/s, respectively. Firing rate traces, velocity traces, and bar plots show mean ± SEM.

Supplementary Tables

Figure	Part	Test	P-value (stim/las	P-value (pre vs p	P-value (stim/las	doF (ANOVA)	F-value (ANOVA)	Cohen's d
2	D (fib)	rmANOVA	1.19E-07	8.03E-01	1.19E-07	2,8	60.057	1.83
2	D (vel)		7.11E-09	9.96E-01	1.09E-09	2,8	57.668	1.74
2	F (fib)		9.54E-07	4.27E-01	4.17E-07	2,8	37.391	1.61
2	F (vel)		1.11E-09	9.96E-01	1.09E-09	2,8	66.612	1.79
2	H (fib)		9.04E-01	8.06E-01	8.34E-01	2,8	0.27308	
2	H (vel)		1.66E-03	8.75E-03	1.02E-05	2,8	27.391	1.43
2	J (spike)		2.89E-02	9.97E-01	5.03E-03	2,10	11.046	1.54
2	J (vel)		3.13E-04	1.25E-01	1.77E-05	2,10	39.742	2.11
3	C (fib)		4.14E-05	9.99E-01	4.18E-05	2,6	25.567	1.66
3	C (vel)		1.33E-05	1.70E-01	6.55E-06	2,6	41.02	0.76
3	E (fib)		3.32E-05	1.11E-01	9.04E-05	2,6	24.39	1.78
3	E (vel)		4.99E-06	4.10E-01	9.82E-07	2,6	27.097	0.78
3	G (fib)		6.68E-06	4.57E-03	1.24E-04	2,7	40.141	5.01
3	G (vel)		1.56E-02	5.13E-04	2.25E-04	2,7	18.622	1.14
3	I (spike)		1.21E-02	3.79E-01	9.49E-03	2,8	15.221	1.87
3	I (vel)		1.28E-03	8.38E-01	1.62E-04	2,8	37.4	2.84
4	C (fib)		8.37E-02	9.54E-02	1.11E-01	2,8	4.5518	
4	C (vel)		1.51E-09	8.72E-01	1.18E-09	2,8	66.475	1.51
4	E (fib)		5.88E-05	1.76E-02	6.47E-05	2,7	23.021	1.17
4	E (vel)		4.14E-07	5.73E-01	6.95E-07	2,7	30.395	1.49
5	C		8.24E-03	8.24E-01	5.48E-03	2,10	10.579	0.67
6	D (high)	WRS	1.48E-11					3.27
6	D (low)		5.42E-01					
7	C	rmANOVA	2.46E-02	1.36E-01	6.89E-01	2,16	4.3163	0.10
7	E		2.40E-02	9.97E-01	4.57E-02	2,16	5.5241	0.11
7	F	WRS	5.13E-01					
7	G		3.88E-02					0.81
7	I	rmANOVA	2.96E-01	2.45E-01	9.87E-01	2,10	1.7004	
7	K		1.27E-08	4.84E-01	1.30E-09	2,10	51.586	1.41
S1	B (rot)	WRS	0.0014 (healthy vs	(PD-STN vs PD+STN)				
S1	B (vel)		0.0000823 (healthy	(PD-STN vs PD+STN)				
S2	C	FT	3.92E-09	6.08E-01	7.41E-07	2	216.1956 (chi^2)	
S2	D		3.53E-09	5.28E-01	1.29E-06	2	214.4129 (chi^2)	
S2	E	WSR	7.15E-01					
S2	H	FT	5.53E-08	7.87E-01	2.01E-06	2	189.3723 (chi^2)	
S2	I		1.46E-08	5.39E-01	4.70E-06	2	195.1556 (chi^2)	
S2	J	WSR	4.68E-01					
S2	M	rmANOVA	9.69E-10	5.11E-07	9.56E-10	2,29	92.27	
S2	N		9.56E-10	2.02E-08	9.56E-10	2,29	164.19	
S2	O	WSR	1.73E-06					
S3	D		3.91E-03					
S3	F (fib)	rmANOVA	8.38E-02	1.18E-03	8.13E-01	2,8	5.6691	
S3	F (rot)	WSR	2.89E-01					
S3	F (vel)	rmANOVA	1.15E-03	1.41E-04	9.63E-03	2,8	22.701	
S3	G	WSR	9.77E-04					
S4	D		7.81E-03					
S4	F (fib)	rmANOVA	3.07E-01	2.65E-01	9.64E-01	2,7	1.6116	
S4	F (rot)	WSR	5.47E-01					
S4	F (vel)	rmANOVA	4.66E-05	2.04E-04	2.64E-02	2,7	24.425	
S4	G	WSR	3.91E-03					
S5	E	ANOVA	9.60E-01				0.00336	
S6	F	rmANOVA	3.26E-02	2.68E-02	6.86E-03	2,12	11.298	
S6	H		6.26E-01	5.81E-03	7.39E-02	2,12	3.6871	
S6	I	WSR	8.10E-01					
S6	J	rmANOVA	6.53E-01	4.55E-01	9.77E-01	2,10	0.75399	
S6	K		6.64E-01	9.31E-01	9.08E-01	2,8	0.31701	
S6	L	WSR	1.60E-02					
S6	M	rmANOVA	5.56E-09	7.33E-01	1.07E-09	2,10	52.8	
S6	N		8.14E-01	9.94E-01	8.21E-01	2,8	0.22566	

Supplementary Table 1. Full statistical results for the indicated figures. rmANOVA=one-way repeated measures Analysis of Variance with post-hoc Tukey multiple-comparisons test. WRS=Wilcoxon Rank Sum test (Mann-Whitney U test). FT=Friedman's test with post-hoc Tukey multiple-comparisons test. WSR=Wilcoxon Signed-Ranks test.

Current Amplitude (μA)	Frequency (Hz)	Pulse Width (μs)	High vs Low Effect
200	60	60	High
200	60	100	High
200	80	60	High
200	100	60	High
200	120	60	High
200	140	60	High
200	160	60	High
225	80	80	High
175	20	50	Low
200	10	120	Low
200	120	20	Low
300	10	60	Low
400	1	120	Low

Supplementary Table 2. Related to Figure 6. Stimulation parameters used in assessing differences between high and low effect stimulation.