

# Original article

## Effect of deep brain stimulation on substantia nigra neurons in a rat model of Parkinson's disease

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**Keywords:** Parkinson's disease; apoptosis; deep brain stimulation; excitatory neurotransmitter

**Background** Parkinson's disease (PD) is a common neurodegenerative disease, which occurs mainly in the elderly. Recent studies have demonstrated that apoptosis plays an important role in the occurrence and development of PD. Subthalamic nucleus deep brain stimulation (STN-DBS) has been recognized as an effective treatment for PD. Recent clinical observations have shown that STN-DBS was able to delay early PD progression, and experiments in animal models have also demonstrated a protective effect of STN-DBS on neurons. However, the correlation between the neuron-protective effect of STN-DBS and the progression of substantia nigra pars compacta (SNc) neuronal apoptosis is still unknown. The aim of this study was to investigate the protective effect and potential mechanism of STN-DBS on SNc neurons in PD rats.

**Methods** After the establishment of a PD rat model by unilateral/2-point injection of 6-hydroxydopamine in the medial forebrain bundle of the brain, DBS by implanting electrodes in the STN was administered. Behavioral changes were observed, and morphological changes of SNc neurons were analyzed by Nissl staining and DNA *in situ* end-labeling. Through extracellular recording of single neuron discharges and microelectrophoresis, the causes of and changes in SNc excitability during STN-DBS were analyzed, and the protective effect and potential mechanism of action of STN-DBS on SNc neurons in PD rats was investigated.

**Results** SNc neuron apoptosis was significantly decreased ( $P < 0.05$ ) in the stimulation group, compared with the sham stimulation PD group. Spontaneous discharges of SNc neurons were observed in normal rats and PD model rats, and the mean frequency of spontaneous discharges of SNc neurons in normal rats ( $40.65 \pm 11.08$  Hz) was higher than that of residual SNc neurons in PD rats ( $36.71 \pm 9.23$  Hz). Electrical stimulation of the STN in rats was associated with elevated excitation in unilateral SNc neurons. However, administering the gamma-aminobutyric acid receptor blocker, bicuculline significantly reduced SNc neuron excitation, but the change in SNc neuron excitation was not present when MK801, a glutamate receptor blocker, was administered.

**Conclusions** High-frequency stimulation of the STN has a protective effect on SNc neurons in PD rats. The possible molecular mechanism may be related to changes in the distribution and metabolism of neurotransmitters in the SNc region.

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Parkinson's disease (PD) is a common neurodegenerative disease, which occurs mainly in the elderly. An epidemiology report published in *Lancet* by Zhang et al<sup>1</sup> demonstrated that the prevalence of PD is 2.1% in China, with a prevalence of 1.7% and 1.6% in men and women over 65 years of age, respectively. With an annual increase in China of 100 000 cases, 1.75 million individuals are estimated to suffer from PD. The pathogenesis of PD and the cause of neuron defects remain unclear. Recent studies demonstrated that apoptosis plays an important role in the occurrence and development of PD. Subthalamic nucleus deep brain stimulation (STN-DBS) has been recognized as an effective treatment for patients with PD, and the main effect is an improvement of symptoms in the middle to late stages of the disease.<sup>2-5</sup> Recent clinical observations have shown that STN-DBS was able to delay early PD progression,<sup>6,7</sup> and experiments in animal models have also demonstrated a protective effect of STN-DBS on neurons.<sup>8</sup> However, the correlation between the neuron-protective effect of STN-DBS and the progression

of substantia nigra pars compacta (SNc) neuron apoptosis is still unknown. In this study, the protective effect and potential mechanism of STN-DBS on SNc neurons in PD rats were investigated.

### METHODS

#### Experimental animals

Thirty-five adult male Sprague-Dawley (SD) rats (Laboratory Animal Center, Academy of Military Medical

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Science) weighing (250±30) g were included in this study. Rats were divided into 4 groups: control ( $n=5$ ), PD model group ( $n=10$ ), sham stimulation group ( $n=10$ ), and stimulation group ( $n=10$ ). The care and handling of animals were conducted in compliance with the *Chinese Animal Welfare Act* and were approved by the responsible governmental agency at the Capital Medical University-Affiliated Beijing Neurosurgical Institute.

### Establishment of PD rat model

To protect noradrenergic neurons, the rats received an intraperitoneal injection of 0.75% desipramine (3.3 ml/kg body weight, China) before surgery. After intraperitoneal anesthesia, rats were fixed in a stereotactic instrument (Stoelting, USA) at the cranial level. Based on the description in the *Rat Brain Atlas*,<sup>9</sup> 2 coordinates of the medial forebrain bundle were positioned: (1) 3.5 mm posterior to the anterior fontanel, 0.5 mm to the right side of the centerline, and 9.0 mm inferior to the dura; (2) 4.4 mm posterior to the anterior fontanel, 1.5 mm to the right side of the centerline, and 7.8 mm inferior to the dura. The skull was opened at these points by a tooth drill. A total of 2.5- $\mu$ l and 3.0- $\mu$ l 6-hydroxydopamine (6-OHDA, Sigma, USA) was injected using a 10- $\mu$ l microinjector (Stoelting) into the 2 openings for the establishment of the PD model.

### Implantation of stimulation electrodes

Electrodes were implanted in rats, in both stimulation groups, on the right side of the STN coordinates, and electrodes were fixed by dental care powder on the day of model construction. From the first day after surgery to the day of sacrifice, the rats received electrical stimulation at the STN every 24 hours. The electrical stimulation was continuous for 60 minutes at 2 V, a wave width of 0.12 ms, and frequency of 130 Hz. Electrical stimulation was repeated twice, with a 10-minute interval between stimulation. Rats in the sham stimulation group received no electrical stimulation.

### Behavioral observations

To induce contralateral rotation behavior, rats in each group received 0.5 mg/kg (0.5 g/L) apomorphine (APO, Sigma) via a subcutaneous injection in the neck. The frequency of contralateral rotation was recorded 10 minutes after APO injection for 30 minutes.

### Recording of neuron discharge and microelectrophoresis

Seven homemade glass microelectrodes (tip diameter, 4–8  $\mu$ m) were prepared. The central tube (resistance, 5–12 M $\Omega$ ) was filled with 0.1% Pontamine sky blue in 3 mol/L NaCl (pH 7.0) solution for the induction of neuron discharge. The peripheral tube (resistance, 20–100 M $\Omega$ ) was infused with the indicated solutions for microelectrophoresis (Table 1).

Microelectrodes were slowly inserted, using a microelectrode propeller, until the tip of the

**Table 1.** Drugs for microiontophoresis

Drugs	Concentration	pH
NaCl	3.0 mol/L	7.0
Glu	1.0 mol/L	8.0
MK-801	10 mmol/L	4.0
GABA	0.2 mol/L	3.5
BIC	10 mmol/L	4.0

Glu: glutamic acid. GABA: gamma-aminobutyric acid. BIC: bicuculline.

microelectrode reached the SNc in the rat brain. Using a 6400 A microelectrophoresis apparatus, the solution in the peripheral tube was electrophoresed to the SNc at electrophoresis currents of 5–100 nA and stagnation currents of 5–10 nA. Except for Glu, other neurotransmitter detection solutions were electrophoresed using a positive charge. Unit neuron discharge was displayed on an oscilloscope after it was mediated and filtered through a microelectrode amplifier and preamplifier, and the discharge pattern was recorded by the Spike2 bio-signal processing system (Cambridge Electronic Design, Cambridge, UK) and stored on a computer. The serial histogram of neuron discharge was then generated. Only signals with stable discharge and a signal-to-noise ratio of >3:1 were recorded. To exclude other possible current effects, discharge changes induced by NaCl injection were not included in data collection. Neuron discharge frequencies of SNc neurons after high-frequency electrical stimulation, before microelectrophoresis and during microelectrophoresis, were calculated.

### Pathological examination

Rats were sacrificed after 2 weeks. The brain was removed, fixed, and paraffin-embedded using routine methods. Continuous coronal sections (6–8  $\mu$ m in thickness) of rat brain were prepared, and the sections were numbered. These sections were processed with Nissl or TUNEL staining (Nanjing Jiancheng Bioengineering Institute, China), and histological changes in the brain were observed under an optical microscope. Image analysis of Nissl-stained slices was performed using the CIAS1000 color pathological analysis system (CIAS1000, Leica, Japan). Each slice was observed under a high-power microscope through 3 visual fields of the SNc, and the average gray value was measured. The number of apoptotic SNc neurons was quantified using the apoptosis index (AI = (apoptotic cells / total cells)  $\times$  100%).

### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation (SD), and were analyzed using *t*-tests and *F*-tests of one-way analysis of variance (ANOVA). All data were analyzed using SPSS 11.0 software (SPSS, IL, USA).  $P < 0.05$  was accepted as statistically significant.

## RESULTS

### Behavioral observations

Contralateral rotation frequency increased with time in

the experimental groups after surgery. The induced rotation behavior occurred earliest in the PD model group, and the rotation frequency was also the highest, but similar to the sham stimulation group. No statistically significant difference ( $P > 0.05$ ) was found among the stimulation, sham stimulation, and PD model groups at week 1. However, contralateral rotation frequency was significantly lower in the stimulation group ( $P < 0.05$ ) compared with the PD model group at week 2 (Table 2).

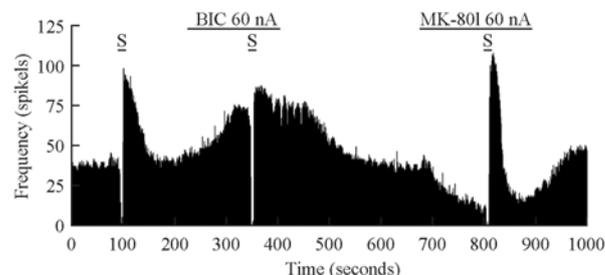
### Nissl staining and TUNEL assay

At week 2, the number of neurons at the injection sites of 6-OHDA in the SNc area was decreased in the PD model group compared with normal control rats. Nissl bodies in neurons were unclear with lighter coloration, and the granule density was lower (Figure 1A and 1B). The gray scale of the images was reduced, and the mean gray scale values were significantly lower compared with the normal control group ( $P < 0.05$ ). TUNEL positive cells were detected in the SNc area of the midbrain in the PD model group (Figure 1C and 1D), and the number of apoptotic neurons was significantly decreased in the stimulation group compared with PD model and sham stimulation groups ( $P < 0.05$ ) (Table 3).

### Electrophysiological results

Spontaneous SNc neuron discharge was observed in the ipsilateral side of normal rats and PD model rats, and the wave width was 1.35–2.30 ms with a discharge frequency of 0.6–49.0 Hz. The average spontaneous discharge from SNc neurons in normal rats was  $(40.65 \pm 11.08)$  Hz, which was slightly higher than that detected from the residual SNc neurons in the PD model rats ( $(36.71 \pm 9.23)$  Hz). Electrical stimulation of the STN in PD model rats was associated with excitation of ipsilateral SNc neurons.

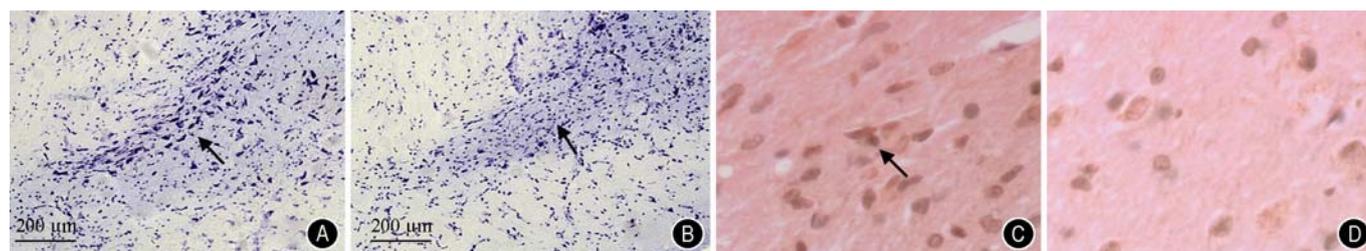
Simultaneous treatment with the gamma-aminobutyric acid (GABA)-receptor blocker, bicuculline (BIC), resulted in a significant reduction in the excitation of SNc neurons. Conversely, treatment with the glutamate receptor blocker, MK-801, resulted in no significant excitatory changes in SNc neurons (Figure 2).



**Figure 2.** Effect of subthalamic nucleus stimulation (STN), and bicuculline (BIC) and MK-801 microelectrophoresis on the electrical activity of SNc neurons. When stimulating the subthalamic nucleus (2 V, wave width 0.12 ms, 130 Hz) the discharge frequency of SNc neurons significantly increased. Simultaneous treatment with BIC resulted in a significant reduction in the excitation of SNc neurons, whereas treatment with MK-801 resulted in no significant changes in the excitation of SNc neurons.

### DISCUSSION

DBS is a surgical treatment method developed in the last decade, and is recognized as a new milestone for treatment of PD since the introduction of levodopa. Through continuous high-frequency stimulation regulating neural network function, DBS realigns the balance in basal ganglia motor circuits, which are the circuits responsible for symptoms in patients with PD. Since beginning treatment of patients with PD using DBS



**Figure 1.** **A:** Nissl staining of substantia nigra pars compacta (SNc) neurons in the normal control group showed a clustered distribution of pyramidal cells (arrow) (Original magnification  $\times 100$ ). **B:** Nissl staining of SNc neurons in the PD model group showed a scarce distribution of SNc neurons (arrow) (Original magnification  $\times 100$ ). **C and D:** Apoptotic cells in SNc neurons detected by TUNEL assays (Original magnification  $\times 400$ ). TUNEL positive neurons showed a dispersed distribution and nuclei condensation with a brownish-yellow color, and irregular- or round-shaped nuclei (arrow). **C:** PD model group, **D:** stimulation group.

**Table 2.** Comparison of the rate of rotation behavior in rats in each group (circles/min, mean $\pm$ SD)

Rotation occurrence time	Normal control group	PD model group	Sham stimulation group	Stimulation group
3 days	–	7.40 $\pm$ 0.12	6.08 $\pm$ 2.13	7.24 $\pm$ 1.43*
1 week	1.23 $\pm$ 0.63	8.72 $\pm$ 1.98	8.22 $\pm$ 2.22	7.12 $\pm$ 1.68
2 weeks	1.11 $\pm$ 1.87	14.54 $\pm$ 2.37	12.78 $\pm$ 2.65	10.11 $\pm$ 0.43*

Compared with PD model group, \* $P < 0.05$ .

**Table 3.** Nissl and TUNEL staining in the brain of rats in each group

Staining	Normal control group	PD model group	Sham stimulation group	Stimulation group
Nissl (gray scale value)	179.16 $\pm$ 12.17	128.29 $\pm$ 11.43	129.31 $\pm$ 14.42*	139.67 $\pm$ 17.22*
TUNEL (%)	4.21 $\pm$ 0.49	70.15 $\pm$ 9.24	67.12 $\pm$ 9.67	37.46 $\pm$ 13.12†

Compared with normal control group, \* $P < 0.05$ . Compared with PD model group, † $P < 0.05$ .

at Beijing Tiantan Hospital in 1998, more than 500 successful surgeries have been performed and positive treatment outcomes have been achieved, indicating the efficacy and safety of DBS treatment for PD.<sup>10</sup> Morphological studies have demonstrated that apoptotic changes are the main characteristics associated with the death of dopamine (DA) neurons in PD patients, and excessive apoptosis of residual DA neurons in the SNc of the midbrain in PD patients is one of the key mechanisms of secondary neural damage. Ziv et al<sup>11</sup> suggested that regulation of apoptosis might be the key to regulation of striatonigral degeneration. In this study, the apoptosis rate of ipsilateral SNc neurons in rats in the stimulation group was lower compared with the PD model group, indicating that electrical stimulation exhibited a protective effect on SNc neurons, which further supports the hypothesis that inhibition of abnormal STN excitation delays loss of SNc neurons.

During the progression of PD, degeneration and defects are observed in DA neurons in the SNc. This leads to dysfunction of multiple nuclei in the basal ganglia motor circuits, resulting in enhancement in the activity of the globus pallidus and substantia nigra pars reticulata (Gpi-SNr) complex. Eventually, the hypothalamic region controlling movement is inhibited and the activity of the motor cortex weakens, which leads to the onset of tremor, rigidity, and loss of movement ability. The pathogenic mechanisms of many neurodegenerative diseases, including Huntington's disease and Alzheimer's disease, involve Glu-mediated excitotoxicity that is considered "the last road" leading to neuronal death. Based on classical basal ganglia circuit theory,<sup>12</sup> a massive amount of Glu secreted by the STN is able to excite DA neurons in the SNc through nerve fiber projections. In PD, the activity of the STN is enhanced and a large amount of Glu is released, which causes secondary damage to residual DA neurons in the SNc. In this study, electrical stimulation of the STN resulted in a significant increase in SNc neuron discharge frequency. Administering BIC through microelectrophoresis significantly reduced SNc neuron excitation, whereas MK-801 resulted in no obvious changes in SNc neuron excitation. These results suggest that excitatory changes in SNc neurons during electrical stimulation of the STN are mainly regulated by GABA-related neurotransmitters, and Glu has a weaker effect. Thus, STN-DBS might reduce Glu in the SNc through the inhibition of abnormal STN activity. Specifically, Glu-mediated neurotoxicity is reduced and the occurrence of neuronal apoptosis is inhibited, which protects DA neurons and alleviates the progression of PD. Previous studies have shown that performing STN-DBS in a 6-OHDA-induced PD rat model resulted in a significant enhancement of DA metabolism in the corpus striatum and an increase in corpus striatum DA release.<sup>13</sup> In a primate PD model,<sup>8</sup> STN-DBS also increased the survival of DA neurons. These results provide evidence that STN-DBS might have a neural protective effect.

Currently, STN-DBS is primarily used in the treatment of progressive PD, which implies that most patients may

only consider surgical treatment when symptoms have progressed to the middle to later stages. Thus, if the neural protective effect of DBS on early stage PD could be verified in humans, the traditional view of DBS as a late-stage treatment should be changed, which could revolutionize the treatment of PD. Through early surgical treatment, it may be possible to stop disease progression, and eventually cure PD in combination with other treatments.

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