題名

Morphologic changes of dendritic spines of striatal neurons in the L-DOPA-induced dyskinesia model

（レボドパ誘発ジスキネジアモデルにおける線条体神経細胞樹状突起上のスパインの形態変化）

脳神経科学領域

神経生理学教育研究分野

西嶌春生　（にしじまはるお）

指導教授

上野　伸哉

Abstract

Back ground: Maladaptive plasticity at corticostriatal synapses plays an important role in the development of L-DOPA-induced dyskinesia. Recently it has been shown that synaptic plasticity is closely linked to morphologic changes of dendritic spines.

Aim: To evaluate morphologic changes of dendritic spines of two types of striatal medium spiny neurons, which project to the internal segment of globus pallidus or the external segment of globus pallidus, in 6-hydroxydopamine-lesioned rats chronically treated with L-DOPA, a model of L-DOPA-induced dyskinesia.

Results: Dendritic spines were decreased and became enlarged in the direct pathway neurons of the model of L-DOPA-induced dyskinesia. The same L-DOPA treatment to normal rats, in which no dyskinesia was observed, also induced enlargement of dendritic spines, but not a decrease in density of spines in the direct pathway neurons.

Conclusion: These results suggest that a loss and enlargement of dendritic spines in the direct pathway neurons plays important roles in the development of L-DOPA-induced dyskinesia.

1. Introduction

The dopamine precursor, L-DOPA, remains the most effective drug to alleviate motor symptoms of Parkinson's disease (PD). However, with the progression of PD and accompanying loss of dopamine neurons, the capacity of brain to restore and maintain physiological levels of dopamine is impaired in the striatum after administration of L-DOPA [1]. This leads to unphysiological pulsatile stimulation of striatal dopamine receptors and results in the development of L-DOPA-induced dyskinesia (LID) that limits L-DOPA utility [2] [3] [4] [5]. The precise mechanisms underlying LID are not clearly understood yet.

LID has been regarded as behavioral expression of maladaptive synaptic plasticity within the basal ganglia [6] [7]. A critical association between LID and abnormal synaptic plasticity at corticostriatal synapses is demonstrated in a rat model of LID. Once long term potentiation (LTP) at corticostriatal synapses induced by high frequency stimulation in the LID model, LTP is not reversed after low frequency stimulation, which indicates lack of depotentiation [6]. In recent years it has been reported that synaptic plasticity is closely linked to morphologic changes of dendritic spines [8] [9]. We have shown that dendritic spines of striatal medium spiny neurons (MSNs) are decreased and enlarged in a rat model of LID, 6-hydroxydopamine (6-OHDA) - lesioned rats repeatedly treated with L-DOPA [10]. Additionally, Zhang et al. have recently shown in the LID model that the number of corticostriatal endings to MSNs are increased and density of mushroom-like spines was greater compared with the control group [11]. Mushroom-like spines have

larger postsynaptic densities and can receive multisynaptic excitatory inputs. Thus the enlargement of spines may be a morphologic basis of the electrophysiological abnormality, lack of depotentiation in MSNs of the LID model described by Picconi et al [6]. However, there are two types of projection neurons in the striatum. One expresses the D1 dopamine receptor and projects to the internal segment of globus pallidus (GPi) and the substantia nigra pars reticulata, forming the first part of the direct pathway of the basal ganglionic circuit [12]. The other expresses the D2 dopamine receptor and projects to the external segment of globus pallidus (GPe), making up the first part of the indirect pathway of the circuit [12]. It remains to be concluded which type of striatofugal projection neurons, neurons of the direct pathway or neurons of the indirect pathway, undergo enlargement of spines in the rat model of LID.

Biochemical examinations have suggested that hypersensitivity of D1 dopamine receptors in striatal neurons might be responsible for expression of LID [13] [14] [15] [16]. Therefore, we hypothesized that dendritic spines of MSNs of the direct pathway change their morphology accounting for the lack of depotentiation in the LID model [6]. To verify the hypothesis, we examined spine pathology of putative direct pathway neurons and indirect pathway neurons in the striatum of the LID model rats.

2. Methods

In total, 42 Wister rats (CLEA, Japan) were used in this study. They were all males, 8 weeks old and weighed 260 to 290 g each at the beginning of the experiment. All efforts were made to minimize the number of animals used and their suffering. All experimentations were carried out in accordance with the Guidelines for Animal Experimentation, Hirosaki University.

The 6-OHDA-lesioning was performed in 26 rats and the lesioning was validated as previously described [17]. We injected 6-OHDA into the right medial forebrain bundle and the dopaminergic denervation was confirmed by apomorphine-induced rotational behavior. We have demonstrated that the rats with this validation were found to lose more than 99% of dopamine in the striatum [18].

The 26 6-OHDA-lesioned rats were randomly allocated to one of two groups. One group received intraperitoneal injections of L-DOPA methyl ester, 50 mg/kg in combination with benserazide, 12.5 mg/kg, twice daily for 2 weeks (LID model, n = 13). The other group received the same dose of saline (PD model, n = 13).

Sixteen control rats received the saline (vehicle control, n = 11) or received L-DOPA treatment as described above (L-DOPA control, n = 5). The dose of L-DOPA in this study was very high and lower L-DOPA doses can induce abnormal involuntary movements (AIMs) [19]. We used high dose of L-DOPA because we previously confirmed that this dose of L-DOPA induced unphysiological fluctuation of extracellular dopamine in the 6-OHDA-lesioned striatum with dyskinesia [17].

To confirm behavioral sensitization after L-DOPA treatment, we measured AIM score according to the method described previously [20]. We measured the score on day 1, 4, and 11 of L-DOPA treatment. All 13 rats which received L-DOPA treatment after 6-OHDA-lesioning showed AIM severity grade ≥ 2 on day 11. The total AIM score of the LID model of day 11 was over twice as that of day 1 (Figure 1).

For visualizing striatal neurons of the direct pathway or the indirect pathway, Fast Blue (Polysciences, Inc., ref No. 74749-42-1) was injected to GPi or GPe of each rat 4 days before sacrifice, respectively. The tracer could be taken up through fibers of passage following injections, however, it has been reported that tracer injections in the rat GPi almost exclusively label the direct pathway neurons [21], and those in the rat GPe mainly label the neurons of the indirect pathway (about 75%) [21]. We chose GPi instead of substantia nigra pars reticulata for retrograde labeling of direct pathway neurons, for we previously confirmed the morphological change in GPi in the LID model and consider GPi to play an important role in emergence of LID [22]. Fast Blue was injected through a stainless steel needle in the GPi (2.3 mm posterior to the bregma, 2.8 mm lateral to the sagittal suture, and 8.0 mm ventral to the periosteum surface) or in the GPe (1.3 mm posterior to the bregma, 3.2 mm lateral to the sagittal suture, and 6.5 mm ventral to the periosteum surface) according to the atlas of Paxinos and Watson [23]. The volume of the tracer was 22.5 μg / 0.75 μL in saline to GPi or 30 μg / 1.0 μL in saline to GPe. In the brain slices of rats the tracer deposits were restricted to the targeted areas (Figure 2). We injected Fast Blue to right GPi and left GPe (n = 5), right GPi (n = 3), or right GPe (n = 3) of the vehicle control rats. We also injected Fast

Blue to right GPi (n = 7) or right GPe (n = 6) of the PD model, right GPi (n = 6) or right GPe (n = 7) of the LID model. With the L-DOPA control we injected Fast Blue to right GPi and left GPe (n = 5). In PD model and LID model rats, Fast Blue was injected to the ipsilateral side of the 6-OHDA-lesion. Fast Blue labeled neurons in the striatum (Str) retrogradely. We could visualize striatal neurons that project to either GPi (Str-GPi neurons; putative direct pathway neurons) or GPe (Str-GPe neurons; putative indirect pathway neurons).

Four days after Fast Blue injection (12 hours after the last treatment), rats were perfused transcardially with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (1200 ml/kg). After perfusion, the brains were taken out from the skull and cut into serial sections (250 μm thick) through the anterior striatum with the aid of Microslicer (D.S.K: DTK-1000; Dosaka EM, Kyoto, Japan)

Individual sections were mounted between Millipore filters (AABG02500, Billerica, MA). The slice was mounted in a perspex dish on a fixed stage microscope (Optiphot2-UD microscope, Nikon, Tokyo, Japan). Then we could observe cell bodies of Str-GPi neurons or Str-GPe neurons labeled by Fast Blue under UV excitation (380-420 nm). We electronically microinjected Lucifer Yellow CH dilithium salt (Sigma L0259-25MG, St Louis, MO) into somata of medium-sized spiny neurons labeled by Fast Blue located at the dorsolateral striatum under visual guidance with continuous negative current through a patch pipette. The dendritic spines were then clearly visible. The sections were mounted onto slides, then coverslipped with Permount, SlowFade Gold Antifade Reagent (S36937, Invitrogen, Tokyo, Japan). The

tissue was examined using a confocal microscopy system (C1si, Nikon, Tokyo, Japan).

Yellow signals (515 / 30 nm) were acquired using 488 nm B excitation from each sample. Fluorescence projection images of somata and dendritic fields were acquired with a 60× oil-immersion lens. We randomly drew 1 to 11 cells from each rat, and 1 to 5 horizontally-projecting dendrites from each cell. Then we determined the analyzed area (20 µm dendrite-length from 50 to 100 µm away from a soma) in each dendrite where spine density is relatively uniform [24] [25]. Projection images of spines in the analyzed areas of each dendrite were acquired with 0.0069-μm^2 pixels and consisted of 15 to 96 images taken at 0.25 µm focal steps. Image stacks were 3D-deconvoluted using the NIS elements imaging software (Nicon, Tokyo, Japan) and volume rendered as 2D-images to facilitate overview of the figures (Figure 3a-c). Image analysis was performed using ImageJ (National Institutes of Health, Bethesda, MD). Estimates of spine density and spine head area were based upon projection 2D-images. Each spine was manually traced. The spine density was expressed as an average spine number per 10 µm of linear dendritic length (Figure 3b). Then we measured cross sectional areas of spine head (Figure 3c). In total, we measured 10231 spines from 258 neurons in 51 striata; 8 striata labeled from GPi in vehicle control rats, 7 striata labeled from GPe in vehicle control rats, 7 striata labeled from GPi in PD model rats, 6 striata labeled from GPe in PD model rats, 6 striata labeled from GPi in LID model rats, 7 striata labeled from GPe in LID model rats, 5 striata labeled from GPi in L-DOPA control rats, and 5 striata labeled from GPe in L-DOPA control rats. Statistics were performed using the computer software program

Ekuseru-Toukei 2008 (Social Survey Research Ingomation Co.,Ltd., Japan) and the Excel (Microsoft, USA). All data were expressed as means ± standard error of the mean. We calculated the average of spine density of each neuron and then the average of each rat. In cross sectional area of spine head we calculated the average of the mushroom-like spines of each dendrite and then the average of each neuron, and each rat. The averages of spine density and the averages of cross sectional area of spine head in each rat were submitted to analyses. The significance of differences in the density of striatal spines or cross sectional areas of spine head labeled from GPi or GPe between the vehicle control, the PD model, the LID model, and the L-DOPA control was evaluated using two-way analysis of variance followed by post hoc comparisons with Tukey-Kramer method. We also evaluated the significance of differences among AIM scores of day 1, day 4, and day 11 with L-DOPA treatment of the LID model or those of the L-DOPA control using Kruskal-Wallis test followed by post hoc comparisons with Steel-Dwass methods. On all analyses differences were considered to be statistically significant at a level of $p < 0.05$.

3. Results

In the PD model spine density of Str-GPe neurons was decreased when compared to the vehicle control (Figure 4b,d,k) ($F_{1,21}$ = 6.9994, p = 0.0151). Spine density was unchanged in Str-GPi neurons in the PD model (Figure 4a,c,k), however the density of spines of Str-GPi neurons in the LID model was significantly decreased when compared to that of the PD control (Figure 4c,e,k) ($F_{1,22}$ = 4.8644, p = 0.0382). L-DOPA treatment to normal rats induced no changes in spine density of either neurons when compared to the vehicle control (Figure 4a,b,g,h,k). Thus, dopaminergic denervation may cause a loss of spines in Str-GPe neurons and dopaminergic denervation plus repeated L-DOPA treatment with development of LID may induce a loss of spines in Str-GPi neurons. Accordingly, spine losses of Str-GPe neurons and Str-GPi neurons may be associated with development of parkinsonian symptoms and of LID, respectively.

Spines of Str-GPi neurons in the LID model significantly became enlarged when compared to the PD model and L-DOPA controls (Figure 4c,e,g,l) ($F_{1,22}$ = 27.3462, p < 0.0001 and $F_{1,22}$ = 10.1836, p = 0.0042, respectively). Additionally, the size of spines in Str-GPi neurons of L-DOPA controls was larger than that of vehicle controls (Figure 4a,g,l) ($F_{1,22}$ = 11.7634, p = 0.0024). As well as Str-GPi neurons, dendritic spines were increased in their size in Str-GPe neurons of the LID model when compared to the PD model (Figure 4b,f,i,l) ($F_{1,21}$ = 11.0495, p = 0.0032). Str-GPe neurons of L-DOPA controls also had enlarged spines when compared to those of vehicle controls (Figure 4b,h,j,l) ($F_{1,21}$ = 7.5251, p = 0.0122). Thus, L-DOPA treatment

appeared to induce hypertrophy of dendritic spines of both Str-GPi and Str-GPe neurons in the LID model and the L-DOPA control.

Dendritic spines of Str-GPi neurons in the LID model and the L-DOPA control became uniformly enlarged (Figure 4e,g). However, some neurons of Str-GPe neurons of the LID model and the L-DOPA controls had normal-sized dendritic spines (Figure 4f,h) and other neurons of them had enlarged spines (Figure 4i,j). Thus, L-DOPA treatment uniformly induced enlargement of spines in Str-GPi neurons. However, Str-GPe neurons consisted of two types of neurons; one with enlarged spines and the other with normal-sized spines after chronic L-DOPA treatment.

4. Discussion

The important results obtained in this study were that dendritic spines of striatal neurons with projection to GPi became enlarged and less dense in 6-OHDA-lesioned rats chronically treated with L-DOPA, a model of L-DOPA-induced dyskinesia. These morphologic changes suggest that the striatal direct pathway neurons are highly responsive to glutamatergic drive from the cortex in the dyskinesia model. Our results fit well with structural modifications, increased corticostriatal contacts to MSNs [11], and electrophysiological plastic abnormality, lack of depotentiation, demonstrated at corticostriatal synapses in the dyskinesia model [6].

4.1. Spine density and L-DOPA-induced dyskinesia

It has been demonstrated that spine density in the striatum decreases in patients with PD [26] [27] and animal models of the disease [28] [29] [30].

Our results showed that dopaminergic denervation appeared to decrease the density of spines in Str-GPe neurons (Figure 4k). Since many of Str-GPe neurons are those of indirect pathway neurons [21], our results were compatible with a previous report in rodents [28]. Dopaminergic denervation induced no significant changes in density of dendritic spines in Str-GPi neurons.

The spine density changes in patients with LID have not been investigated yet. The LID model in the present study showed a decrease in spine density in Str-GPi neurons. The projection neurons from the striatum to the GPi

exclusively consist of direct pathway neurons [21]. L-DOPA treatment following dopaminergic denervation appeared to decrease spine density in the direct pathway neurons (Figure 4a,e,k). The decrement of dendritic spines of direct pathway neurons is the firstly described pathological synaptic change in LID models, and could be associated with the emergence of LID.

4.2. Spine enlargement and L-DOPA-induced dyskinesia

Repeated L-DOPA treatment following dopaminergic denervation increased the size of spine head of both Str-GPi and Str-GPe neurons when compared to the PD model rats (Figure 4l). Since the projection neurons from the striatum to the GPi exclusively consist of the direct pathway neurons [21], we could conclude that dendritic spines of the direct pathway neurons are enlarged in the LID model. On the other hand, although projections from the striatum to the GPe are mainly axons of the indirect pathway, some projections (about 25%) are collaterals from the direct pathway neurons [21]. The findings that some Str-GPe neurons had enlarged spines and others had normal-sized spines (Figure 4f,i) suggest that spine enlargement in Str-GPe neurons in the LID model could be due to the enlargement of spines of the direct pathway neurons retrogradely labeled via collaterals to GPe. Thus, from these results involvement of the indirect pathway in the development of LID remains to be concluded.

Picconi et al. [6] have demonstrated the lack of depotentiation in rats with LID. Additionally, this lack of depotentiation is modified by administration of the D1 dopamine receptor agonist [6], suggesting the

electrophysiological phenotype was linked to the synaptic plasticity of the direct pathway neurons. It has been demonstrated that enlarged spines become more sensitive to glutamate [31]. Thus the enlargement of dendritic spines in the direct pathway neuron could fit with the increased multisynaptic excitatory inputs to MSNs from cortex [11], hypersensitivity to glutamate demonstrated in the dopamine-denervated striatum [32] [33] and lack of depotentiation in the rat model of LID [6].

Repeated L-DOPA treatment administered to normal rats also increased spine size of both Str-GPi and Str-GPe neurons (Figure 4g,h,j,l) although L-DOPA controls did not display dyskinesia (Figure 1). Some Str-GPe neurons of the L-DOPA controls had normal-sized spines as in the LID model (Figure 4h,j). Str-GPe neurons with enlarged spines in L-DOPA controls also might be those of direct pathway. This observation indicates that only spine enlargement is not enough to induce LID. It has been reported that L-DOPA treatment induces dyskinesias in normal monkeys [34]. Pulsatile stimulation of striatal dopamine receptors is essential for induction of LID [2] [3] [4] [5]. We have shown that striatal extracellular dopamine concentration unphysiologically fluctuates after high dose L-DOPA treatment even in the striatum of normal rats [18]. Fluctuation of dopamine in the normal striatum after the L-DOPA treatment in the present study might be enough to induce spine enlargement but not enough to induce dyskinesia.

Spine morphology could change depending on the dendritic branching pattern [24] [26], and the dendritic branching patterns are different depending on the compartmental organization of the striatum [35] [36]. Striatum has a mosaic organization composed of striosome and matrix compartment. The

matrix compartment gives rise to both direct and indirect pathway neurons, whereas the striosome compartment has been considered to involve direct pathway neurons alone. However, a recent study indicates that not only matrix but also striosome compartments contain both direct and indirect pathway neurons [35]. We did not take striosome/matrix organization into consideration in the present study. Str-GPi neurons, putative direct pathway neurons in our study might contain MSNs in striosome and matrix compartment. Detailed anatomical examinations are needed to elucidate exact changes of spine morphology.

5. Conclusion

Dopaminergic denervation alone induced no significant changes in the density and morphology of dendritic spines in MSNs of the direct pathway. Dopaminergic denervation plus chronic L-DOPA treatment induced a spine loss and spine enlargement in the direct pathway neurons with development of dyskinesia. L-DOPA treatment to rats without dopaminergic denervation induced spine enlargement only. Thus the spine enlargement accompanied by a spine loss in the direct pathway neurons might be the characteristics for priming of LID.

Acknowledgement

This study was supported by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (no. 22590952) to Masahiko Tomiyama.

We especially thank Mr. Tomofumi Oga, Ms. Takayo Ito, and Dr. Paul Hollister for their excellent technical advice and Ms. Saeko Osanai for her kind support in our laboratory.

References

1. Cenci MA: **Dopamine dysregulation of movement control in L-DOPA-induced dyskinesia**. *Trends Neurosci* 2007, **30**(5):236-243.

2. Olanow CW, Obeso JA: **Preventing levodopa-induced dyskinesias**. *Ann Neurol* 2000, **47**(4 Suppl 1):S167-176; discussion S176-168.

3. Olanow CW, Obeso JA, Stocchi F: **Continuous dopamine-receptor treatment of Parkinson's disease: scientific rationale and clinical implications**. *Lancet Neurol* 2006, **5**(8):677-687.

4. Pavese N, Evans AH, Tai YF, Hotton G, Brooks DJ, Lees AJ, Piccini P: **Clinical correlates of levodopa-induced dopamine release in Parkinson disease: a PET study**. *Neurology* 2006, **67**(9):1612-1617.

5. Calabresi P, Di Filippo M, Ghiglieri V, Tambasco N, Picconi B: **Levodopa-induced dyskinesias in patients with Parkinson's disease: filling the bench-to-bedside gap**. *Lancet Neurol* 2010, **9**(11):1106-1117.

6. Picconi B, Centonze D, Hakansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P: **Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia**. *Nat Neurosci* 2003, **6**(5):501-506.

7. Picconi B, Paille V, Ghiglieri V, Bagetta V, Barone I, Lindgren HS, Bernardi G, Angela Cenci M, Calabresi P: **l-DOPA dosage is critically involved in dyskinesia via loss of synaptic depotentiation**. *Neurobiol Dis* 2008, **29**(2):327-335.

8. Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H: **Structural basis of long-term potentiation in single dendritic spines**. *Nature* 2004, **429**(6993):761-766.

9. Kasai H, Fukuda M, Watanabe S, Hayashi-Takagi A, Noguchi J: **Structural dynamics of dendritic spines in memory and cognition**. *Trends Neurosci* 2010, **33**(3):121-129.

10. Nishijima H, Arai A, Kimura T, Mori F, Yamada J, Migita K, Wakabayashi K, Baba M, Ueno S, Tomiyama M: **Drebrin immunoreactivity in the striatum of a rat model of levodopa-induced dyskinesia**. *Neuropathology* 2013, **33**(4):391-396.

11. Zhang Y, Meredith GE, Mendoza-Elias N, Rademacher DJ, Tseng KY, Steece-Collier K: **Aberrant restoration of spines and their synapses in L-DOPA-induced dyskinesia: involvement of**

corticostriatal but not thalamostriatal synapses. *J Neurosci* 2013, **33**(28):11655-11667.

12. DeLong MR, Wichmann T: **Circuits and circuit disorders of the basal ganglia**. *Arch Neurol* 2007, **64**(1):20-24.

13. Gerfen CR: **D1 dopamine receptor supersensitivity in the dopamine-depleted striatum animal model of Parkinson's disease**. *Neuroscientist* 2003, **9**(6):455-462.

14. Santini E, Valjent E, Fisone G: **Parkinson's disease: levodopa-induced dyskinesia and signal transduction**. *FEBS J* 2008, **275**(7):1392-1399.

15. Bateup HS, Santini E, Shen W, Birnbaum S, Valjent E, Surmeier DJ, Fisone G, Nestler EJ, Greengard P: **Distinct subclasses of medium spiny neurons differentially regulate striatal motor behaviors**. *Proc Natl Acad Sci U S A* 2010, **107**(33):14845-14850.

16. Iravani MM, McCreary AC, Jenner P: **Striatal plasticity in Parkinson's disease and L-dopa induced dyskinesia**. *Parkinsonism Relat Disord* 2012, **18 Suppl 1**:S123-125.

17. Tanaka H, Kannari K, Maeda T, Tomiyama M, Suda T, Matsunaga M: **Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats**. *Neuroreport* 1999, **10**(3):631-634.

18. Maeda T, Kannari K, Suda T, Matsunaga M: **Loss of regulation by presynaptic dopamine D2 receptors of exogenous L-DOPA-derived dopamine release in the dopaminergic denervated striatum**. *Brain Res* 1999, **817**(1-2):185-191.

19. Lindgren HS, Andersson DR, Lagerkvist S, Nissbrandt H, Cenci MA: **L-DOPA-induced dopamine efflux in the striatum and the substantia nigra in a rat model of Parkinson's disease: temporal and quantitative relationship to the expression of dyskinesia**. *J Neurochem* 2010, **112**(6):1465-1476.

20. Cenci MA, Lundblad M: **Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice**. *Curr Protoc Neurosci* 2007, **Chapter 9**:Unit 9 25.

21. Deng YP, Lei WL, Reiner A: **Differential perikaryal localization in rats of D1 and D2 dopamine receptors on striatal projection neuron types identified by retrograde labeling**. *J Chem Neuroanat* 2006, **32**(2-4):101-116.

22. Tomiyama M, Mori F, Kimura T, Ichinohe N, Wakabayashi K, Matsunaga M, Baba M: **Hypertrophy of medial globus pallidus and substantia nigra reticulata in 6-hydroxydopamine-lesioned rats treated with L-DOPA: implication for L-DOPA-induced dyskinesia in Parkinson's disease**. *Neuropathology* 2004, **24**(4):290-295.

23. Paxinos G, Watson C: *The rat brain in stereotaxic coordinates*. 6th edition. Amsterdam ; Boston ;: Academic Press/Elsevier; 2007.

24. Wilson CJ: **Understanding the neostriatal microcircuitry: high-voltage electron microscopy**. *Microsc Res Tech* 1994, **29**(5):368-380.

25. Neely MD, Schmidt DE, Deutch AY: **Cortical regulation of dopamine depletion-induced dendritic spine loss in striatal medium spiny neurons**. *Neuroscience* 2007, **149**(2):457-464.

26. Stephens B, Mueller AJ, Shering AF, Hood SH, Taggart P, Arbuthnott GW, Bell JE, Kilford L, Kingsbury AE, Daniel SE *et al*: **Evidence of a breakdown of corticostriatal connections in Parkinson's disease**. *Neuroscience* 2005, **132**(3):741-754.

27. Zaja-Milatovic S, Milatovic D, Schantz AM, Zhang J, Montine KS, Samii A, Deutch AY, Montine TJ: **Dendritic degeneration in neostriatal medium spiny neurons in Parkinson disease**. *Neurology* 2005, **64**(3):545-547.

28. Day M, Wang Z, Ding J, An X, Ingham CA, Shering AF, Wokosin D, Ilijic E, Sun Z, Sampson AR *et al*: **Selective elimination of glutamatergic synapses on striatopallidal neurons in Parkinson disease models**. *Nat Neurosci* 2006, **9**(2):251-259.

29. Villalba RM, Lee H, Smith Y: **Dopaminergic denervation and spine loss in the striatum of MPTP-treated monkeys**. *Exp Neurol* 2009, **215**(2):220-227.

30. Soderstrom KE, O'Malley JA, Levine ND, Sortwell CE, Collier TJ, Steece-Collier K: **Impact of dendritic spine preservation in medium spiny neurons on dopamine graft efficacy and the expression of dyskinesias in parkinsonian rats**. *Eur J Neurosci* 2010, **31**(3):478-490.

31. Holtmaat A, Svoboda K: **Experience-dependent structural synaptic plasticity in the mammalian brain**. *Nat Rev Neurosci* 2009, **10**(9):647-658.

32. Silverdale MA, Kobylecki C, Hallett PJ, Li Q, Dunah AW, Ravenscroft P, Bezard E, Brotchie JM: **Synaptic recruitment of AMPA glutamate receptor subunits in levodopa-induced dyskinesia in the MPTP-lesioned nonhuman primate**. *Synapse* 2010, **64**(2):177-180.

33. Cenci MA, Konradi C: **Maladaptive striatal plasticity in L-DOPA-induced dyskinesia**. *Prog Brain Res* 2010, **183**:209-233.

34. Togasaki DM, Tan L, Protell P, Di Monte DA, Quik M, Langston JW: **Levodopa induces dyskinesias in normal squirrel monkeys**. *Ann Neurol* 2001, **50**(2):254-257.

35. Fujiyama F, Sohn J, Nakano T, Furuta T, Nakamura KC, Matsuda W, Kaneko T: **Exclusive and common targets of neostriatofugal projections of rat striosome neurons: a single neuron-tracing study using a viral vector**. *Eur J Neurosci* 2011, **33**(4):668-677.

36. Kawaguchi Y, Wilson CJ, Emson PC: **Intracellular recording of identified neostriatal patch and matrix spiny cells in a slice preparation preserving cortical inputs**. *J Neurophysiol* 1989, **62**(5):1052-1068.

Figure legends

Figure 1

The abnormal involuntary movement (AIM) scores in 6-hydroxydopamine (6-OHDA) -lesioned rats treated with L-DOPA (L-DOPA-induced dyskinesia (LID) model) and non-lesioned rats treated with L-DOPA (L-DOPA control).

The LID model developed AIMs. The AIM score significantly increased along with the treatment. On the other hand the L-DOPA control displayed no AIMs.

＊; $p < 0.05$　＊＊; $p < 0.01$

LID; L-DOPA-induced dyskinesia

Figure 2

Examples of Fast Blue injection sites in rats' brains.

a; A injection site in the internal part of globus pallidus (GPi).

b; A injection site in the external part of globus pallidus (GPe).

Images were taken using light field microscopy, and the injection sites are indicated by the black circles. The Fast Blue deposits were located in the targeted areas, GPi or GPe.

In the present study, to simplify discussions of similarities between primates and rodents, we use the same terminology for rodents as commonly used in primates for the two subdivisions of the globus pallidus. The structure commonly called the globus pallidus in rodents is instead called the GPe, while the structure commonly called the entopeduncular nucleus in rodents is instead called the GPi, for widely accepted homology.

Figure 3

Confocal microscopy and Image analysis.

The representational confocal laser scanning images of a medium-sized spiny neuron visualized by Lucifer Yellow. We measured density and the size of spine head on dendrites from 50 to 100 µm away from cell bodies (Figure 3a). An average number of spines per 10 µm of linear dendritic length was expressed as density of spines (Figure 3b, zoomed up image of the white box in Figure 3a). Then we measured the area of spine heads in 2-dimensional reconstructed images (Figure 3c, zoomed up image of the white box in Figure 3b).

Figure 4

Photomicrographs of dendritic spines labeled by Lucifer Yellow of striatum (Str) -globus pallidus internus (GPi) neurons (a, c, e, g) and Str-globus pallidus externus (GPe) neurons (b, d, f, h, i, j). The averages of spine density and cross sectional areas of spine head.

a; striatum-internal segment of the globus pallidus (Str-GPi) neurons of the vehicle control.
b; striatum-external segment of the globus pallidus (Str-GPe) neurons of the vehicle control.
c; Str-GPi neurons of the Parkinson's disease (PD) model.
d; Str-GPe neurons of the PD model.
e; Str-GPi neurons of the L-DOPA-induced dyskinesia (LID) model.
f; Str-GPe neurons with normal-sized spines of the LID model.
g; Str-GPi neurons of the L-DOPA control.
h; Str-GPe neurons with normal-sized spines of the L-DOPA control.
i; Str-GPe neurons with enlarged spines of the LID model.
j; Str-GPe neurons with enlarged spines of the L-DOPA control.
k; The averages of spine density of Str-GPi neurons (left) and Str-GPe neurons (right).
l; The averages of spine head area of Str-GPi neurons (left) and Str-GPe neurons (right).

Scale bar; 5 μm
＊; $p < 0.05$　＊＊; $p < 0.01$

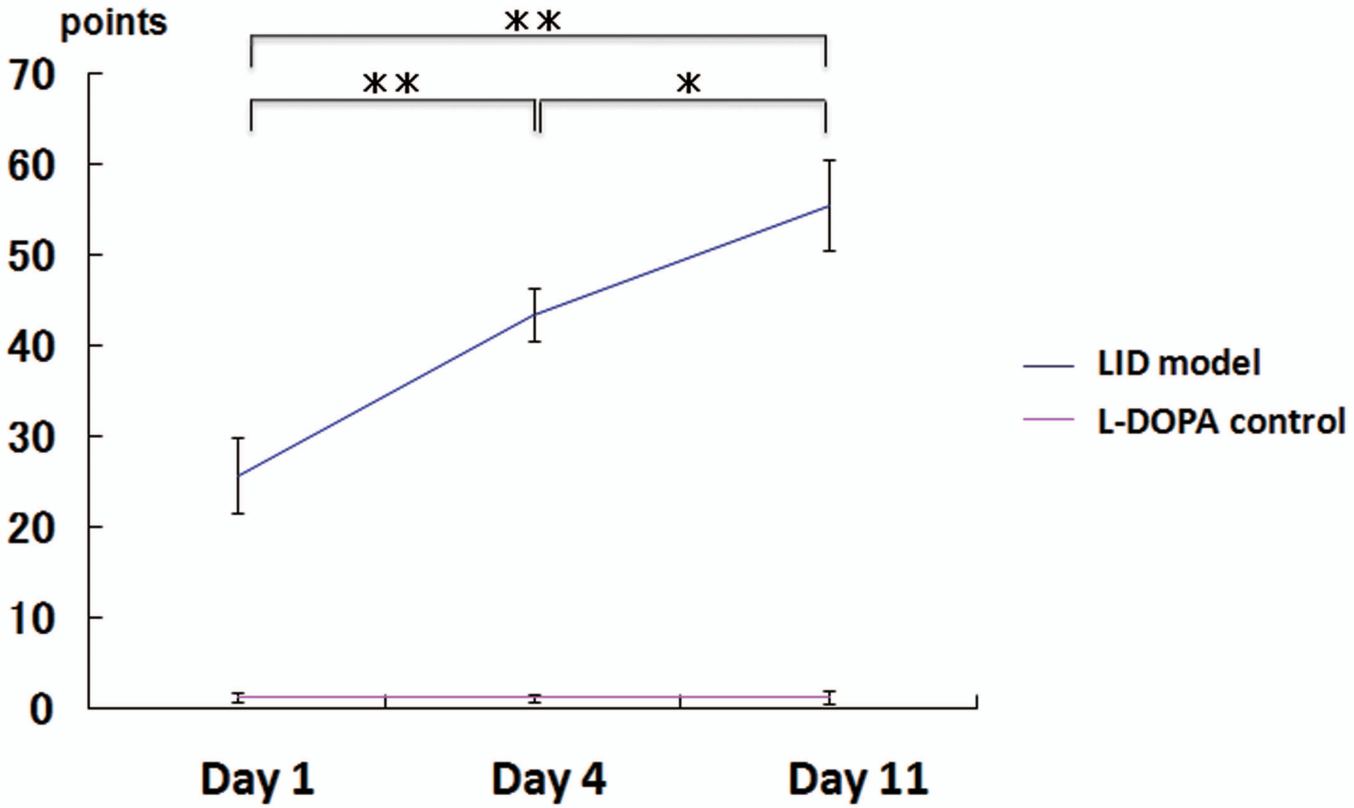
Str; striatum

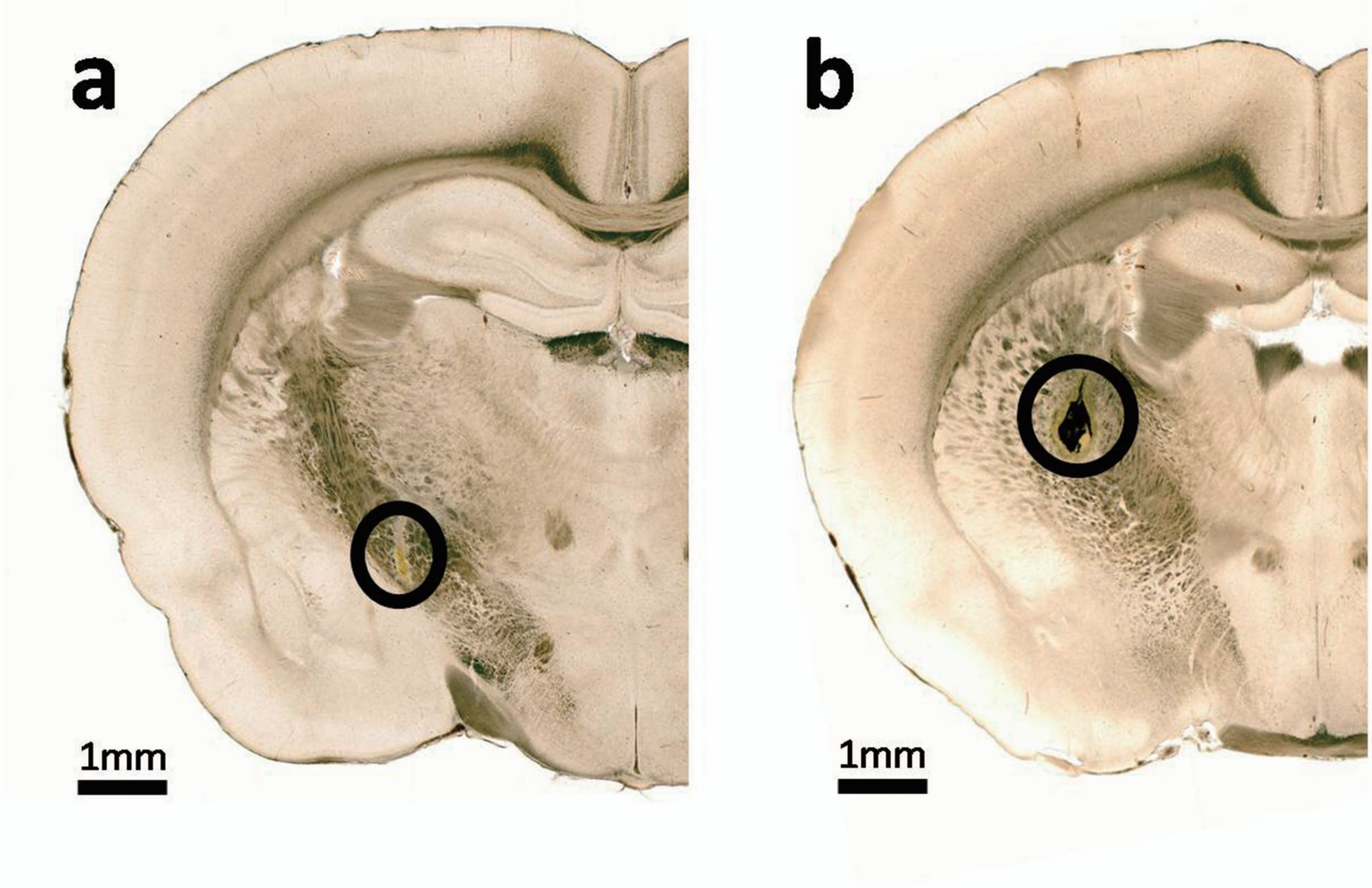
GPi; internal segment of the globus pallidus

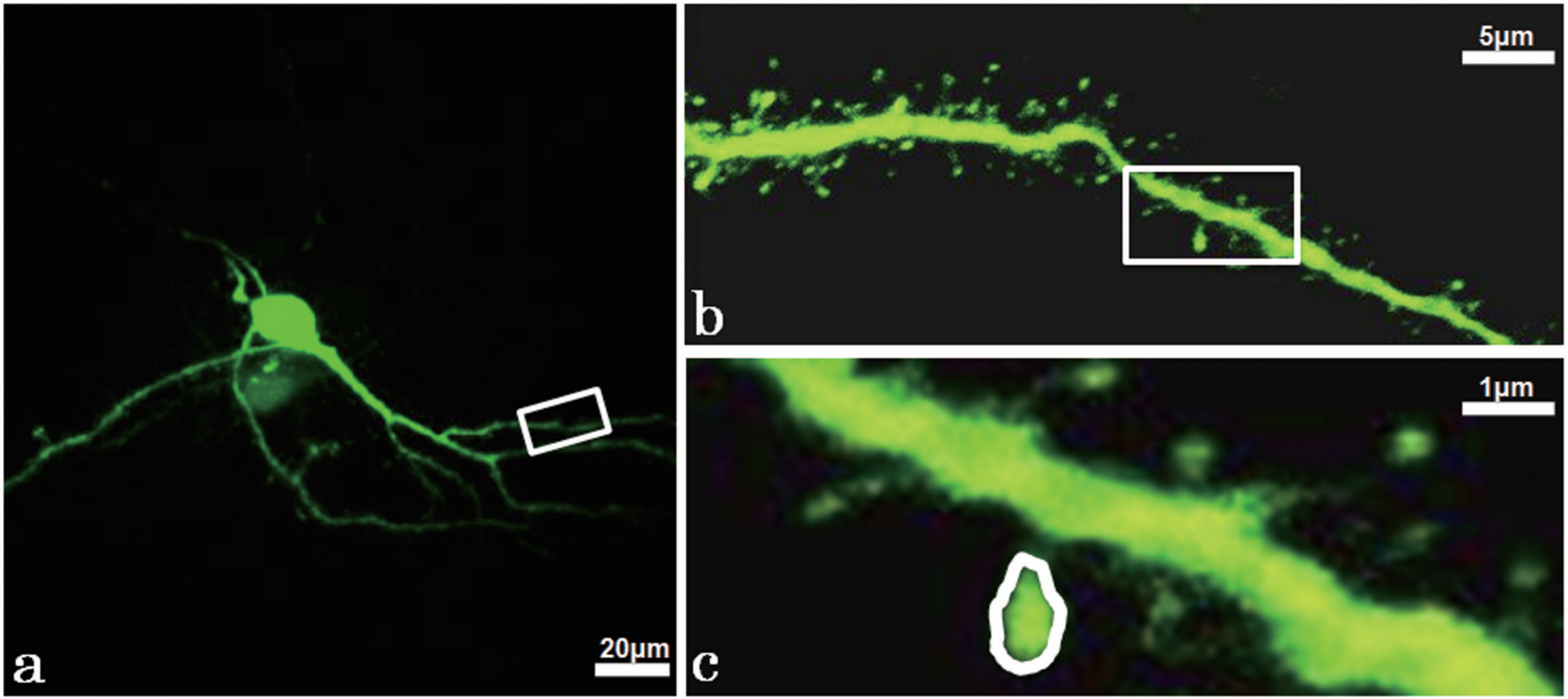
GPe; external segment of the globus pallidus

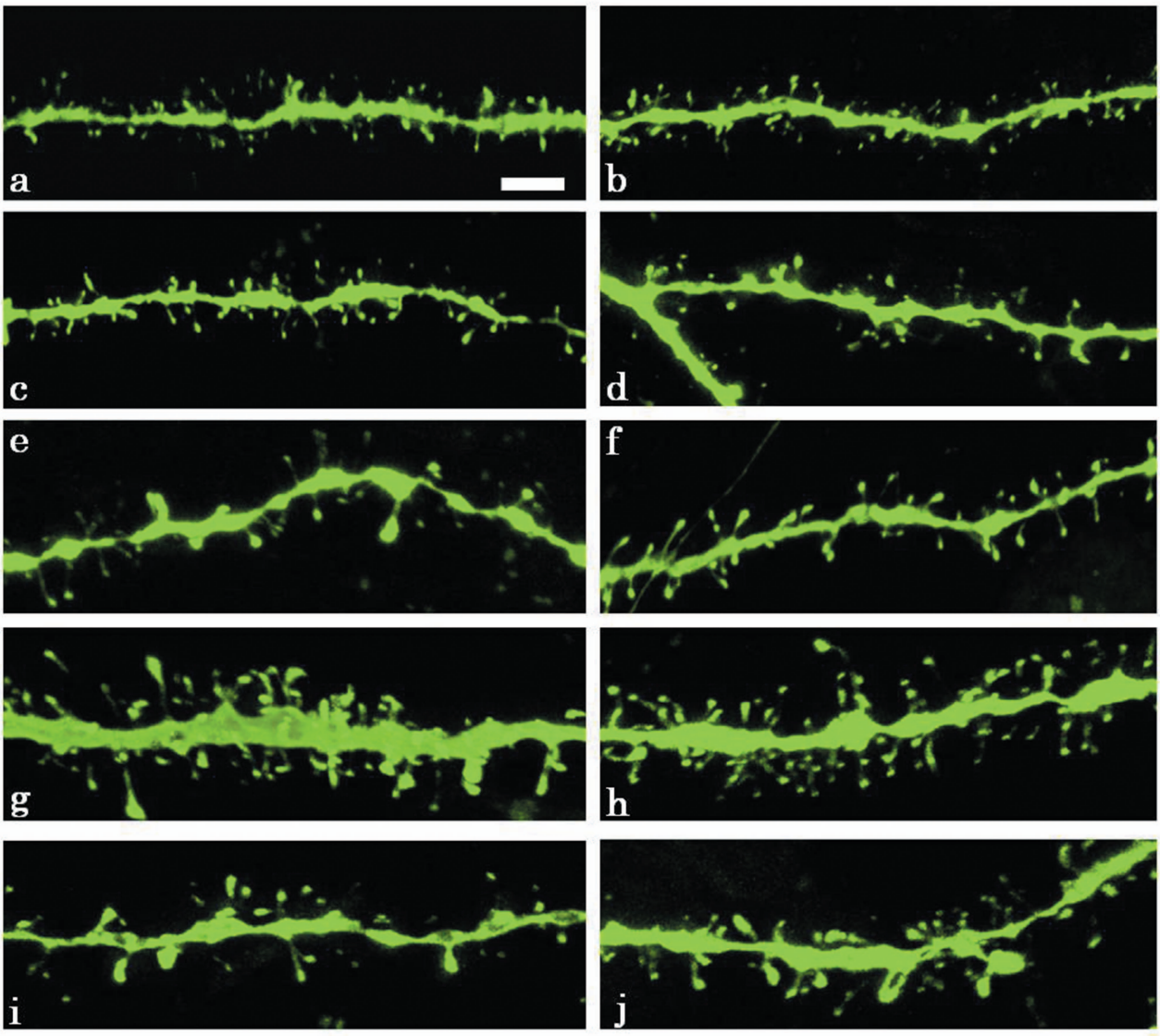
PD; Parkinson’s disease

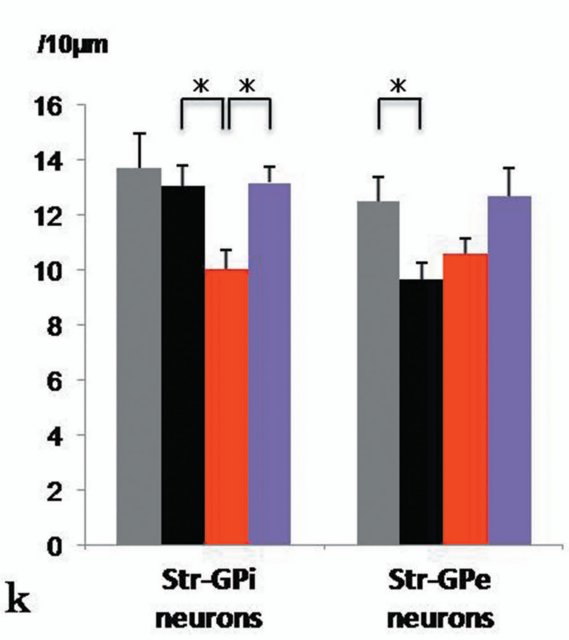
LID; L-DOPA-induced dyskinesia


points
70
60
50
40
30
20
10
0
**
**
*
Day 1
Day 4
Day 11
LID model
L-DOPA control

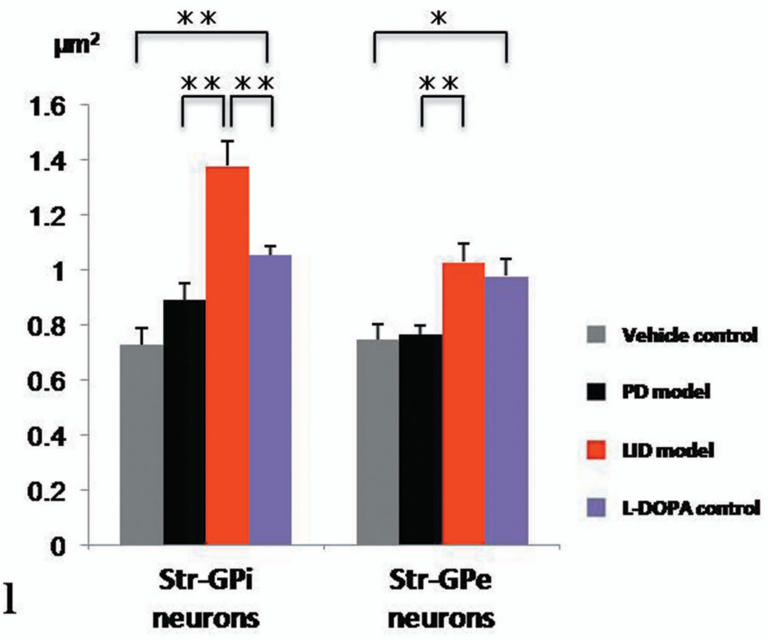

a
1mm
b
1mm


a
20μm
b
5μm
c
1μm


a
b
c
d
e
f
g
h
i
j


/10μm
16
14
12
10
8
6
4
2
0
Str-GPi neurons
Str-GPe neurons

k


μm²
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Str-GPi neurons
Str-GPe neurons
Vehicle control
PD model
LID model
L-DOPA control

l