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

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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.

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**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.

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\cite{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\cite{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\cite{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\cite{6}. Animals injected with 6-OHDA unilaterally develop side-biased motor impairments that can be observed through behavioral tests\cite{7}. Furthermore, a rat model that mimics preclinical and clinical stages of PD was developed in which behavioral changes were
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 (MBF) 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. 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. 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% ascorbic acid, 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 bandwidth 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. Brain samples were fixed and sectioned 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 overnight 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 their nigral population compared to the control group, indicating a significant loss of dopaminergic neurons. The extent of cell loss varied significantly between different concentrations of 6-OHDA, with the highest concentration leading to the greatest loss of TH-positive cells, thus confirming the graded nature of the model.

In conclusion, the use of this 6-OHDA-lesioned PD mouse model allows for the investigation of neurophysiological changes at each stage of PD and provides a valuable tool for studying the progression of dopaminergic cell loss and the development of potential therapeutic strategies. Further studies are needed to elucidate the underlying mechanisms and to translate findings to the clinic.
eral swings than the control group (4 and 2 EBSTs, there was no significant difference between the control slow recovery over the remaining test period (Fig. 2A). The significant weight loss during the first 2 weeks post-lesion and Groups treated with 6 and 4 weight was monitored continuously for the following 5 weeks.

In the control group (12.1 ± 3.3; Fig. 1A).

All animals were weighed before 6-OHDA lesioning and their weight was monitored continuously for the following 5 weeks. Groups treated with 6 and 4 μg/2 μl 6-OHDA showed significant weight loss during the first 2 weeks post-lesion and slowly recovered over the remaining test period (Fig. 2A). The apomorphine-induced rotation test performed 3 weeks post-lesion clearly indicated an increase in net contralateral rotations in the 6-OHDA treated groups (Fig. 2B). Mice administered 6 μg/2 μl 6-OHDA exhibited the greatest number of contralateral turns (18.6 ± 0.6 turns/min), followed by the groups treated with 4 and 2 μg/2 μl 6-OHDA (12.6 ± 0.6 and 6.3 ± 0.9 turns/min, respectively). Control mice had a mean of 0.1 ± 0.7 contralateral rotations. Cylinder tests were performed to test forelimb asymmetry (Fig. 2C). Impaired forepaw use during wall contact significantly decreased in 6-OHDA treated groups, with the 6 μg/2 μl 6-OHDA treated mice showing the most unbalanced forelimb use, followed by the groups treated with 4 and 2 μg/2 μl 6-OHDA, and finally the control group (29.0 ± 3.0, 36.2 ± 2.8, 45.1 ± 2.1, and 60.2 ± 3.0%, respectively). In EBSTs, there was no significant difference between the control and 2 μg/2 μl 6-OHDA groups (p > 0.05; Fig. 2D). However, 6 and 4 μg/2 μl 6-OHDA treated mice performed notably more ipsilateral swings than the control group (p < 0.05). Head position tests (Fig. 2E) revealed that 6 and 4 μg/2 μl 6-OHDA treated mice positioned their head toward the ipsilateral side to a greater degree than 2 μg/2 μl 6-OHDA treatment or control groups (53.6 ± 2.8, 47.3 ± 3.4, 34.6 ± 3.3, and 30.7 ± 0.1 s at week 1, respectively). However, this tendency decreased over the 5-week test period. Balance beam tests (Fig. 2F) indicated a significant association between the concentration of 6-OHDA and beam traversal time. Mice treated with 6 and 4 μg/2 μl 6-OHDA took an average of 84.3 ± 5.0 and 71.2 ± 5.7 s, respectively, to cross the beam, whereas mice treated with 2 μg/2 μl 6-OHDA and control groups took less than 1 min to cross the same distance (52.2 ± 5.4 and 19.1 ± 1.5 s, respectively).

In the electrophysiological recordings of STN neurons, increased firing rates (spikes/s) were observed in the groups treated with 6-OHDA (Fig. 3A). Whereas the firing rates of STN neurons in the control group remained below 10 spikes/s throughout the experiment, 6-OHDA treated groups exhibited significantly higher firing rates (p < 0.05). There was no significant difference in firing rates between the groups treated with 6-OHDA at 1 and 4 weeks post-lesion. However, at 7 weeks post-lesion, firing rates in the group treated with 6 μg/2 μl 6-OHDA significantly increased (25.7 ± 2.3 spikes/s), followed by those in the groups treated with 4 and 2 μg/2 μl 6-OHDA (18.4 ± 1.2 and 12.9 ± 1.2 spikes/s, respectively), thus showing a clear relationship between the concentration of 6-OHDA administered and changes in STN neuronal firing rate in each group (Fig. 3B).

In the present study, a mouse model was developed by injecting different concentrations of 6-OHDA into the MFB. The four groups showed significant changes in motor function, dopaminergic cell loss in the SNC, and neuronal firing rates in the STN, thus mimicking each progressive stage of human PD. In the behavioral tests, motor deficits showed a strong association with the 6-OHDA concentrations treated in each group. In the cylinder test, forelimb asymmetries clearly indicated that mice treated with 6 μg/2 μl 6-OHDA avoided the use of the contralateral forelimb [17]. The results of the EBST contradict those of a previous study [16], as the groups treated with 6 and 4 μg/2 μl 6-OHDA exhibited a greater number of ipsilateral swings (in contrast to the contralateral swings observed in the cited study); however, ipsilateral curling responses were observed in a rat model of PD [8], which supports the present.

Fig. 1. SNC TH-immunohistochemistry after 6-OHDA lesion. Different concentrations of 6-OHDA were injected into the right MFB to generate a mouse model of each progressive stage of PD: (A) saline (control), (B) 2 μg/2 μl 6-OHDA treatment, (C) 4 μg/2 μl 6-OHDA treatment, and (D) 6 μg/2 μl 6-OHDA treatment. (E) Significant differences in the percentage of TH-cell loss (%) were observed among the four groups (all 6-OHDA treated groups vs. control, p < 0.05).
Fig. 2. Changes in both weight and behavioral characteristics following 6-OHDA lesion. (A) Significant weight loss was observed in the groups treated with 6 and 4 μg/2 μl 6-OHDA during the first 2 weeks post-lesion. (B) Net contralateral turns after apomorphine administration showed clear between-group differences (all 6-OHDA treated groups vs. control, \( p < 0.05 \)). (C) During wall contact, significant decrease in the percentage of contralateral forelimb use was observed (all 6-OHDA treated groups vs. control, \( p < 0.05 \)). (D) During EBSTs, mice treated with high 6-OHDA concentrations performed increased ipsilateral swings compared to the control group (6 and 4 μg/2 μl 6-OHDA vs. control, \( p < 0.05 \)). (E) The net head positioning during 1 min revealed head position bias toward the ipsilateral side in groups treated with high 6-OHDA concentrations (6 and 4 μg/2 μl 6-OHDA vs. control, \( p < 0.05 \)). (F) Significant difference in beam traversal time was observed between 6-OHDA treated groups and saline treated group (\( p < 0.05 \)).

findings. Head-position tests also clearly distinguished the groups treated with 6 and 4 μg/2 μl 6-OHDA from the groups treated with 2 μg/2 μl 6-OHDA or saline. It is important to note that in both tests, the group administered 2 μg/2 μl 6-OHDA showed amelioration of the motor disabilities over time, eventually showing comparable responses to those of the control group. Balance beam tests were performed to test balance ability, which is affected in PD patients with clinical stages of 2–3 [18,19]. Notably, the 6-OHDA lesion clearly affected the traversal time, indicating behavioral changes in balance ability in accordance with dopaminergic cell loss [20]. Additionally, apomorphine-induced rotation tests also showed a strong association with nigral cell loss in each group in the PD model [21,22].

The present study is the first to analyze electrophysiological characteristics of STN neurons in a mouse model mimicking each progressive stage of human PD. Notably, STN neurons in the group treated with 2 μg/2 μl 6-OHDA exhibited lower firing rates than those in the groups treated with 6 and 4 μg/2 μl 6-OHDA, which is consistent with data from human PD [11]. However, the firing rates were significantly higher than those in the control group. Moreover, a previous study reported that the mean discharge rate of STN neurons increases following 6-OHDA lesion in rats, resulting
in STN hyperactivity [23]. The present results therefore support the view that 6-OHDA lesion increases STN neuronal firing in rodents and that increased firing rates have a strong association with the size of the lesion or disease progression. Additionally, the results presented herein indicated increases in the mean firing rate similar to those in an MPTP-treated primate model of PD [24]. Thus, the model herein approximates PD pathophysiology and could be used to study therapeutic applications and neurophysiological mechanisms at each stage of PD, particularly through the examination of various electrophysiological characteristics, including firing frequency, burst index, and interspike interval coefficient of variability.

Although previous studies have provided preclinical and clinical rat models of PD [8], mouse models are often preferred because transgenic mice are more easily generated [14]. Using the methodology presented above, significant changes in both behavioral and electrophysiological aspects can be identified in accordance with dopaminergic cell loss in the SNc. Therefore, the mouse model of PD presented herein will be useful for investigating the pathogenesis of PD and associated neurophysiological mechanisms.

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