

## Unilateral Ibotenic Acid Lesions of the Prefrontal Cortex Reduce Rotational Behavior in 6-hydroxydopamine-lesioned Rats

Daniel Gonzalez<sup>a</sup>, Osamu Miyamoto<sup>b</sup>, Tetsuo Touge<sup>c</sup>, Kazunori Sumitani<sup>d</sup>,  
Shigeki Kuriyama<sup>a</sup>, and Toshifumi Itano<sup>b\*</sup>

Departments of <sup>a</sup>Internal Medicine, <sup>b</sup>Neurobiology, <sup>c</sup>Health Sciences, and <sup>d</sup>Sports Physiology,  
Faculty of Medicine, Kagawa University, Miki-cho, Kagawa 761-0793, Japan

Rats with 6-hydroxydopamine (6-OHDA)-induced lesions of the substantia nigra are used as a model of Parkinson's disease (PD), and these "lesioned" rats exhibit a rotational behavior when further injected with apomorphine (APO). We examined whether lesions in the prefrontal cortex (PFC) could modify the rotational behavior in PD model rats. Rats initially received unilateral lesions of the substantia nigra by 6-OHDA injection, and then their rotational behavior was measured. Two PFC lesions were achieved by intracerebral infusions of ibotenic acid, followed by measurement of APO-induced rotation. Rotation was reduced by approximately 30% after PFC injury. The PFC may have functional influences on the basal ganglia and may be involved in the pathophysiology of the rotational behavior of PD model rats.

**Key words:** Parkinson model rat, rotational behavior, ibotenic acid, 6-hydroxydopamine, prefrontal cortex

**P**arkinson's disease (PD) is a neurodegenerative disorder mainly characterized by degeneration in the dopaminergic neurons of the substantia nigra pars compacta (SNc), and can be modeled experimentally in animals using a specific neurotoxin for catecholaminergic neurons, such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [1]. The loss of nigral neurons produces functional modifications that involve all components of the basal ganglia circuitry. In this respect, increased activity of the subthalamic nucleus (STN) plays a fundamental role in the pathophysiology of PD. Indeed, an STN lesion has been shown to reduce parkinsonian symptoms in rodent [2] and

monkey models [3] of PD. Moreover, high frequency stimulation of the STN was found to alleviate parkinsonian motor symptoms in patients with disabling akinetic-rigidity [4]. Patel *et al.* also reported that unilateral subthalamotomy by radiofrequency ablation significantly improved parkinsonian symptoms in their patients [5]. In the classic model of the ganglia basal network, the enhanced activity of the STN neurons is thought to be the direct consequence of pathological hypo-activity of the external segment of the globus pallidus (GP) [6]. However, the hyperactivity of the STN may also be explained by other sources of excitation or disinhibition that can influence this nucleus, such as the cerebral cortex and parafascicular nucleus of the thalamus [7].

The cerebral cortex and the basal ganglia are functionally related through multi-synaptic loop circuits [8]. In rats, the major cortical projections to

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\*Corresponding author. Phone: +81-87-898-5111; Fax: +81-87-891-2251  
E-mail: toshi@kms.ac.jp (T. Itano)

the STN are from the motor and pre-motor areas [9], which influence the activity of the STN-GP network directly via excitatory projections. However, anatomical and electrophysiological findings have shown that the STN also receives direct excitatory afferents from the medial division of the prefrontal cortex (PFC) [9, 10]. Stimulation of the PFC has been shown to influence the discharge of STN cells by the induction of 2 excitatory peaks, often separated by a brief inhibitory peak [10]. In fact, medial PFC and STN disconnection induces behavioral deficits [11], which suggests a reciprocal functional interaction between the 2 areas. Moreover, during effective STN stimulation, movement-related increases in cerebral blood flow are higher in the supplementary motor area, cingulate cortex and dorsolateral PFC, which may indicate the dominant role of non-primary motor areas in the control of movement in parkinsonian patients [12]. Nevertheless, little is known about the influence of the PFC on the STN in pathological conditions such as PD.

We hypothesized that the PFC may be related to the hyperactivity of the STN, and that impairment of the PFC may improve behavioral abnormality in PD. To test our hypothesis, we used a well-established rodent model of PD, which involves unilateral destruction of the SNc by injection of 6-OHDA.

## Materials and Methods

**Parkinson model rat.** Male Sprague-Dawley rats weighing around 250 g were housed in cages under controlled conditions with a constant temperature ( $23 \pm 1$  °C), a 12-h light/dark cycle, and *ad libitum* access to drinking water and food. All procedures were conducted in accordance with the requirements of the Kagawa University Animal Committee, and every effort was made to minimize both the number of animals used and their suffering. The animals were deeply anaesthetized with sodium pentobarbital (40 mg/kg i.p.) and placed in a stereotaxic holder fitted with non-traumatic ear bars. Following local injection of xylocaine, the scalp was retracted to expose the skull, and craniotomies were made directly above the target region of the brain. The drugs were injected via a stainless steel cannula (27-gauge) connected to a microdrive pump (model 200 series; KD Scientific, Holliston, MA, USA).

Each rat received an injection of 2  $\mu$ l of 6-OHDA solution (10  $\mu$ g/ $\mu$ l) (Sigma-Aldrich, St. Louis, MO, USA) in the right SNc (stereotaxic coordinates; A = 5.2 mm anterior to the bregma, L = 2.0 mm on the right side lateral to the midline, V = 7.8 mm below the skull). Following the injection, the rat was sutured and returned to its cage. PD model rats exhibit contralateral rotation upon administration of a dopamine agonist, such as apomorphine (APO), resulting from denervation supersensitivity [13]. To evaluate the completeness of the 6-OHDA lesion, rats were tested with a subcutaneous injection of APO (0.5 mg/kg in saline solution; Sigma-Aldrich) 3 weeks after the 6-OHDA lesioning. Only animals exhibiting at least 5 contralateral rotations/min in 60 min were used in subsequent experiments. This criterion selected lesioned rats with greater than 95% depletion of striatal dopamine and tyrosine hydroxylase [14]. Rotational behavior was measured as previously reported [15]. Briefly, rats were placed in a plastic chamber and rotations were detected electronically using a system controlled by light-activated silicon-controlled rectifiers for 60 min after APO injection.

**PFC lesions.** The rats were divided into 3 groups: an experimental group (n = 7, injection of ibotenic acid into the PFC), a control-1 group (n = 7, injection of ibotenic acid into the parietal cortex) and a control-2 group (n = 4, injection of saline into the PFC). In the experimental group and control-2 group, 2  $\mu$ l of ibotenic acid solution (2.5  $\mu$ g/ $\mu$ l) or saline was infused into 2 sub-areas of the right PFC at the following coordinates (A = +3.2 mm, L = 0.7 mm, V = 2 mm, and AP = +3.2 mm, L = 0.7 mm, V = 5 mm), respectively. In the control-1 group, 4  $\mu$ l of ibotenic acid solution was injected into the parietal cortex (A = +2.2 mm, L = 4.4 mm, V = 3.4 mm). One week after PFC lesioning, the rotational behavior was tested using the same procedure as described above.

**Immunohistochemistry.** All rats from the experimental and control groups were deeply anaesthetized with sodium pentobarbital (60 mg/kg i.p.) and perfused transcardially with 0.02 M phosphate buffered saline (PBS, pH 7.4), followed by a fixative of 4% paraformaldehyde in PBS. The brains were removed and cryoprotected in 30% (w/v) sucrose at 4 °C until they sank. Frozen sections (20- $\mu$ m thick-

ness) at the SNc and striatum levels were cut coronally using a freezing microtome, mounted on glass slides and processed for tyrosine hydroxylase (TH) immunostaining ( $n = 3$  in each group). Sections were incubated with TH antiserum (1/200, polyclonal antibody; Chemicon, Temecula, CA, USA) overnight at 4 °C. This was followed by incubation for 1 h in secondary biotinylated anti-rabbit IgG and for 30 min with avidin-biotin complex (Vectastain, Elite ABC kit; Vector Laboratories, Burlingame, CA, USA), followed by reaction for 3–5 min with 0.05% diaminobenzidine-0.03% hydrogen peroxidase. Hematoxylin and eosin staining of the PFC and SNc was also performed to verify the location and to assess the extent of lesion-induced neuronal loss.

**Statistical analysis.** The number of rotations is reported as a percentage of the pre-PFC-lesioned value. Data are expressed as the mean  $\pm$  s.d. The statistical significance of differences was assessed using Friedman's  $\chi^2$  r-test followed by the Wilcoxon  $t$ -test. Values of  $p < 0.05$  were considered to be statistically significant. For all data collection, the experimenters were blinded to the group identities.

## Results

TH staining labeled the dopamine neurons of the substantia nigra and ventral tegmental area (VTA) bilaterally in control rats, and there was also a heavily stained terminal plexus within both sides of the striatum (Figs. 1A, C). The injection of 6-OHDA induced a loss of TH immunoreactive cells in the SNc and in the associated terminal plexus in the striatum on the side ipsilateral to the lesion (Figs. 1B, D). PFC lesions produced by the ibotenic acid injection showed a cavity, marked gliosis and a loss of neurons (Fig. 2).

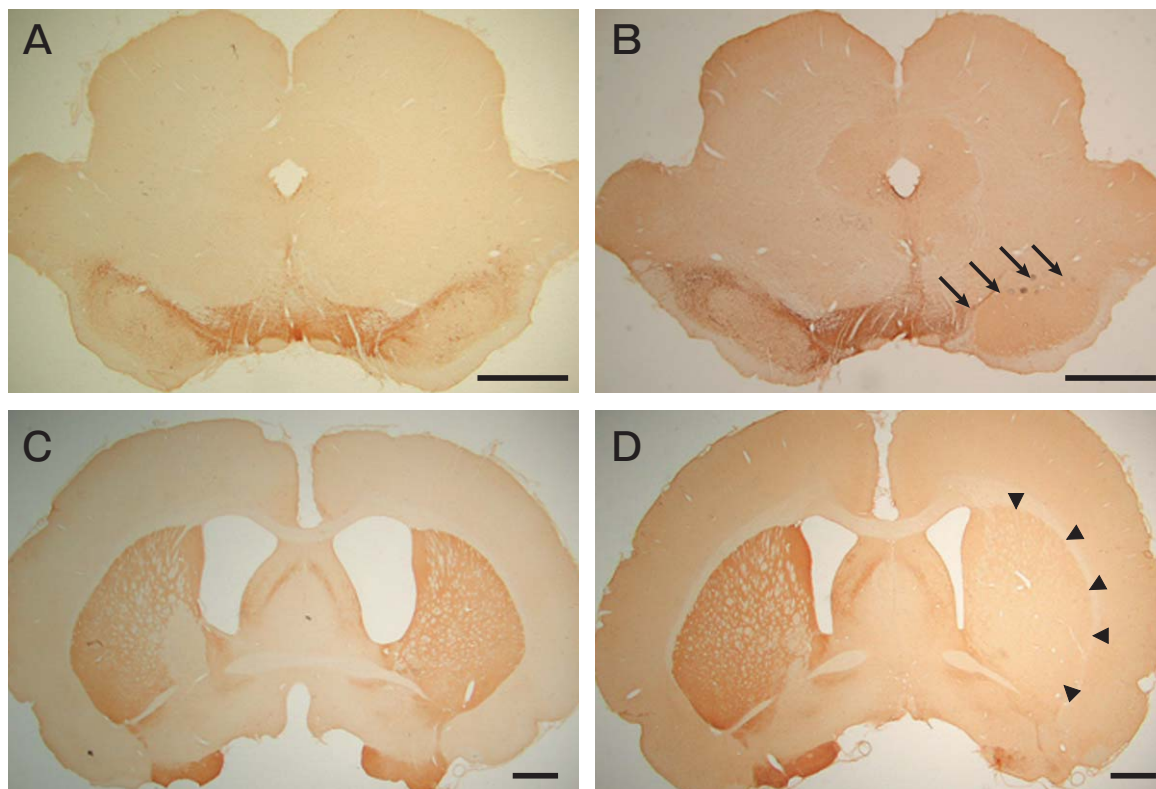
Apomorphine induced a rotational behavior contralateral to the lesion side in all Parkinson model rats during the session. The rats showed progressive enhancement of rotational behavior following the first day's session, which then stabilized. This phenomenon may be related to the development of sensitization induced by repeated administration of APO [15]. Measurements were taken over 4 consecutive days after rats began exhibiting stable rotational behavior. Following PFC-lesioning with ibotenic acid, rotational behavior significantly decreased (more than 30

% reduction) compared with that of the pre-PFC-lesion rats in the experimental group (Fig. 3). On the other hand, no significant reduction was observed after the lesioning of the parietal cortex in the control-1 group or saline injection into the PFC in the control-2 group.

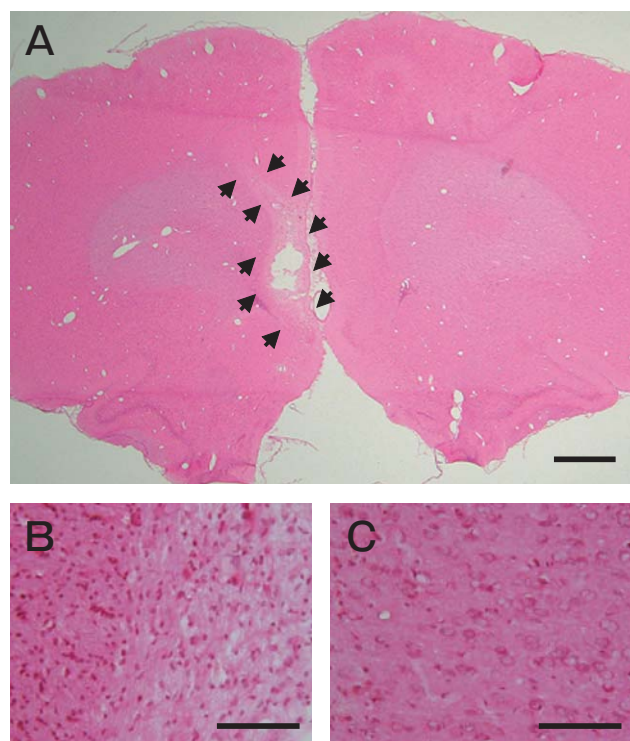
## Discussion

The present study indicated that the cortical neurons in PFC could influence the activity of the STN via the cortico-subthalamic pathway in 6-OHDA lesioned rats. These results, together with the similar findings of previous studies, indicate that the PFC and STN regions are electrophysiologically related. Stimulation of the PFC influences the STN neurons through a direct excitatory projection [10]. Moreover, the PFC may influence the activity of STN cells through an indirect disinhibitory circuit that involves the ventral striatum, the core of the nucleus accumbens, and the ventral pallidum [10, 17]. Stimulation of the medial prefrontal cortex has also been shown to induce the expression of Fos-positive cells in the striatum and STN, which may reflect the level of the afferent synaptic activity in the basal ganglia [18]. Interestingly, the cortex also sends projections (especially from the frontal and cingulate areas) to the SNc and VTA [19]. On the other hand, afferents to prefrontal and cingulate cortex were found to arise from the mesencephalic dopaminergic neurons [20]. Thus, the cortico-subthalamic pathway may represent only a part of a complex network of functional interactions among the prefrontal cortex, basal ganglia and nigrostriatal DA systems.

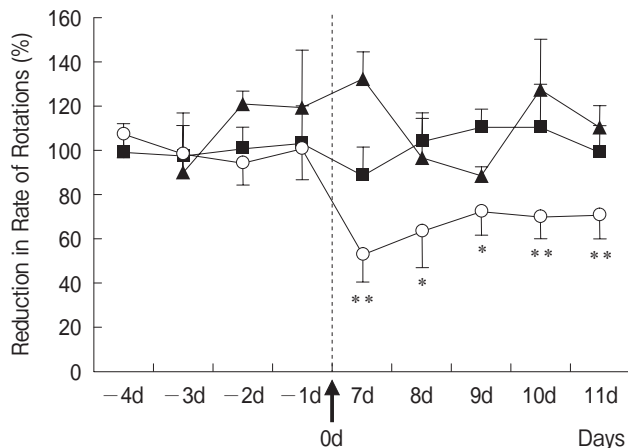
Our results demonstrate for the first time that ibotenic acid-induced lesions of the PFC can reduce APO-induced rotations in 6-OHDA-lesioned rats. As the projections of cortical neurons have an excitatory effect [21] on the STN, these inputs may produce STN hyperactivity after 6-OHDA lesioning of the SNc. Indeed, blockade of the glutamatergic cortico-STN transmission is known to suppress the early excitation of pallidal neurons, whereas interference with the GABAergic pallido-STN neurotransmission has little effect on early excitation [22]. Based on these findings, the cortico-subthalamo-pallidal pathway is thought to deliver powerful excitatory effects



**Fig. 1** Photomicrographs showing TH immunolabeling of the substantia nigra pars compacta (SNc) (**A**, **B**) and striatum (**C**, **D**) of normal control (**A**, **C**) and Parkinson model rats (**B**, **D**). Note the complete loss of TH immunolabeling within the SNc (**B**, arrows) and striatum (**D**, arrowheads) of the lesioned side compared with the contralateral side in the Parkinson model rats. Scale bars = 1 mm.



**Fig. 2** Photomicrographs of the coronal section stained with hematoxylin and eosin showing ibotenic acid lesions in the prefrontal (PFC) area (arrowheads in **A**). A cavity, marked gliosis and a loss of neurons were observed in the PFC area of the ibotenic acid injection side (**B**) but not in the contralateral side (**C**). Scale bars = 1 mm in **A** and 100  $\mu\text{m}$  in **B**, **C**.



**Fig. 3** Changes in apomorphine-induced rotation in the control-1 (parietal cortex-lesioned parkinsonian model, ■), control-2 (PFC-lesioned with saline parkinsonian model, ▲) and experimental rats (PFC-lesioned with ibotenic acid parkinsonian model, ○). The dotted vertical line divides the figure into the periods before and after PFC lesioning by ibotenic acid injection. Zero day (0d) indicates the day of the PFC lesioning. Each bar represents the mean  $\pm$  s.d. \* $p < 0.05$ ; \*\* $p < 0.01$  compared with before PFC lesioning (-1d).

from cortical areas to the pallidum [23]. Thus, disruption of the influence of the PFC on the STN may help explain our results, through reduction of the excitatory signals from the cortex to the STN.

The present study showed that PFC lesions produced an approximately 30% reduction in the number of rotations compared with the value before PFC lesioning in PD model rats. One possible explanation for this finding is that the PFC areas projecting to the STN may innervate only a restricted region on the STN [9]. Additionally, the cortico-STN-pallidum pathway may not be the only source of excitation of the basal ganglia. For example, the neurons in the pedunculopontine nucleus and parafascicular nucleus of the thalamus that project to the subthalamic nucleus are hyperactive after nigrostriatal dopaminergic denervation in rats [24]. Moreover, that fact that there was no reduction in rotational behavior following saline injection to the PFC indicates that some degree of destruction of the PFC is necessary to influence STN activity. Taken together, these results indicate that the hyperactivity of the STN in Parkinson's disease may be due not only to hypoactivity of the globus pallidus but also to strong excit-

atory inputs from other cerebral structures.

Our work confirms that the PFC plays a significant role in cortico-basal ganglia circuits and demonstrates the importance of the cortico-subthalamic disconnection in the reduction of abnormal behavior in PD model rats. Disruption of the excitatory influence of the frontal cortex on the STN may bring about important clinical advantages, since it is possible to influence the activity of the basal ganglia from regions of the brain more superficial than the STN. In this regard, Ikeguchi *et al.* [25] showed that successive repetitive transcranial magnetic stimulation of the frontal cortex improved parkinsonian symptoms. Moreover, high frequency electric stimulation of this area has been shown to result in fewer medical complications than deep lesioning or stimulation of the STN in Parkinson's patients [26]. Finally, our findings reinforce the notion that mechanisms other than inhibitory control of the subthalamic nucleus by the lateral globus pallidus may underlie the regulation of subthalamic activity. Further experiments using high frequency stimulation of the PFC are required to complement this study from the viewpoint of a potential alternative clinical treatment for Parkinson's disease.

In conclusion, apomorphine-induced rotational behavior was observed in rats having unilateral lesions of the substantia nigra (Parkinson model rats). Additional lesions in the medial prefrontal cortex significantly reduced the rotational behavior in the Parkinson model rats. The prefrontal cortex might control the activity of the basal ganglia and play a crucial role in Parkinson's patients.

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