Supplementary Material

Serotonergic Regulation of Synaptic Dopamine Levels Mitigates L-DOPA-Induced Dyskinesia in a Mouse Model of Parkinson's Disease

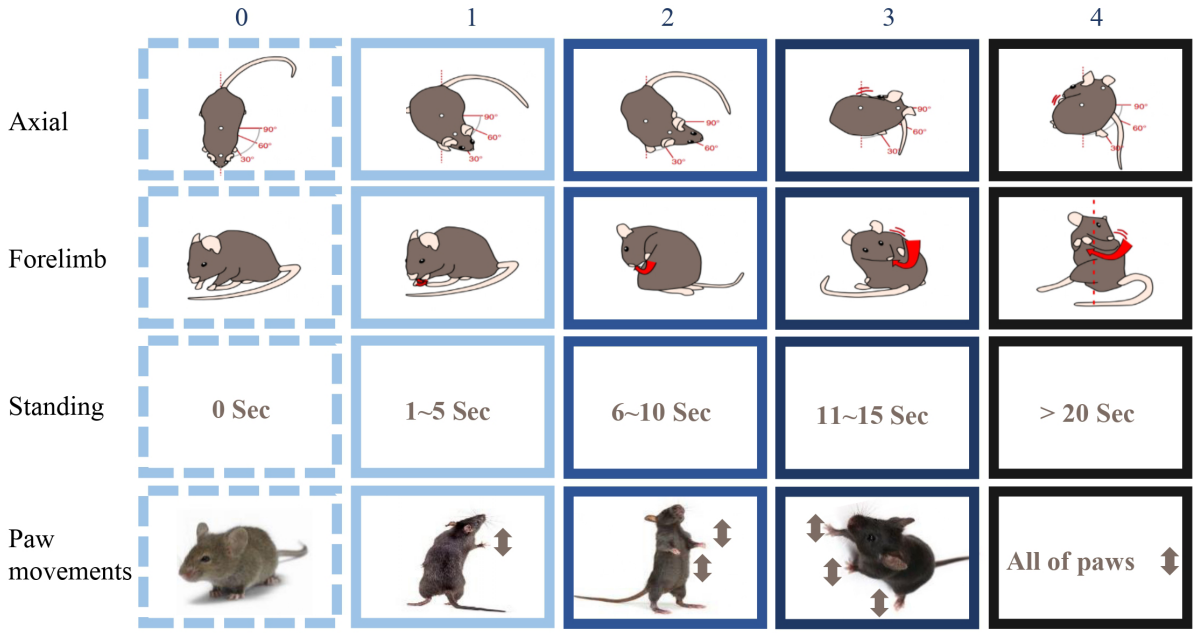
Supplementary Table 1. Numbers of control and MitoPark mice used.

	Group	Behavioral tests (AIM test + Locomotor activity)	FSCV	HPLC	WB	PET	Total
WT	WT	3	12	15	5	5	40
MP	MP	8	12	15	5	5	45
	MP LID	38	12	23	17	16	106

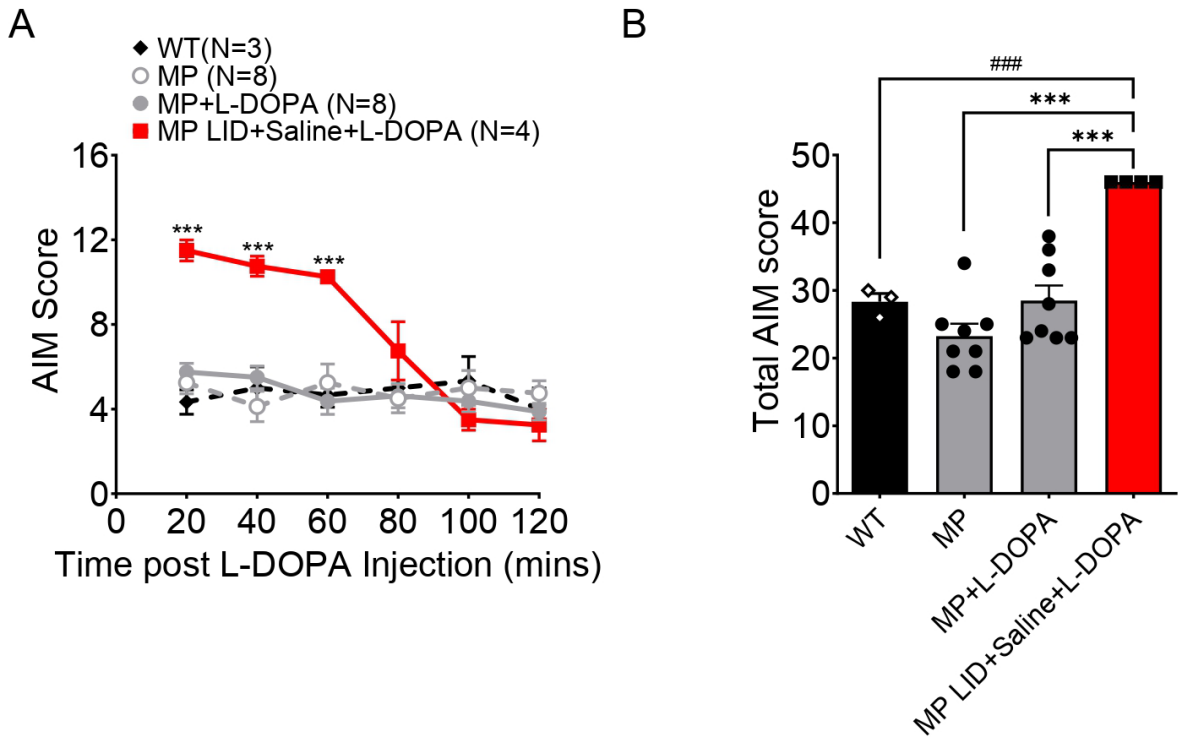
Supplementary Table 2. The linear regression analysis revealed a correlation between abnormal dyskinesia induced by L-DOPA and neurotransmitter levels.

	Total AIM		Axial		Limbs		Stand		Vertical movement	
	R squared	P value	R squared	P value	R squared	P value	R squared	P value	R squared	P value
DA	0.9791	0.0924	0.000158	0.992	0.6907	0.3755	0.7645	0.3226	0.9375	0.1608
DOPAC	0.6999	0.3691	0.187	0.7153	0.9761	0.0988	**0.9948**	**0.0459***	0.5975	0.4375
HVA	0.8666	0.2381	0.2354	0.6775	0.1986	0.7059	0.2688	0.653	0.9307	0.1696
DA turnover rate	0.09047	0.8055	0.8201	0.2789	0.7441	0.3376	0.6685	0.3906	0.03869	0.874
5-HT	0.3259	0.6132	0.5452	0.4712	0.9488	0.1454	0.9061	0.1983	0.2299	0.6817
5-HIAA	0.4543	0.5291	0.4137	0.5552	0.9908	0.0613	0.9682	0.1142	0.3491	0.5976
5-HT turnover rate	0.5365	0.4767	0.3341	0.6077	**0.9998**	**0.0089***	0.9906	0.0618	0.4293	0.5452
DA/5-HT	0.9874	0.0716	0.05884	0.844	0.4382	0.5395	0.5211	0.4865	**1**	**0.0032***
DA turnover rate/5-HT turnover rate	0.4719	0.5179	0.3963	0.5665	0.9938	0.05	0.9741	0.1029	0.3661	0.5863

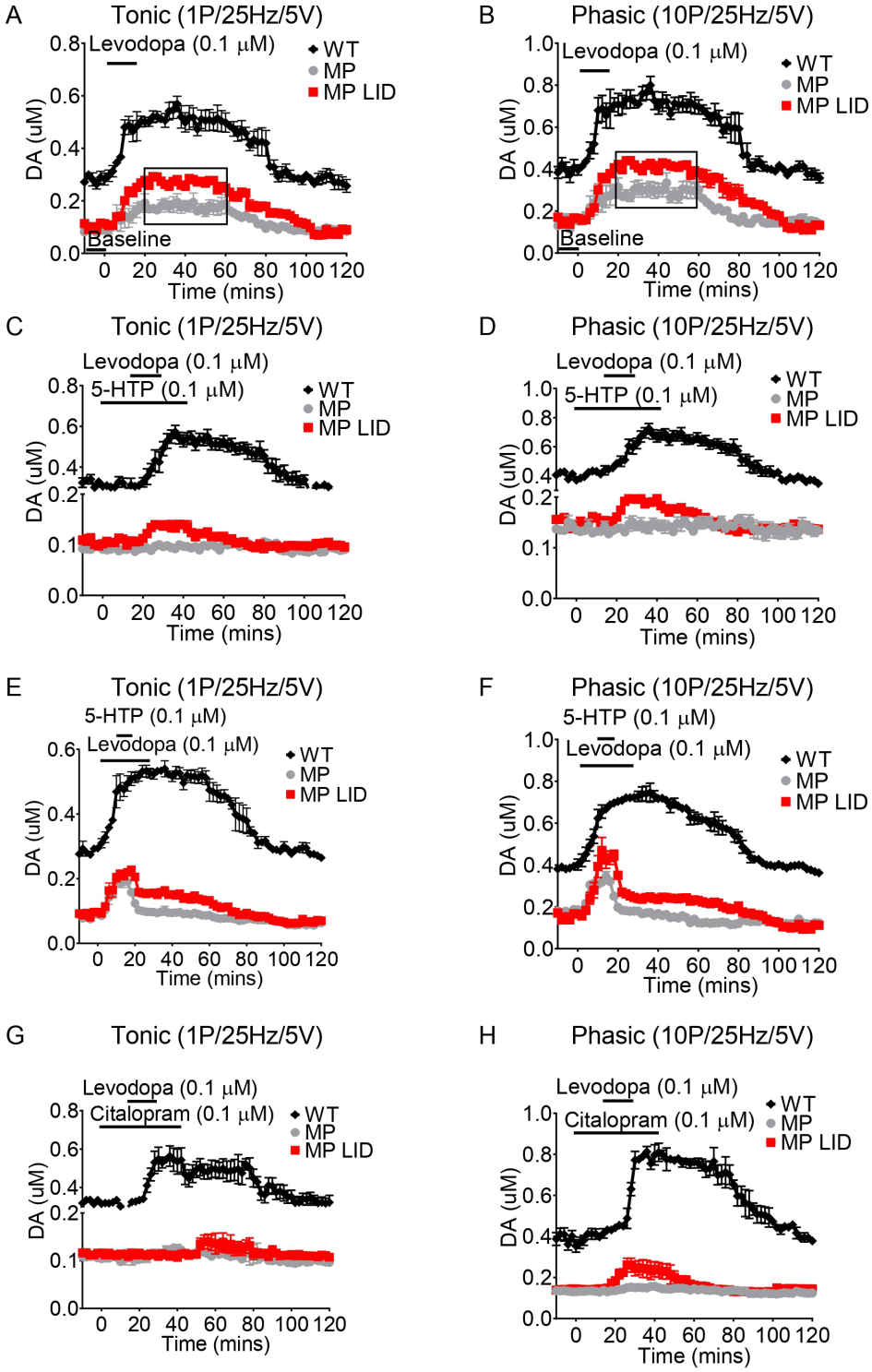
Supplementary Figure 1. Abnormal involuntary movement (AIM) analysis. The AIM scoring used in this study modified the AIM behavior grading of the mouse proposed by Ding and Sebastianutto. The modified AIM behavior grading was used to score four behaviors in experimental animals within one minute: Axial, Forelimb, Standing, and Paw movements. Torsion of the neck/upper trunk by at least 30°, 60°, 90°, and > 90° leads to mice losing their balance, correlating with scores of 1, 2, 3, and 4, respectively. Forelimb movement scores range from 1 to 4, indicating a tiny displacement of the forepaw around a fixed position, large movements causing visible displacement of the entire forelimb, significant displacement of the entire forelimb with visible engagement of shoulder muscles, and vigorous limb displacement crossing over the midline of the body, respectively. Standing involves continuous standing for 1 to 5, 6 to 10, 11 to 15, and exceeding 20 seconds, representing scores of 1, 2, 3, and 4, respectively. Abnormal paw movements are scored by observing the number of paws on the surface of the cylinder wall, with one or both front paws scoring 1 and 2 points, and two front paws and one or both hind paws scoring 3 and 4 points. Each of the four items was recorded once per minute. The highest score achieved within 1 minute based on the above grading is considered the score for this item. The AIM score is calculated as the sum of the four items within a 1-min time frame.



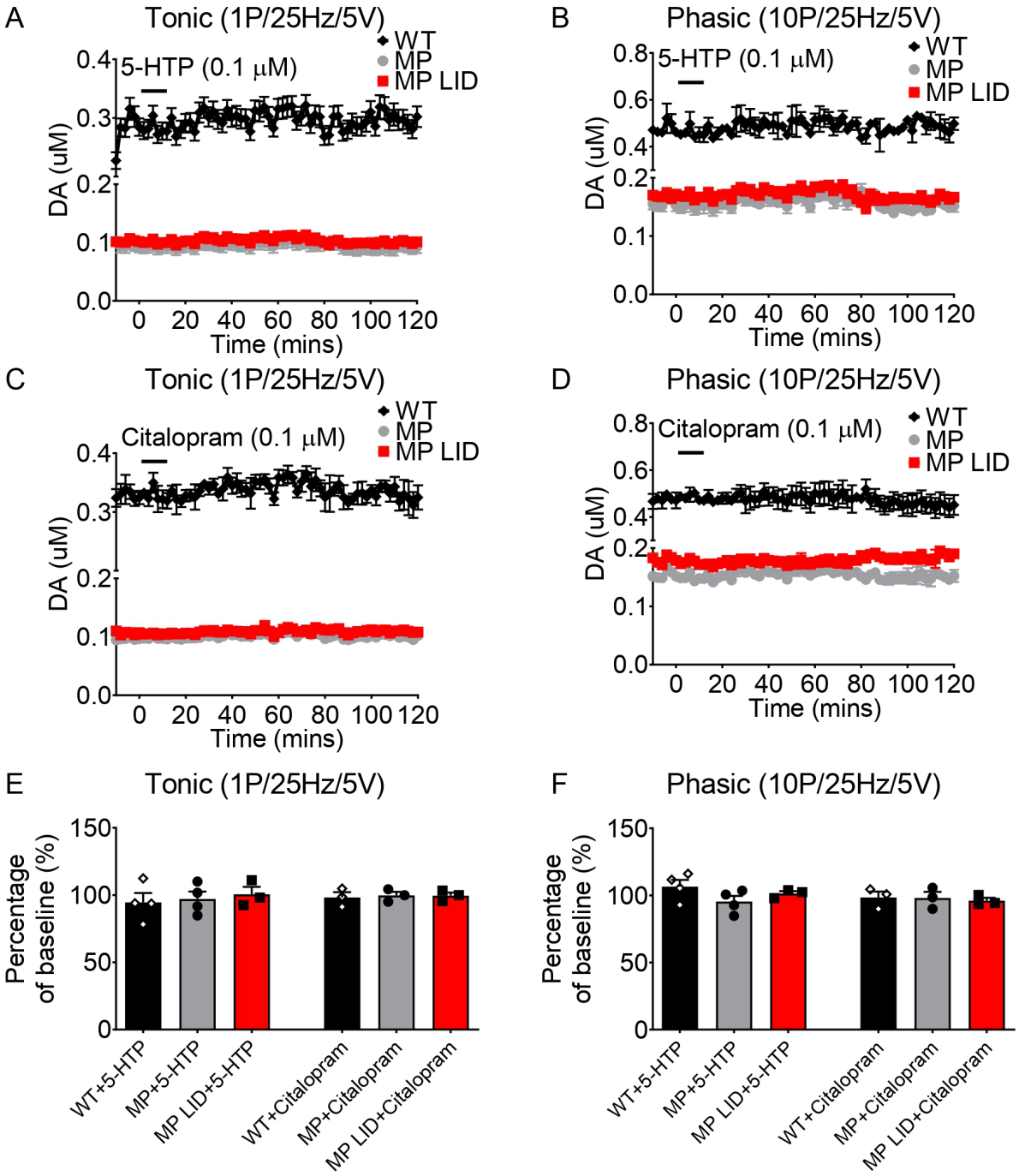
Supplementary Figure 2. AIM scores in MP mice with/without L-DOPA treatment. A) The AIM score in the MP LID group exhibited a significant increase following the injection of L-DOPA (represented by the red line; MP LID + Saline + L-DOPA). Two-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test for multiple comparisons. MP+L-DOPA compared to MP LID+Saline+L-DOPA, *** $p<0.001$. B) The cumulative AIM score at all recording time points showed a significant increase following L-DOPA injection in the MP LID groups. One-way ANOVA followed by a Bonferroni post hoc test for multiple comparisons. MP+L-DOPA compared to MP LID+Saline+L-DOPA, *** $p<0.001$; WT compared to MP LID+Saline+L-DOPA, ### $p<0.001$.



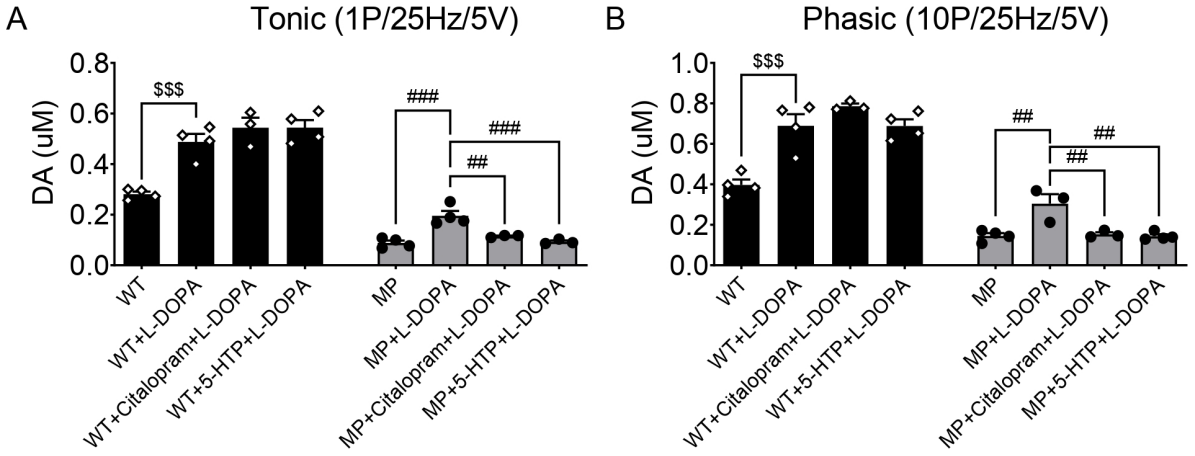
Supplementary Figure 3. The effects of perfusing L-DOPA, 5-HTP, and Citalopram on dopamine release in the dorsal striatum were investigated through FSCV. Perfusing L-DOPA alone effectively increased the release of tonic (A) and phasic (B) in WT, MP, and MP LID mice. Prior perfusion of 5-HTP followed by L-DOPA reperfusion did not affect DA release of tonic (C) and phasic (D) in WT mice, whereas it significantly inhibited DA release in dopamine-deficient MP and MP LID mice, especially in MP mice. Concurrent perfusion of 5-HTP during L-DOPA action also significantly reduced DA release (E, F) in MP and MP LID mice. After prior perfusion with Citalopram followed by L-DOPA reperfusion, the increase in DA release by L-DOPA infusion in tonic (G) and phasic (H) in MP and MP LID mice was reduced, whereas WT mice were unaffected.



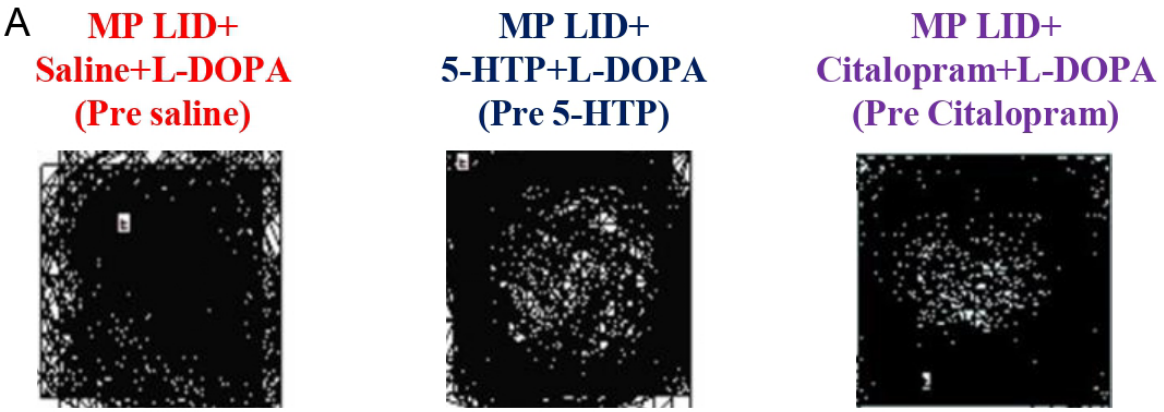
Supplementary Figure 4. DA release in striatal slices of WT, MP and MP LID mice measured by FSCV. In all three groups of mice, including the WT, untreated MP, and MP LID groups, the only administration of 5-HTP did not result in any significant change in tonic release (A). (B) Similar effects of 5-HTP on phasic DA release were found in all three groups of mice. Infusion of Citalopram (C, D) alone did not have a significant effect on tonic or phasic DA release. The infusion of 5-HTP, or Citalopram in WT and MP striatal slices did not have any significant effect on either tonic (E) or phasic (F) dopamine release shown as percentage of change from baseline.

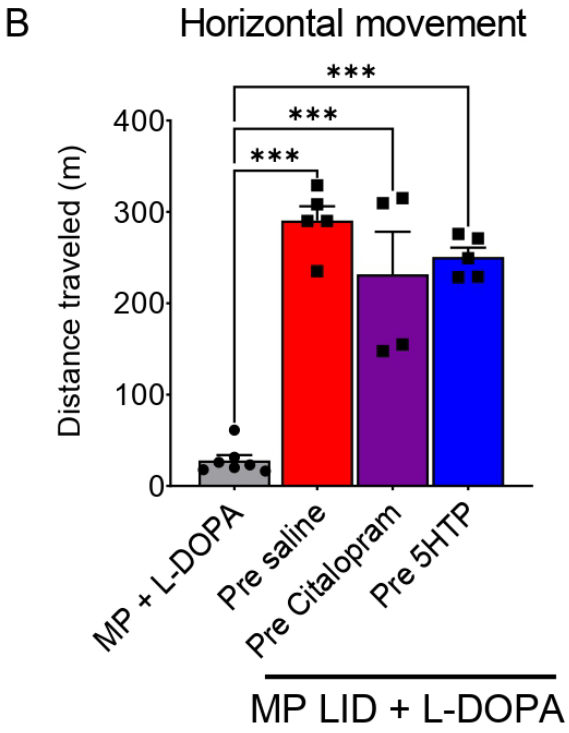


Supplementary Figure 5. FSCV was employed to determine the effects of the 5-HTP and Citalpram on dopamine release following L-DOPA infusion in the dorsal striatum. A, B) The infusion of 5-HTP or Citalopram in the dorsal striatum significantly downregulated the L-DOPA-increased DA release in MP mice with marked dopaminergic denervation ($p<0.01$). However, in WT mice, the infusion of these serotonin modulators in the dorsal striatum did not affect DA release following L-DOPA infusion. One-way ANOVA followed by a Bonferroni post hoc test for multiple comparisons. ## $p<0.01$, ### $p<0.001$, compared to MP+L-DOPA; \$\$\$ $p<0.001$, compared to WT+L-DOPA.

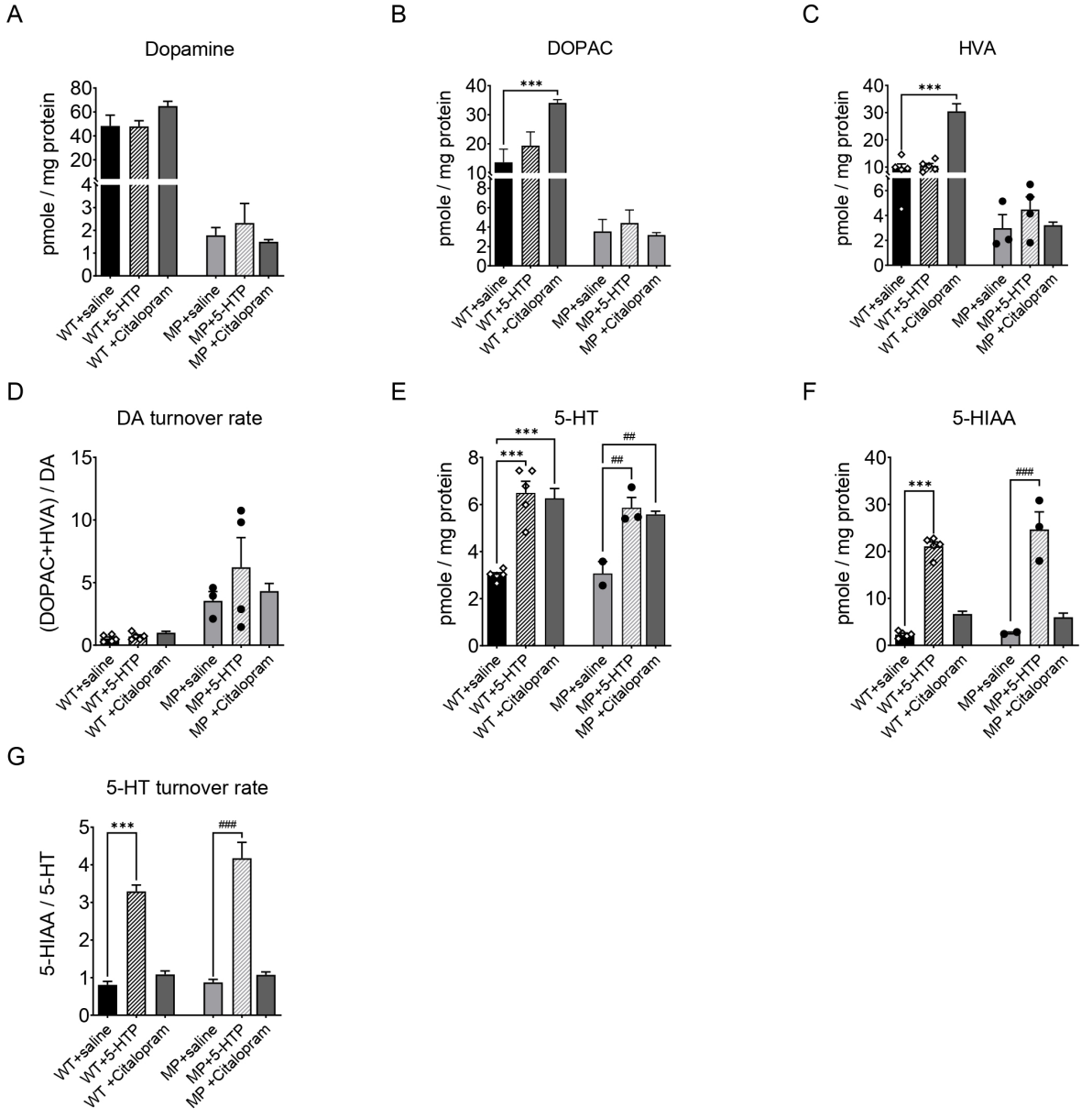


Supplementary Figure 6. Pretreatment with either 5-HTP or Citalopram did not influence horizontal mobility in MP LID mice. A) Representative recording maps of horizontal ambulation analyzed for 60 min following L-DOPA injection. B) There were no significant differences in horizontal movement observed among the Pre 5-HTP, Pre Citalopram and Pre saline group ($F_{3, 21} = 1.21$, $p=0.33$). One-way ANOVA followed by a Bonferroni post hoc test for multiple comparisons. ***$p<0.001$, compared to Pre saline.





Supplementary Figure 7. HPLC measurements of DA and serotonin in the striatum with either 5-HTP or Citalopram treatment. The administration of 5-HTP in WT and MP mice did not have a significant effect on the dopamine concentration (A), DOPAC concentration (B), HVA concentration (C), or DA turnover rate (D). In WT mice, pretreatment with Citalopram led to increased DOPAC and HVA levels, while DA concentration and turnover rate remained unaffected. Moreover, in MP mice, Citalopram pretreatment did not influence the concentration of DA or its metabolites. However, the administration of 5-HTP not only increased serotonin concentration in WT mice (E), but also 5-HIAA concentration in the striatum of both WT and MP mice (F). The administration of 5-HTP also increased serotonin turnover (G) in the dorsal striatum of WT or MP mice. In contrast, pretreatment with Citalopram increased serotonin concentrations in both WT and MP mice, with no changes observed in 5-HIAA levels and 5-HT turnover rate. One-way ANOVA followed by a Bonferroni post hoc test for multiple comparisons. *** $p<0.001$ WT+saline compared to WT+ saline; ## $p<0.01$, ### $p<0.001$, compared to MP+ saline.



Supplementary Figure 8. Linear regression analysis revealed that the level of abnormal dyskinesia induced by L-DOPA injection correlated with the dopamine pathway markers. The total AIM score was linearly correlated with dopamine concentration (Coefficient of determination $r^2 = 0.8323$), but not with the (B) DA turnover rate, (C) 5-HT turnover rate, and (D) 5-HT concentration in MP LID mice. (E) The coefficient of determination r^2 of the linear regression between the standing values in the AIM score and DOPAC concentration reached 0.9948 in MP LID mice. (F) The limb values in the AIM score show a linear regression relationship with the 5-HT turnover rate (Coefficient of determination $r^2 = 0.9998$) in MP LID mice.