SUPPLEMENTAL INFORMATION

Figure S1

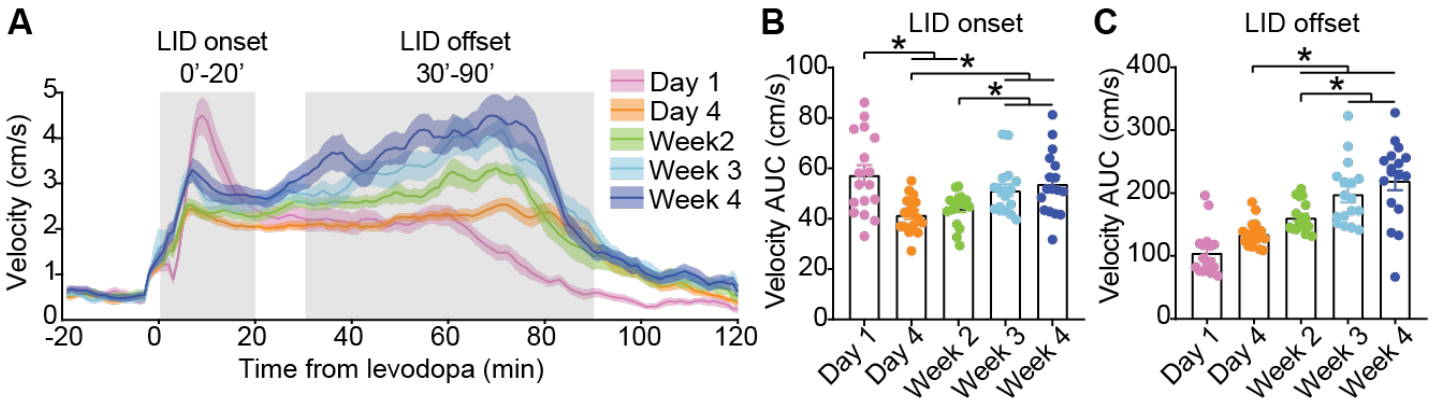

Figure S1 (related to Figure 1). Changes in locomotor velocity after repetitive levodopa. (A) Average locomotor velocity in response to IP injection of 5mg/kg levodopa for different treatment timepoints. **(B-C)** Area under the curve (AUC) of velocity traces during LID onset (B), and offset (C) phases. [RM one-way ANOVA, B: $p=0.0004$, post-hoc $^*p<0.05$; C: $p<0.0001$, post-hoc $^*p<0.05$]. Data shown as mean±SEM. N=17.

Figure S2

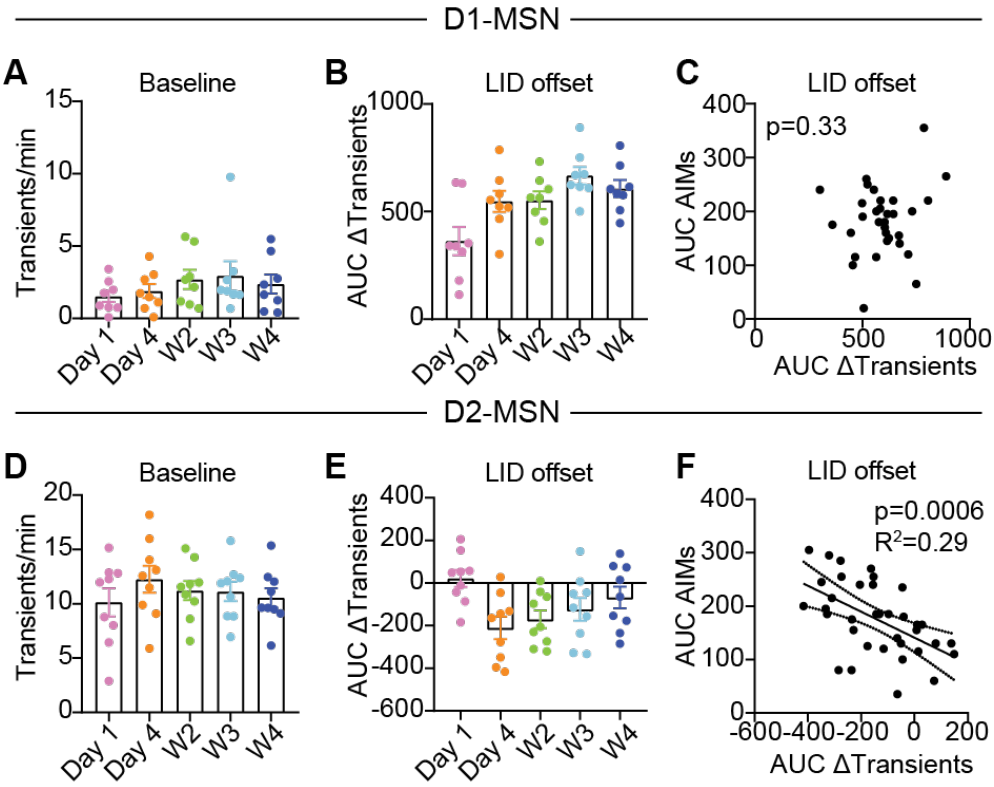

Figure S2 (related to Figure 2). More rapid LID offset relates to the recovery of D2-MSN activity. **(A)** Average baseline rates of D1-MSN GCaMP transients 30 min before levodopa injection. [RM one-way ANOVA, p=0.2270]. **(B)** Area under the curve of D1-MSN ΔTransients during LID offset. [RM one-way ANOVA, ns]. **(C)** Correlation of D1-MSN ΔTransients with AIMs during LID offset. **(D)** Average baseline rates of D2-MSN GCaMP transients 30 min before levodopa injection. [RM one-way ANOVA, p=0.1573]. **(E)** Area under the curve of D2-MSN ΔTransients during LID offset. [RM one-way ANOVA, p=0.0436]. **(F)** Correlation of D2-MSN ΔTransients with AIMs during LID offset. N=8 D1-Cre and N=9 A2a-Cre. Each dot is one mouse. Data shown as mean±SEM (A, C).

Figure S3

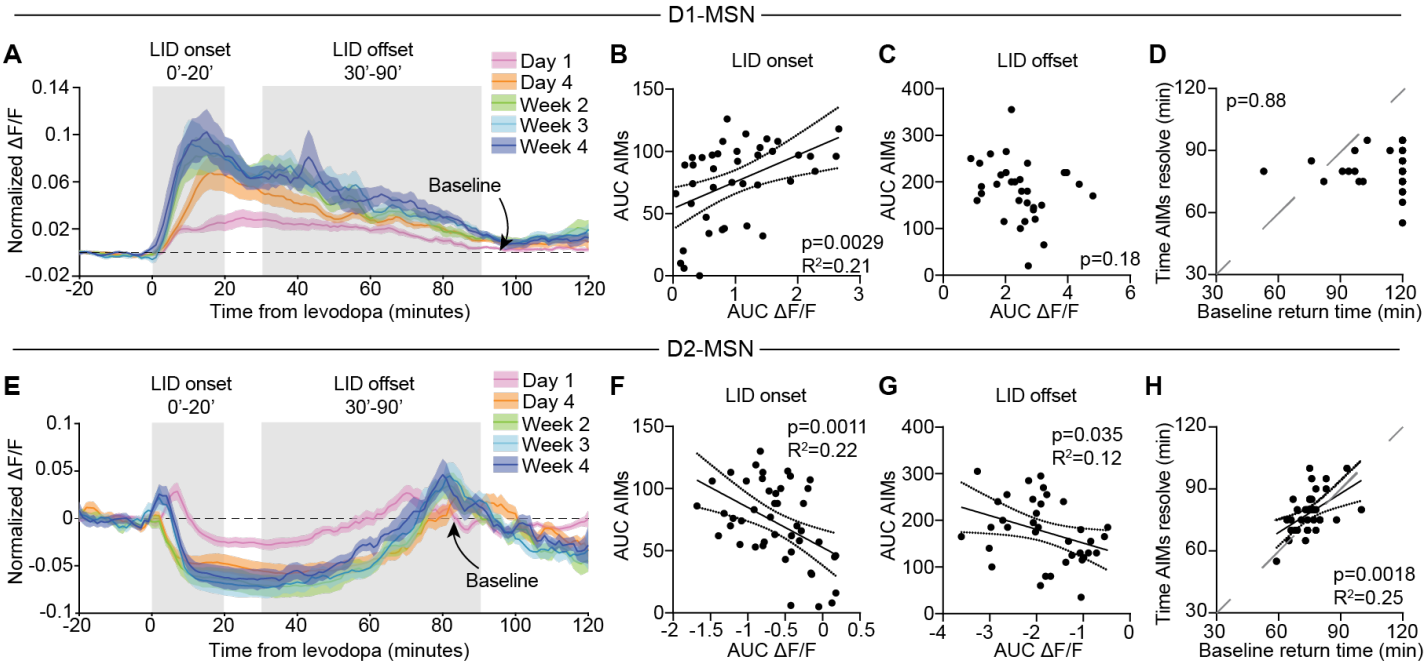

Figure S3 (related to Figure 2). Changes in striatal activity parallel alterations in dyskinesia during repetitive levodopa treatment. GCaMP fiber photometry in DLS D1-MSNs (A-D) or D2-MSNs (E-H) of 6-OHDA mice treated with levodopa. **(A)** D1-MSN ΔF/F response to levodopa with baseline subtraction across treatment timepoints. LID onset and offset phases are shown in grey. **(B-C)** Correlation between AIMs and D1-MSN ΔF/F during LID onset (B) and offset (C) phases. **(D)** Correlation between AIMs resolution time and D1-MSN ΔF/F return to baseline. **(E)** D2-MSN ΔF/F response to levodopa with baseline subtraction across treatment timepoints. **(F-G)** Correlation between AIMs and D2-MSN ΔF/F during LID onset (F) and offset (G) phases. **(H)** Correlation between AIMs resolution time and D2-MSN ΔF/F return to baseline. N=9 A2a-Cre and N=8 D1-Cre mice. Data shown as mean±SEM (A, E).

Figure S4

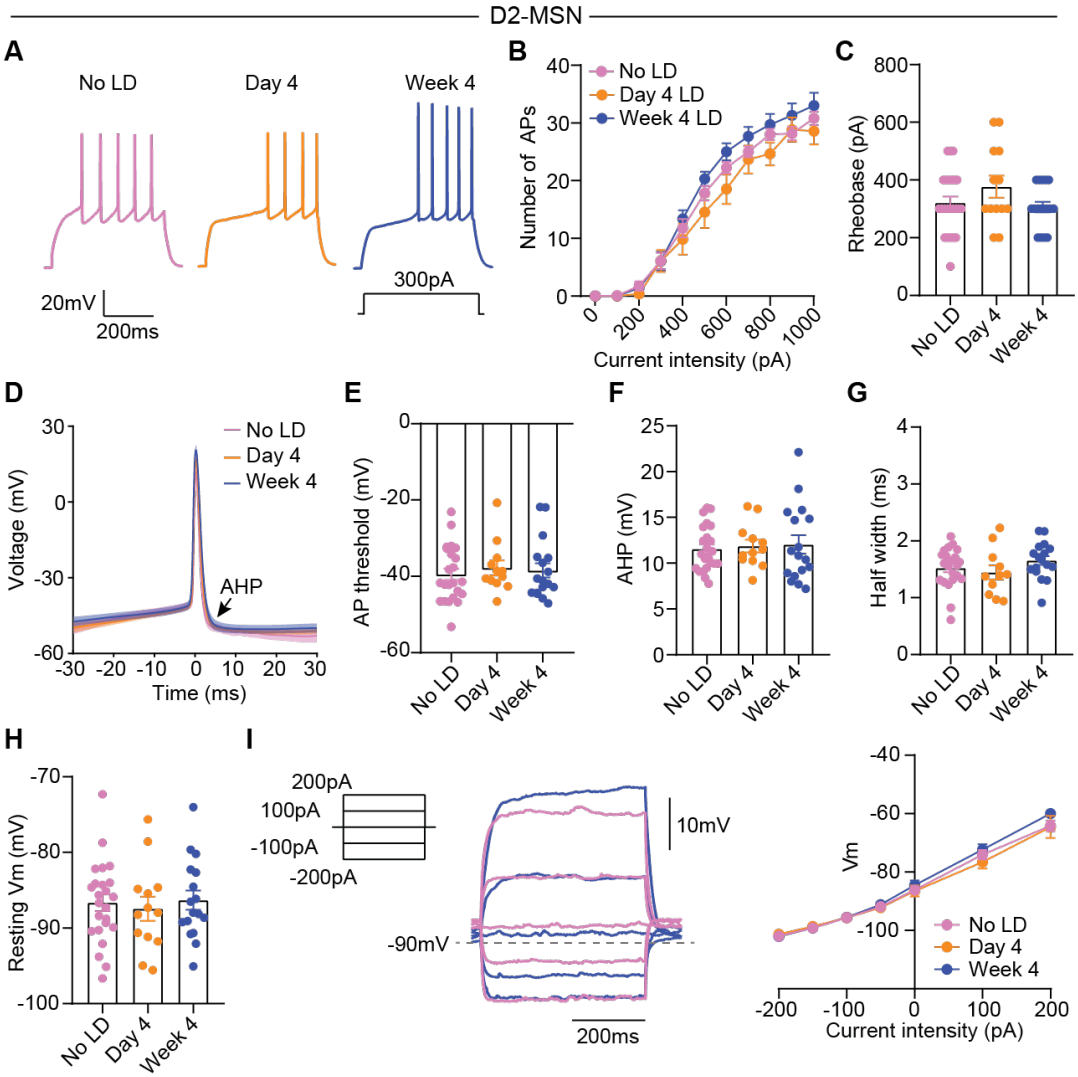

Figure S4 (related to Figure 6). D2-MSN intrinsic excitability remains unchanged after repetitive levodopa. (A) Voltage responses to current injection in D2-MSN after different levodopa treatment durations. **(B)** Number of action potentials (APs) in response to depolarizing current steps. [Two-way ANOVA, ns]. **(C)** D2-MSN rheobase across treatment duration. [Kruskal-Wallis, ns]. **(D)** Average action potential shape. **(E-H)** Action potential threshold (E), afterhyperpolarization (AHP, F), half width (G) and resting membrane potential (H). [Kruskal-Wallis, ns]. **(I)** Voltage deflection to current injections. [Two-way ANOVA, ns]. No LD: n=23-24, N=11; Day 4: n=12-13, N=6; Week 4: n=17, N=5. Data shown as mean±SEM. n=cells; N=mice. Each dot represents one cell.

Table S1. Experimental design and statistical analysis of all key experiments

Experiment	Fig	Test	N (mice)	n (cells)	p-value	Significant post-hoc	p-value	Planned sample size
AIM AUC 0-20 min	1c	RM one-way ANOVA	17	-	<0.0001	Day 1 vs rest	<0.0001	N = 10 mice
AIM AUC 30-90 min	1d	RM one-way ANOVA	17	-	<0.0001	Day 4 vs week 2 Day 4 vs week 3 Day 4 vs week 4 Week 2 vs week 3 Week 2 vs week 4	0.0486 <0.0001 <0.0001 0.0285 0.0205	N = 10 mice
Time AIMs resolve	1e	RM one-way ANOVA	17	-	<0.0001	Day 4 vs week 2 Day 4 vs week 3 Day 4 vs week 4	0.0043 0.0001 0.0002	N = 10 mice
Velocity AUC 1-20 min	S1b	RM one-way ANOVA	17	-	0.0004	Day 1 vs Day 4 Day 1 vs week 2 Day 4 vs week 3 Day 4 vs week 4 Week 2 vs week 3 Week 2 vs week 4	0.0095 0.0179 0.0088 0.0010 0.0243 0.0111	N = 10 mice
Velocity AUC 30-90 min	S1c	RM one-way ANOVA	17	-	<0.0001	Day 4 vs week 2 Day 4 vs week 3 Day 4 vs week 4 Week 2 vs week 3 Week 2 vs week 4	0.0020 0.0008 <0.0001 0.0150 0.0021	N = 10 mice
D1 AUC ΔTransients for LID onset	2f	RM one-way ANOVA	8	-	0.0003	Day 1 vs day 4 Day 1 vs week 2 Day 1 vs week 3 Day 1 vs week 4	0.0466 0.0031 0.0010 0.0004	N = 10 mice
Time D1 ΔTransients return to baseline	2g	RM one-way ANOVA	8	-	0.5063	-	-	N = 10 mice
D1 Correlation AIMs vs ΔTransients for LID onset	2h	Pearson	8 mice x 5 timepoints	-	0.0008 R2=0.26	-	-	N = 10 mice
D1 correlation Time AIMs resolve vs ΔTransients return to baseline	2i	Pearson	8 mice x 4 timepoints	-	0.4819	-	-	N = 10 mice
D2 AUC ΔTransients for LID onset	2l	RM one-way ANOVA	9	-	0.0001	Day 1 vs day 4 Day 1 vs week 2 Day 1 vs week 3	0.0026 0.0040 0.0342	N = 10 mice
Time D2 ΔTransients return to baseline	2m	RM one-way ANOVA	9	-	0.0047	Day 4 vs week 4	0.0344	N = 10 mice
D2 Correlation AIMs vs ΔTransients for LID onset	2n	Pearson	9 mice x 5 timepoints	-	<0.0001 R2=0.39	-	-	N = 10 mice
D2 correlation Time AIMs resolve vs ΔTransients return to baseline	2o	Pearson	9 mice x 4 timepoints	-	<0.0001 R2=0.43	-	-	N = 10 mice
D1 baseline transient frequency	S2a	RM one-way ANOVA	8	-	0.2270	-	-	N = 10 mice
D1 AUC ΔTransients for	S2b	RM one-way	8	-	0.0471	-	-	N = 10 mice

LID offset		ANOVA						
D1 Correlation AIMs vs ΔTransients for LID offset	S2c	Pearson	8 mice x 4 timepoints	-	0.3314	-	-	N = 10 mice
D2 baseline transient frequency	S2d	RM one-way ANOVA	9	-	0.1573	-	-	N = 10 mice
D2 AUC ΔTransients for LID offset	S2e	RM one-way ANOVA	9	-	0.0436	-	-	N = 10 mice
D2 Correlation AIMs vs ΔTransients for LID offset	S2f	Pearson	9 mice x 4 timepoints	-	0.0006 R2=0.29	-	-	N = 10 mice
D1 Correlation AIMs vs ΔF/F for LID onset	S3b	Pearson	8 mice x 5 timepoints	-	0.0029 R2=0.21	-	-	N = 10 mice
D1 Correlation AIMs vs ΔF/F for LID offset	S3c	Pearson	8 mice x 4 timepoints	-	0.1833	-	-	N = 10 mice
D1 Correlation Time AIMs resolve vs ΔF/F returns to baseline	S3d	Pearson	8 mice x 4 timepoints	-	0.8803	-	-	N = 10 mice
D2 Correlation AIMs vs ΔF/F for LID onset	S3f	Pearson	9 mice x 5 timepoints	-	0.0011 R2=0.22	-	-	N = 10 mice
D2 Correlation AIMs vs ΔF/F for LID offset	S3g	Pearson	9 mice x 4 timepoints	-	0.0350 R2=0.12	-	-	N = 10 mice
D2 Correlation Time AIMs resolve vs ΔF/F returns to baseline	S3h	Pearson	9 mice x 4 timepoints	-	0.0018 R2=0.25	-	-	N = 10 mice
Number of c-Fos+ cells	3b	Kruskal-Wallis	Day 1: 4 Day 4: 5 Week 4: 5	-	0.8626	-	-	N = 5 mice/group
Number of c-Fos+/tdTom+ cells	3c	Kruskal-Wallis	Day 1: 4 Day 4: 5 Week 4: 5	-	0.2683	-	-	N = 5 mice/group
ΔFR of putative D1 MSN	3g	Kruskal-Wallis	Day 1: 10 Day 4: 10 Week 4: 14	Day 1: 55 Day 4: 48 Week 4: 157	0.0009	Day 1 vs day 4 Day 1 vs week 4	0.0179 0.0007	N = 5 mice/group n = 20 units/group
ΔFR of putative D2 MSN	3j	Kruskal-Wallis	Day 1: 10 Day 4: 10 Week 4: 14	Day 1: 17 Day 4: 29 Week 4: 47	0.9213	-	-	N = 5 mice/group n = 20 units/group
Peak GRAB-DA2h	4e	RM one-way ANOVA	8	-	0.3776	-	-	N = 10 mice
D1 mEPSC frequency	5b	Kruskal-Wallis	no LD: 11 day 4: 6 week 4: 6	no LD: 30 day 4: 22 week 4: 24	<0.0001	no LD vs day 4 no LD vs week 4	0.0141 <0.0001	N = 5 mice/group n = 20 cells/group
D1 mEPSC amplitude	5c	Kruskal-Wallis	no LD: 11 day 4: 6 week 4: 6	no LD: 30 day 4: 22 week 4: 24	0.0158	no LD vs week 4	0.0150	N = 5 mice/group n = 20 cells/group
D2 mEPSC frequency	5e	Kruskal-Wallis	no LD: 10 day 4: 6 week 4: 6	no LD: 32 day 4: 20 week 4: 25	0.5896	-	-	N = 5 mice/group n = 20 cells/group
D2 mEPSC	5f	Kruskal-	no LD: 10	no LD: 32	0.5135	-	-	N = 5

amplitude		Wallis	day 4: 6 week 4: 6	day 4: 20 week 4: 25				mice/group n = 20 cells/group
D1 responses to depolarizing currents	6b	Two-way ANOVA	no LD: 9 day 4: 6 week 4: 6	no LD: 22 day 4: 13 week 4: 15	Treatment factor: 0.0001 Interaction : <0.0001	No LD vs week 4 (200, 300, 400, 500 pA) Day 4 vs week 4 (300, 400, 500 pA)	<0.05 <0.05	N = 5 mice/group n = 15 cells/group
D1 rheobase	6c	Kruskal-Wallis	no LD: 9 day 4: 6 week 4: 6	no LD: 22 day 4: 13 week 4: 15	<0.0001	No LD vs week 4 Day 4 vs week 4	<0.0001 0.0253	N = 5 mice/group n = 15 cells/group
D1 action potential threshold	6e	Kruskal-Wallis	no LD: 9 day 4: 6 week 4: 6	no LD: 21 day 4: 13 week 4: 15	0.0578	-	-	N = 5 mice/group n = 15 cells/group
D1 AHP	6f	Kruskal-Wallis	no LD: 9 day 4: 6 week 4: 6	no LD: 21 day 4: 13 week 4: 15	<0.0001	No LD vs week 4 Day 4 vs week 4	<0.0001 0.0064	N = 5 mice/group n = 15 cells/group
D1 half width	6g	Kruskal-Wallis	no LD: 9 day 4: 6 week 4: 6	no LD: 21 day 4: 12 week 4: 15	0.0021	No LD vs week 4	0.0013	N = 5 mice/group n = 15 cells/group
D1 resting membrane potential	6h	Kruskal-Wallis	no LD: 9 day 4: 6 week 4: 6	no LD: 22 day 4: 13 week 4: 15	0.7714	-	-	N = 5 mice/group n = 15 cells/group
D1 subthreshold voltage deflection	6i	Two-way ANOVA	no LD: 9 day 4: 6 week 4: 6	no LD: 22 day 4: 13 week 4: 15	interaction : <0.0001	No LD vs week 4 (at 100pA)	0.0211	N = 5 mice/group n = 15 cells/group
D2 responses to depolarizing currents	S4b	Two-way ANOVA	no LD: 11 day 4: 6 week 4: 5	no LD: 24 day 4: 13 week 4: 17	Treatment factor: 0.1264	-	-	N = 5 mice/group n = 15 cells/group
D2 rheobase	S4c	Kruskal-Wallis	no LD: 11 day 4: 6 week 4: 5	no LD: 24 day 4: 13 week 4: 17	0.3731	-	-	N = 5 mice/group n = 15 cells/group
D2 action potential threshold	S4e	Kruskal-Wallis	no LD: 11 day 4: 6 week 4: 5	no LD: 23 day 4: 12 week 4: 17	0.6441	-	-	N = 5 mice/group n = 15 cells/group
D2 AHP	S4f	Kruskal-Wallis	no LD: 11 day 4: 6 week 4: 5	no LD: 23 day 4: 12 week 4: 17	0.8176	-	-	N = 5 mice/group n = 15 cells/group
D2 half width	S4g	Kruskal-Wallis	no LD: 11 day 4: 6 week 4: 5	no LD: 22 day 4: 11 week 4: 17	0.2539	-	-	N = 5 mice/group n = 15 cells/group
D2 resting membrane potential	S4h	Kruskal-Wallis	no LD: 11 day 4: 6 week 4: 5	no LD: 24 day 4: 13 week 4: 17	0.7945	-	-	N = 5 mice/group n = 15 cells/group
D2 subthreshold voltage deflection	S4i	Two-way ANOVA	no LD: 11 day 4: 6 week 4: 5	no LD: 24 day 4: 13 week 4: 17	interaction : 0.3247	-	-	N = 5 mice/group n = 15 cells/group
1μM DA-evoked GIRK current	7d	Kruskal-Wallis	no LD: 7 day 4: 7 week 4: 8	no LD: 16 day 4: 15 week 4: 14	0.1274	-	-	N = 5 mice/group n = 12 cells/group

100μM DA-evoked GIRK current	7e	Kruskal-Wallis	no LD: 7 day 4: 7 week 4: 8	no LD: 16 day 4: 15 week 4: 14	0.5348	-	-	N = 5 mice/group n = 12 cells/group
DA-evoked GIRK current ratio	7f	Kruskal-Wallis	no LD: 7 day 4: 7 week 4: 8	no LD: 16 day 4: 15 week 4: 14	0.01	No LD vs Day 4 No LD vs Week 4 Day 4 vs Week 4	>0.9999 0.0098 0.0879	N = 5 mice/group n = 12 cells/group

AIM: Abnormal Involuntary Movements
AUC: Area Under the Curve
LID: Levodopa-Induced Dyskinesia
FR: Firing Rate
mEPSC: miniature excitatory postsynaptic current
AHP: afterhyperpolarization
DA: dopamine