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Short Communication

Morphological dendritic spine changes of medium spiny neurons in the nucleus accumbens in 6-hydroxydopamine-lesioned rats treated with levodopa

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ABSTRACT

The mechanisms of dopamine dysregulation syndrome (DDS) in Parkinson's disease (PD) remain unclear, although it is known that the nucleus accumbens (NAc) plays a role in its development. Based on the hypothesis that DDS and levodopa-induced dyskinesia (LID) share a pathophysiological basis, we investigated dendritic spine morphology of medium spiny neurons (MSNs) in the NAc of a rat model of LID, because spine enlargement in MSNs of the caudate/putamen has been proposed to be a morphological hallmark of LID. Spines of NAc MSNs also became enlarged in the LID model. This result suggests that excitatory supersensitivity of MSNs in the NAc is involved in the development of DDS, similar to what occurs in the caudate/putamen in LID.

Dopamine dysregulation syndrome (DDS) is an iatrogenic disturbance that may complicate long-term therapy of Parkinson's disease (PD). DDS refers to the addictive patterns of usage of levodopa and, less commonly, apomorphine. The prevalence of DDS in PD patients is estimated to be 3%–4% (Evans and Lees, 2004; Pezzella et al., 2005). A previous study hypothesized that progressive dopaminergic denervation reduces presynaptic dopamine storage mechanisms, which exacerbates the pulsatile effects of levodopa-derived dopamine on limbic structures (Giovannoni et al., 2000). In fact, evidence of maladaptation and sensitization in DDS has been reported as enhanced levodopa-induced dopamine release in the nucleus accumbens (NAc) (Evans et al., 2006), the structure that plays a central role in the development of DDS. However, the molecular mechanisms of DDS remain unclear, largely because there are no validated animal models that replicate the clinical features of DDS (Bastide et al., 2015). Levodopa-induced dyskinesia (LID) and DDS are motor and behavioral complications, respectively, that accompany long-term levodopa treatment. DDS is usually concomitant with LID (Giovannoni et al., 2009; Pezzella et al., 2005). PD patients with LID display enhanced levodopa-derived dopamine release in the caudate and putamen (CPu) (de la Fuente-Fernandez et al., 2004), similar to that which occurs in the NAc of patients with DDS (Evans et al., 2006). These results indicate that dopaminergic overstimulation is a common feature of both LID and DDS in response to levodopa (Giovannoni et al., 2000), suggesting that the CPu in LID and the NAc in DDS share a common molecular abnormality (Linazasoro, 2009; Voon et al., 2009).

Several studies have demonstrated that the dendritic spines of medium spiny neurons (MSNs) in the CPu change their morphology in LID models (Fieblinger et al., 2014; Nishijima et al., 2014; Suarez et al., 2016; Suarez et al., 2014; Zhang et al., 2013). These morphological changes suggest maladaptive MSN excitatory synapses in the CPu. MSNs are the primary neurons in both the NAc and CPu. Thus, it may be useful to also examine spine morphology of MSNs in the NAc in LID models, to provide a better understand-

Abbreviations: DDS, dopamine dysregulation syndrome; PD, Parkinson's disease; NAc, nucleus accumbens; LID, levodopa induced dyskinesia; CPu, caudate and putamen; MSNs, medium spiny neurons; MFB, medial forebrain bundle; 6-OHDA, 6-hydroxydopamine; AIM, abnormal involuntary movement; LTP, long-term potentiation; LTD, long-term depression.

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ing of the mechanisms involved in DDS. The NAc comprises two functionally and anatomically distinct subregions: the core and the shell (Zahm, 2000; Ito et al., 2004)). In the present study, we evaluated spine morphology of MSN dendritic spines in the NAc core and shell in 6-hydroxydopamine (OHDA)-lesioned PD model rats, repeatedly treated with levodopa, to determine the morphological basis of DDS.

In total, 27 male Wistar rats (CLEA Japan Inc., Tokyo, Japan) were used. The rats were housed in a temperature-controlled room (around 25 °C) with a 12-h day/night cycle, with free access to food and water. All efforts were made to minimize the number of animals used and their suffering. This study was approved by the Hirosaki University Animal Experimentation Committee.

At 10 weeks of age, the rats were underwent stereotactic infusion of 6-OHDA or saline into the right medial forebrain bundle (MFB), as previously described (Nishijima et al., 2014), to create unilateral dopaminergic denervation and sham-operation animals, respectively (Fig. 1A). Two weeks after the operation the rats were challenged with apomorphine to evaluate dopaminergic denervation (Maeda et al., 1999a,b) (Fig. 1B).

We treated five 6-OHDA-lesioned hemiparkinsonian rats with saline (PD-Saline group), eight 6-OHDA-lesioned hemiparkinsonian rats with chronic levodopa (PD-Levodopa group), six sham-operated control rats with chronic levodopa (Control-Levodopa group), and eight sham-operated control rats with saline (Control-Saline group) (Fig. 1B). Beginning 6 weeks after the apomorphine challenge, the 6-OHDA-lesioned and sham-operated rats received 50 mg/kg levodopa methyl ester with 12.5 mg/kg benserazide, a dopa decarboxylase inhibitor (PD-Levodopa group and Control-Levodopa group), or saline (PD-Saline group and Control-Saline group), twice daily for 14 consecutive days (Nishijima et al., 2014) (Fig. 1B). To evaluate the effects of levodopa, we measured abnormal involuntary movement (AIM) scores on days 1, 4, and 11 (Nishijima et al., 2014) (Fig. 1C).

At 20 weeks of age, the rats were anesthetized with sodium pentobarbital (75 mg/kg, intraperitoneally), and intracardially perfused with 4% paraformaldehyde 12 h after the last treatment. Brains were removed and serial 250-μm-thick coronal sections through the NAc were prepared. Sections were dipped in DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) to stain NAc neuronal nuclei. Individual sections were prepared as previously described (Nishijima et al., 2014). MSNs in the NAc core and shell were identified according to diagrams from Paxinos's rat brain atlas (Fig. 2A). Lucifer yellow was injected into MSN cell bodies with DAPI-stained nuclei in the NAc core and shell of the operated hemisphere (Fig. 2A–C) (Nishijima et al., 2014).

Confocal laser scanning microscopy procedures were performed as previously described (Nishijima et al., 2014). We measured spine density and size on the dendrite, 50–100 μm distal to the cell body (Fig. 2C). Image stacks were 3D-deconvoluted using NIS-Elements software (Nikon) and were volume rendered as 3D images to facilitate figure overviews. All spines were 3D reconstructed again, and spine density and volume were quantified, using Neurolucida software (Micro Bright Field, Inc.).

All the drugs except for Nembutal (Dainippon Sumitomo Pharma, Osaka, Japan) were obtained from Sigma (San Diego, CA, USA).

Quantitative data were evaluated using the Shapiro-Wilk test to determine whether they followed a normal distribution. Spine densities and volumes of spine heads were analyzed using non-parametric tests (Kruskal–Wallis test, followed by the Steel–Dwass multiple comparison test).

All rats that received levodopa treatment after 6-OHDA lesioning (PD-Levodopa group) displayed AIMs during the treatment period (Fig. 1C). The total AIM score in the PD-Levodopa group on day 11 was more than twice the score on day 1. However, control

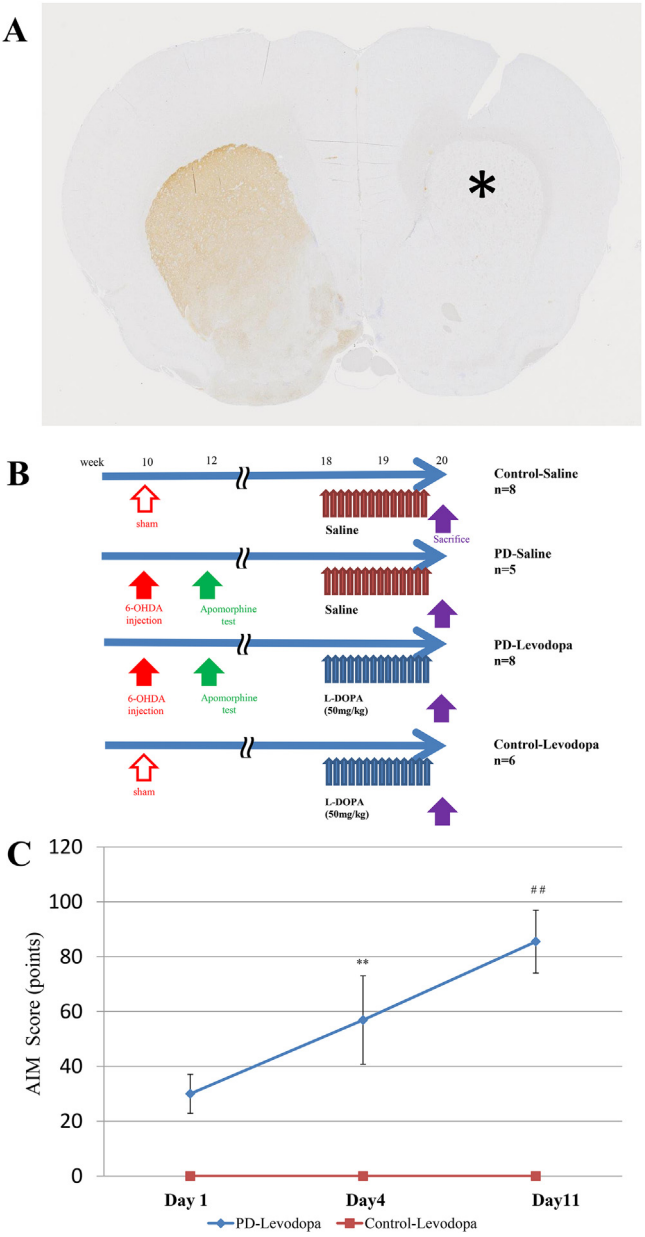

Fig. 1. (A). Tyrosine hydroxylase immunohistochemistry from a section through the nucleus accumbens of a 6-hydroxydopamine-lesioned rat. The lesioned side of the striatum, not stained by tyrosine hydroxylase, is indicated by an asterisk (*). (B). Time chart and experimental design. We injected 6-hydroxydopamine (6-OHDA) or saline into the medial forebrain bundle at 10 weeks of age to establish a hemiparkinsonian model (PD-Levodopa group; PD-Saline group) or sham-operated model (Control-Saline group; Control-Levodopa group), respectively. At 12 weeks of age, 6-OHDA-treated rats were challenged with an apomorphine injection to confirm dopaminergic denervation. The PD-Levodopa group and Control-Levodopa group received daily levodopa treatment, and the PD-Saline group and Control-Saline group received daily saline injections, from 18 weeks. At 20 weeks, all rats were sacrificed and processed for pathological examinations. (C), Changes in abnormal involuntary movement (AIM) scores of control rats treated with levodopa (Control-Levodopa group) or unilaterally 6-OHDA-lesioned rats treated with levodopa (PD-Levodopa group) on days 1, 4, and 11. Control-Levodopa group rats showed no AIM, whereas rats in the PD-Levodopa group showed progressive deterioration of dyskinesias following the commencement of levodopa treatment. (Kruskal–Wallis test followed by Steel–Dwass multiple comparison test: $^{**}P < 0.001$ vs. day 1, $^{\#\#}P < 0.001$ vs. day 4). Control-Levodopa group rats showed no AIMs.