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Short communication

A time-course study of behavioral and electrophysiological characteristics in a mouse model of different stages of Parkinson's disease using 6-hydroxydopamine

Sunghee Estelle Park[a,b,1], Kang-Il Song[a,c,1], Jun-Kyo Francis Suh[a], Dosik Hwang[c], Inchan Youn[a,d,*]

[a] Biomedical Research Institute, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea
[b] Department of Mechanical Engineering, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 136-701, Republic of Korea
[c] Department of Electrical and Electronic Engineering, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, Republic of Korea
[d] Korea University of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea

HIGHLIGHTS

- TH-positive cell loss in the SNc was observed in accordance with 6-OHDA treatment.
- Mice treated with higher 6-OHDA concentrations exhibited degenerated motor symptoms.
- STN neuronal firing rates showed comparable results to those of human PD.
- The mouse model mimics the unique characteristics of each progressive stage of human PD.

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ABSTRACT

Parkinson's disease (PD) is characterized by abnormal motor symptoms and increased neuronal activity in the subthalamic nucleus (STN) as the disease progresses. We investigated the behavioral and electrophysiological characteristics in a mouse model mimicking the progressive stages of human PD (early, moderate, and advanced) by injecting 6-hydroxydopamine (6-OHDA) into the right medial forebrain bundle (MFB) at three different concentrations (2, 4, and 6 μg/2 μl). Significant changes in motor symptoms were demonstrated between groups in association with relative TH-positive cell loss in the substantia nigra pars compacta (SNc). Moreover, electrophysiologically assessed changes in the mean neuronal firing rate in the STN neurons were comparable to those in the early to advanced stages of human PD. Thus, the mouse model presented herein replicates the unique characteristics of each progressive stage of PD, in both motor and neurophysiological aspects, and therefore can be useful for further investigations of PD pathology.

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc), resulting in motor symptoms and nonmotor symptoms [1,2]. The basal ganglia mediate these motor symptoms, and patients in advanced stages of PD usually undergo deep brain stimulation (DBS) to electrically modulate the activity of the basal ganglia [3,4]. Because studies on animal models of PD have revealed hyperactivity in the subthalamic nucleus (STN) neurons, high-frequency DBS targeting the STN is considered an effective treatment for motor disabilities in PD patients [5].

To examine the characteristics of PD symptoms and dopaminergic neuronal degenerations, 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway is widely used in rodents because it closely approximates the symptoms of human PD [6]. Animals injected with 6-OHDA unilaterally develop side-biased motor impairments that can be observed through behavioral tests [7]. Furthermore, a rat model that mimics preclinical and clinical stages of PD was developed in which behavioral changes were

* Corresponding author at: Biomedical Research Institute, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea. Tel.: +82 2 958 5913; fax: +82 2 958 5909.
E-mail address: iyoun@kist.re.kr (I. Youn).
[1] These authors contributed equally to this paper.

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shown in accordance with the degree of cell loss [8]. Another study developed a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model mimicking the early stages of PD [9]; however, side-biased behavioral tests could not be performed as MPTP injections cause bilateral Parkinsonism [10].

Although current 6-OHDA- or MPTP-lesioned graded rodent models of PD demonstrate postural and motor changes, the potential electrophysiological changes occurring at each stage remain largely unknown [8,9]. A recent study analyzed neuronal recordings from the STN of early-stage PD patients and found that STN neurons showed significantly lower firing rates in early PD than in advanced PD [11]. Ideally, animal models should demonstrate neuronal firing rates that correspond to those at each stage of PD. Additionally, because transgenic mouse models are widely available, it would be beneficial to develop a mouse model of different PD stages for combination with genetic methodologies to investigate detailed mechanisms.

In the present study, different concentrations of 6-OHDA were injected intrastriatally into the right medial forebrain bundle (MFB) to generate a mouse model that replicates each progressive stage of human PD. Time-course behavioral tests consisting of a cylinder test, head position test, elevated body swing test (EBST), and a balance beam test were performed to investigate motor deficits according to the dosage of 6-OHDA. STN neuronal recordings were performed to compare changes in neuronal firing rates between PD groups. Nigral cell loss was observed through tyrosine hydroxylase (TH) immunohistochemistry and analyzed to confirm the progress of dopaminergic cell loss.

Twenty-eight adult male C57BL/6 mice (20–25 g, Charles River Laboratories International, Korea) were used in this study. The mice were housed five to a cage under a 12:12 h light–dark cycle and had free access to food and water. All experiments were performed in accordance with recommendations for the care and use of laboratory animals by the Ethical Committee of the Korea Institute of Science and Technology. To achieve graded unilateral lesions of the nigrostriatal pathway, mice were divided into four groups ($n = 7$ for each group). Mice were deeply anesthetized using a Zoletil/Rompun cocktail (0.1 ml/100 g, i.p.) and placed in a stereotaxic frame (David Kopf Instruments, USA) with nose and ear bars specially adapted for mice to constrain head movements. Mice received either saline or 6-OHDA (Sigma–Aldrich, USA) dissolved in saline with 0.1% ascorbic acid at three different concentrations (2, 4, and 6 μg/2 μl) to provide a control group and to mimic early, moderate, and advanced stages of human PD, respectively. A total volume of 2 μl was injected into the right MFB (−1.2 mm AP, +1.2 mm ML, and −5.0 mm DV) [12] using a 5-μl Hamilton microsyringe with an infusion rate of 0.5 μl/min. The needle was left in place for an additional 5 min before being slowly retracted. All animals were given a week to recover before behavioral tests were conducted over the next 5 weeks.

All animals underwent five sets of behavioral tests consisting of a cylinder test, head position test, EBST, and balance beam test. Tests were performed 1, 2, 3, 4, and 5 weeks post 6-OHDA lesion and body weights were measured at the beginning of each test set. Behavioral tests were modified from previously described test paradigms. To analyze forelimb use during wall contact, mice were individually placed in a clean glass cylinder (1.5 cm diameter; 14.5 cm height) [13]. For accurate assessment, mice were habituated to the environment 10 min before being videotaped, and movements in the cylinder were recorded for 3 sessions, each session containing a 1 min recording period. Average forelimb use with weight-bearing wall contact was scored, and contralateral (impaired) forelimb use was expressed as a percentage of the total forelimb use [14].

For head position tests, the direction of head movement was monitored for 3 sessions, as described above. The net head position bias was calculated by recording movements greater than 10° (either left or right) from a neutral position and was expressed as the total time that the head was positioned ipsilaterally minus the total time that the head was positioned contralaterally [8,15].

EBSTs were performed by adapting a previously described paradigm for rats. Mice were held by the base of their tail, vertically 2 cm from the ground for 60 s, and swings were recorded whenever the animal moved its head greater than 10° to either the left or the right side from a neutral position [8,14,16]. The percentage of swings to the ipsilateral side relative to the total number of swings was calculated.

Balance beam tests were performed using a narrow beam (10 mm width; 1 m length) elevated 80 cm from the ground. Animals were individually placed at the end of the beam and a dark cage was placed on the other end. The total time each mouse took to cross the beam was measured, and whenever the animal refused to complete the test in less than 2 min, a time of 2 min was recorded [8]. Tests were repeated three times for every session and the results were averaged.

Apomorphine-induced rotation tests were performed once at 3 weeks post 6-OHDA lesion by injecting 0.3 mg/kg apomorphine hydrochloride (Sigma–Aldrich, USA) dissolved in 0.1% ascorbate saline solution, subcutaneously. Individual mice were placed in a glass bowl (20 cm diameter) and habituated for 10 min before testing. Rotational behavior was recorded for the next 30 min and the number of ipsilateral and contralateral turns was recorded and expressed as net contralateral turns/min. All measurements were analyzed by an investigator blinded to the experimental groups.

To investigate the neurophysiological characteristics of 6-OHDA treated mice, electrode recordings were performed 1, 4, and 7 weeks post-lesion. Mice were anesthetized using a Zoletil/Rompun cocktail (0.1 ml/100 g, i.p.) and an electrode was placed at the right STN (−1.9 mm AP, +1.7 mm ML, and ∼−4 mm DV) to obtain neural signals with an acquisition system (Neuralynx, USA). Signals were measured for 2 min with bandpass filtering at 300–5 kHz. Single-unit firing patterns were extracted from STN recordings using Spike Sorter 3D (Neuralynx, USA) with a KlustaKwik principle component analysis, and NeuroExplorer 4 (Nex Technologies, USA) was used to calculate the mean firing rate. Results were expressed as average mean firing rate of each group. Brains were removed and fixed in 4% paraformaldehyde/0.1 M phosphate-buffered saline (PBS, pH 7.4) overnight. The brains were then placed in 30% sucrose/0.1 M PBS for 48 h before 30-μm thick free-floating coronal sections were prepared for TH immunohistochemistry.

For TH immunohistochemistry, sections were rinsed three times in 0.5% bovine serum albumin (Sigma–Aldrich, USA) in PBS and incubated for 2 h in 0.2% Triton X-100 and 1% bovine serum albumin (at room temperature). Sections were then incubated overnight with rabbit anti-TH antibody (1:500, ThermoFisher Scientific, USA) at 4 °C. After three washes, sections were incubated with biotinylated anti-rabbit antibody (1:200, Vector Laboratories, USA) for 2 h at room temperature, followed by incubation with avidin-biotin-peroxidase complex (ABC-Elite kit, Vector Laboratories, USA) for 2 h. Visualization reactions were performed with 3,3′-diaminobenzidine (DAB, Dako, Denmark) and sections were rinsed, mounted on gelatinized slides, dehydrated in ethanol solution (70–100%), cleared with xylene, and cover-slipped. TH-positive cell loss was expressed as the percentage of TH-positive cells on the lesioned side relative to TH-positive cells on the contralateral side.

For statistical analysis, one-way ANOVA analysis followed by Tukey's post-hoc test was performed to compare between the four groups at each time point and $p < 0.05$ was considered statistically significant. Data are expressed as mean ± S.E.M.

Nigral dopaminergic cell loss in the SNc following 6-OHDA injection was analyzed by TH immunohistochemistry. All 6-OHDA treated groups exhibited significantly fewer TH-positive cells in