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The 6-OHDA Model of Parkinson's Disease: Mechanisms, Applications, and Limitations

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Abstract. Parkinson's disease (PD) is the second most prevalent progressive neurodegenerative disorder, in which the dopaminergic neurons are lost. Genetic and environmental factors are involved in the onset of PD. Symptoms such as tremor, balance problems, nerve pain, muscle stiffness, and mental issues are the most common. Despite the progress made in the understanding of the features of PD, the precise mechanisms responsible for the neuronal degeneration are not well understood. Consequently, animal models such as the 6-hydroxydopamine (6-OHDA) rodent models play a crucial role in studying mechanisms of neurodegeneration and developing therapeutic approaches. The models are made by stereotactic injection of 6-OHDA into striatum to mimic key features of human PD by destroying dopaminergic neurons. The mechanisms, applications and limitations of the 6-OHDA model were explored. Furthermore, analysis indicated that 6-OHDA selectively causes oxidation stress and impairment of mitochondrial function in dopaminergic neurons, thus mimicking various motor symptoms of PD. The acute toxicity and the absence of α-synuclein aggregation and Lewy body formation indicate that it does not recapitulate non-motor symptoms of human PD significantly. Overall, 6-OHDA can still be considered a useful model for preclinical testing of therapeutics. However, aspects of PD pathology such as aggregation of α-synuclein and disease progression as well as non-motor changes, are not well represented. Further work is needed in establishing more clinically simultaneous models or using genetic and α-synuclein-based models in combination with the 6-OHDA model to enhance translational results.

Keywords: Parkinson's disease; 6-OHDA; Striatum; Substantia nigra; Dopamine neuron.

1. Introduction

Parkinson's disease (PD) is the second common neurodegenerative disease after Alzheimer's disease. Neurons in the substantia nigra of the brain are responsible for producing dopamine, and PD patients are found to have lost more than half of the dopamine-producing cells in this area [1]. Animal models provide a great tool for understanding cellular pathways, pathologies, and the development of medical therapies in the field of PD research. The most frequently used rodent model for PD symptoms is the 6-hydroxydopamine (6-OHDA) model, which is injected into the striatum and/or substantia nigra. This causes a loss of dopamine (DA) and manifests PD-like symptoms [2].

However, concerns regarding to what extent the model mimics motor and non-motor PD symptoms exist, and limitations of this model are unclear. This makes the development of the 6-OHDA model and a detailed understanding of its shortcomings essential to make the best use of the model in PD research. This study investigates behavioural and pathological aspects in both healthy and PD-like mice [3]. Behavioural tests such as open field test, gait analysis and pole test are conducted to evaluate motor and non-motor impairments. Moreover, Welch's t-test is done to analyse all data, whereas p-value and mean values are calculated to determine the existence of a significant difference between the healthy and 6-OHDA-treated mice. Aside from that, gait test measures stride length, pressure, walking velocity, interlimb coordination and paw placement consistency. In addition to behavioural tests, loss of dopaminergic neurons is detected by TH immunofluorescence staining. Samples are taken from the brain tissues (substantia nigra, corpus striatum, and hippocampus). By characterizing the extent to which the 6-OHDA models represent PD features, this study seeks to assess its applicability in understanding PD disease mechanisms and developing possible therapeutic approaches, which will provide references for future preclinical studies.

Finally, both single dose unilateral 6-OHDA into the striatum and into the substantia nigra were effective in inducing motor changes in animals, and the protocol would be useful for the study of motor changes in PD patients. However, the models poorly reflect human PD as these non-motor symptoms have not been well developed. In general, the 6-OHDA models are useful in the development of drug and diagnostic strategies for PD, however, the models need to be refined to create more comprehensive models better representing disease course and non-motor outcomes to translate findings from the experimental models to clinical use.

2. Methodology

2.1. Animals

Fifteen C56BL6/J mice were used for the experiments. Five mice were involved in each group (control, striatum, substantia nigra). Three of the mice in the substantia nigra group (SN) died during the first week after injection, one of the mice in the striatum group (ST) died, and one of the mice in the control group (CT) died. Mice were fed and housed in a light/dark cycle room. Ethical issues with animals were considered and dealt with by obtaining institutional approval and following animal welfare guidelines.

2.2. Surgical Procedure

A syringe containing a 2.5 mg/mL solution of 6-OHDA is filled. The mouse is secured on a stereotaxic device. The scalp is incised, and the surface of the skull is gently cleaned with a cotton swab to expose the bregma and lambda points. Next, the stereotaxic apparatus is adjusted so that the heights of bregma and lambda are aligned, ensuring the skull is level. Moreover, a small hole in the skull above the required area is bored using a dental drill. For SN group, the injection site is the substantia nigra region (AP −2.90 mm; ML +1.10 mm; DV −4.50 mm). For ST group, the injection site is the striatum (AP +0.4mm; ML 1.8mm; DV -3.5mm) (Fig. 1). After the procedure is performed, the syringe remains in position for 5 minutes for proper diffusion of the solution. Then, it is gradually removed, followed by suturing and disinfection.

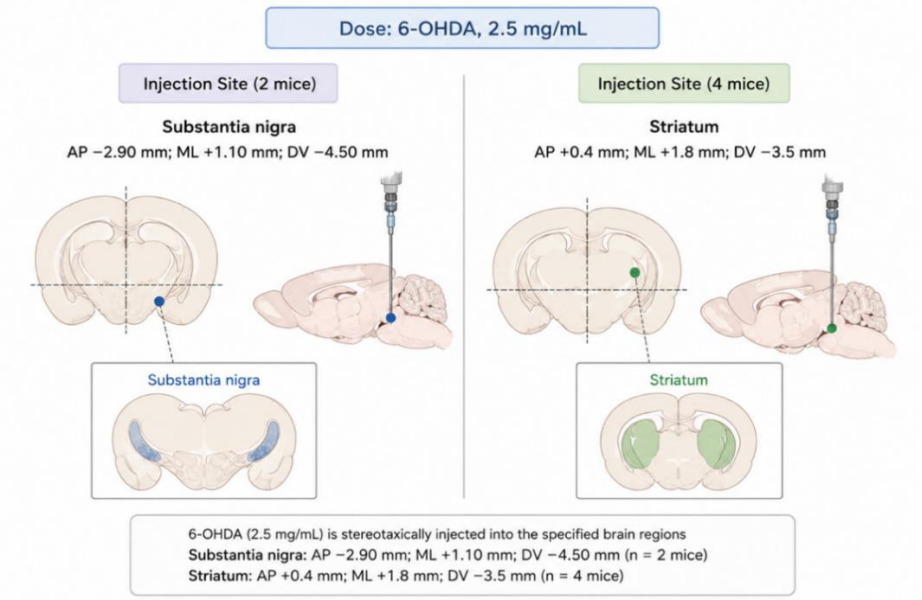

Figure 1. Injection Sites

2.3. Open Field Test

Experiments were carried out under dim lighting and quiet conditions while avoiding visual contact between the animals and the conductor. At the beginning of the experiment, each mouse was placed in the lower right corner of the chamber. Locomotor activity within the chamber was automatically

recorded using an analysis software for 5 minutes. Upon completion, the mouse was removed from the chamber, and the chamber was sanitized, and any faeces or urine residues were removed.

2.4. Pole Test

A pole with a diameter of 2 cm and 75 cm tall was prepared. Before testing, mice should be trained to climb. Each mouse was initially placed at the base of the pole, and moved upward so that the animal became accustomed to the apparatus and climbing procedure. Motor performance was assessed by measuring the time required for a mouse to descend from the top of the pole until both forepaws reach ed the ground. In this experiment, each mouse was placed on a rough wooden sphere connected to the upper end of a rough-surfaced cylindrical wooden pole. Three trials were conducted for each mouse and the turning time and total climbing time were recorded. The mean climbing times were calculated. These measurements were used to evaluate the motor coordination, balance, and muscular function in mice.

2.5. Gait Analysis

A translucent glass walkway was constructed, and a dark enclosed chamber was set up at the end of the walkway. A high-speed camera was placed under the walkway and linked to a computer with the TOP-Gait software. This software was calibrated by setting the detection region for the walkway and detecting the forepaws, hind paws, and tail movements. Mice were given free exploration time for 10 minutes before the tests. Each mouse was put in the start of the walkway and allowed to freely move toward the dark chamber. Three to five valid runs (continuous movement without stopping or turning) of each animal were recorded. The paw prints and gait parameters were automatically recorded and analysed by the software [4].

2.6. TH Assessment via Immunofluorescence

All animals were used for brain tissues, including the striatum, substantia nigra and hippocampus. They were embedded in OCT compound and cryosectioned at 8 µm thickness. Following this, they were washed with PBS (pH 7.4) and treated with antigen retrieval followed by blocking for 30 min at room temperature with 3% BSA. The sections were then stained for 1 h at 37°C with anti-TH primary antibody (1:100) and incubated with HRP-conjugated secondary antibody for 1 h at room temperature. After washing with PBS, a red fluorescent detection reagent was applied for 10 minutes and the nuclei were counterstained with DAPI for 10 minutes. In addition, anti-fade medium was used to mount sections. Finally, the sections were observed under a microscope and a multi-channel fluorescence scanner was used while capturing images (Fig. 2).

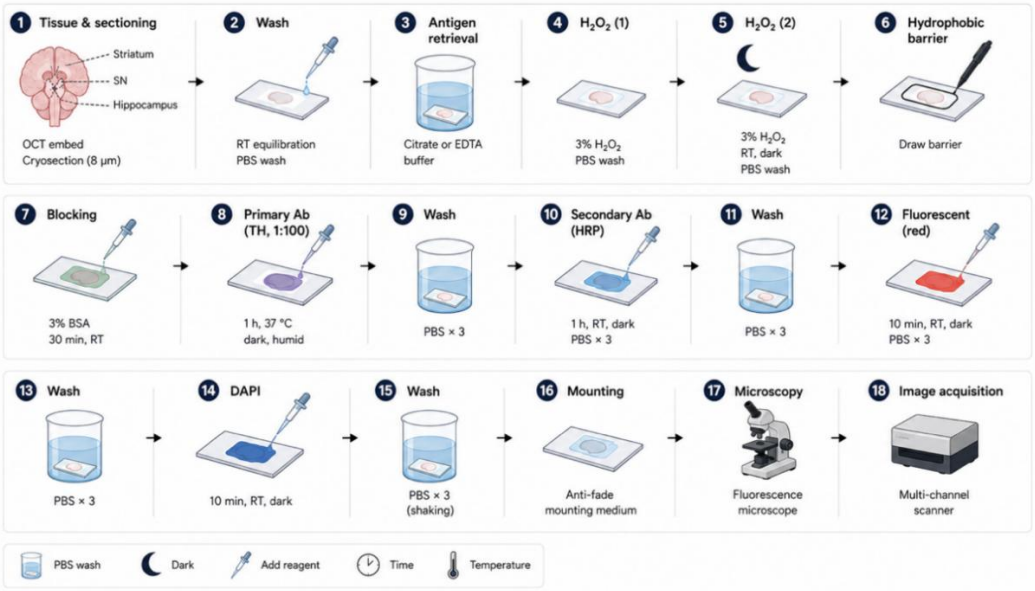

Figure 2. Pathological Test Procedure

2.7. Statistical Analysis

Data are expressed as mean ± standard deviation (SD) and were analysed using GraphPad Prism version 6.0 software (GraphPad Software Inc., San Diego, CA, USA). Statistical analyses were carried out by comparing the means of different groups using one-way ANOVAs. Welch's t-test was used to analyze all data, with $P < 0.05$ considered significant.

3. Results

3.1. Non-motor Behavioural Assessments of the 6-OHDA Model

Nonmotor symptoms (NMS) are commonly exhibited by PD patients, such as depression, cognitive impairments, fatigue, and anxiety. It is found that above 98.6% of the PD patients have reported the presence of at least one NMS [5]. These phenotypes are studied in the 6-OHDA PD animal models. In the open field test, by comparing with the control group, neither the ST nor the SN group showed significant differences in the time spent in the centre region (Fig. 3A). Moreover, comparing the time spent in the edge area, only the SN group showed a slightly significant difference (Fig. 3B). Although it has been reported in other studies that 6-OHDA resulted in anxiety-like behaviours, this experiment suggests that 6-OHDA does not promote any obvious NMS in mice. Therefore, it is arguable whether stress is directly influenced by dopaminergic degeneration. Locomotor activities are recorded, and paths of the animals are shown (Fig. 3C-E).

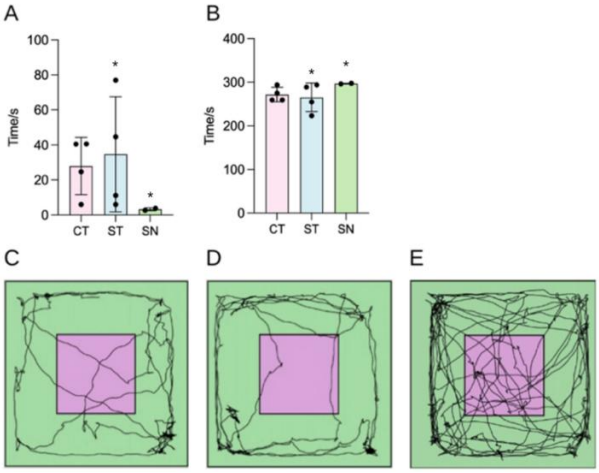

Figure 3. Non-motor Symptom Results

Non-motor behaviour was assessed through conducting an open field test. Average time spent in the center area by the three groups (A). Average time spent in edged area by the three groups (B). *$P < 0.05$ compared with control. Locomotor activity of ST group with coding number 2 (C). Locomotor activity of SN group with coding number 2 (D). Locomotor activity of CT group with coding number 3 (E).

3.2. Motor Behavioural Assessment of the 6-OHDA Model

Gait analysis (Fig. 4A-F) and pole test (Fig. 4G-I) confirmed the motor dysfunction of 6-OHDA. The SN group and the ST group had significant motor deficits in altered locomotor patterns as seen in gait analysis. The gait performances of ST and SN groups were significantly decreased compared to the control groups with respect to the average run duration, average velocity, and average step length (Fig. 4D-F), suggesting impaired gaits. Next, both lesion groups spent a significantly longer time descending the pole than healthy mice in all three pole test trials. These findings together showed that lesioned animals (6-OHDA injected into either striatum or substantia nigra) exhibited profound motor impairments, a hallmark of the PD pathology.

Gait analysis provides insight into changes in motor functions. SN coding number 2 (A). ST coding number 2 (B) CT coding number 3 (C). Average run duration (D). Average velocity (E). Average

step length (F). All three aspects showed significant difference between experiment groups and control groups. Motor behavior is assessed by carrying out pole test. Trial 1 total climbing time (G). Trial 2 total climbing time; (H). Trial 3 total climbing time (I). *P < 0.05, **P < 0.01 compared with control.

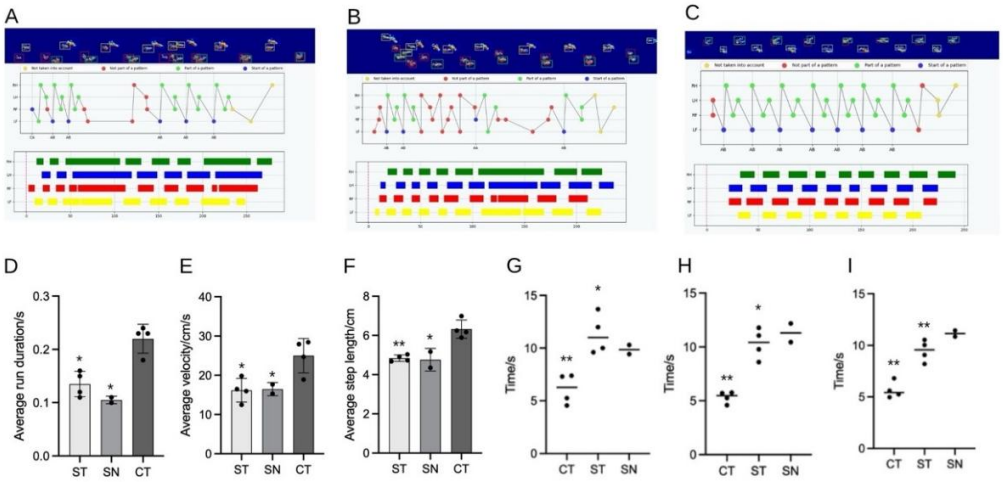

Figure 4. Motor Assessment Results

3.3. Pathological Analysis Tests

It is observed significant loss of TH+ fibres in the substantia nigra and striatum, as well as decrease in fluorescence intensity of TH+ neurons in these two areas of mice treated with 6-OHDA, compared with the control groups (Fig. 5).

Immunofluorescence representative micrographs showed labelling of TH+ (red) from the SNpc and striatum of 6-OHDA-lesioned mice (A, B). Quantification of the striatum coverage by TH+ staining were shown (C, E). Quantification of the SNpc coverage by TH+ staining were shown (D, F). *P < 0.05, **P < 0.01, ***P < 0.001 compared with control.

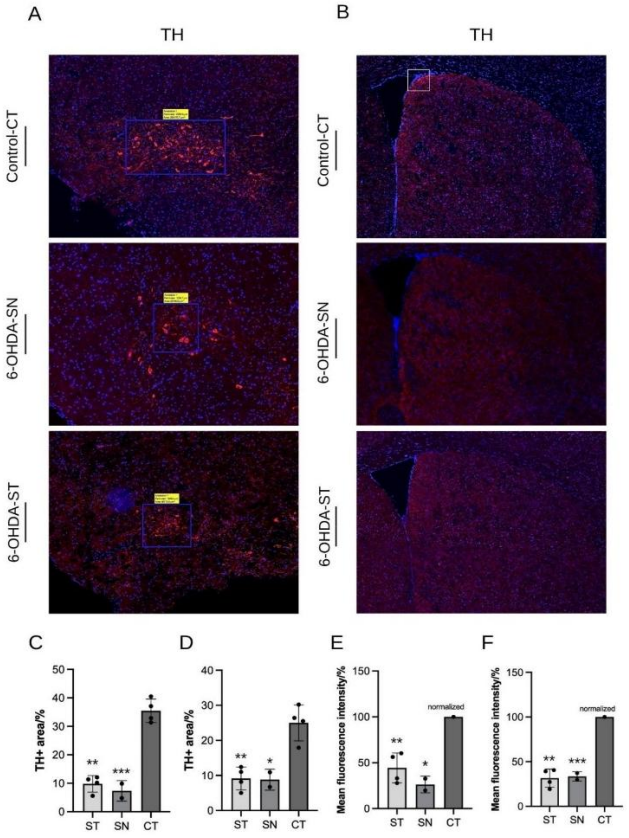

Figure 5. TH+ Staining Immunofluorescence Results

4. Discussion

Rodent animal models are an important component of drug discovery and development in the pre-clinical phase. Therefore, well-established and reliable behavioural assays are needed to evaluate disease-related impairments and the effectiveness of new therapeutic treatments for neurodegenerative diseases.

Previous research have explored motor behaviours [6, 7]. For this study, both motor and non-motor phenotypes in 6-OHDA induced PD mouse models were created with lesions in either the striatum or the substantia nigra. The relationship of DA depletion and deficits in motor functions was confirmed by understanding the PD pathologies and by conducting TH assessment via immunofluorescence and behavioural tests [8]. Motor abnormalities were found in both ST and SN groups. Gait analysis showed that locomotor performance was significantly reduced with decreased run duration ($P < 0.05$ for both ST and SN vs controls) and velocity ($P < 0.05$ for both ST and SN vs controls), and a shorter step length (ST vs CT, $P < 0.01$; SN vs CT, $P < 0.05$). These findings are similar to the bradykinesia and gait abnormalities generally seen in PD patients. Also, the ST group had significantly longer descent times in all three trials ($P < 0.01$) and the SN group had significantly longer descent times in Trials 1 and 2 ($P < 0.05$) and a much stronger effect in Trial 3 ($P < 0.01$).

In the open field test, however, there was no significant difference between the time spent in the central area of the open field for the ST group, compared with the CT group (ST vs CT, $P > 0.05$), or the SN group compared with the CT group (SN vs CT, $P > 0.05$). The behavioural profile did not strongly suggest the presence of anxiety-like phenotypes, although there was a significant increase in edge-zone preference in the SN group ($P < 0.05$). These results are in contrast with some earlier reports of non-motor deficits after 6-OHDA administration which may reflect variations in lesion size, injection site, and/or experimental length. Thus, non-motor alterations were limited based on the findings, raising concerns about the ability of 6-OHDA to model anxiety features.

Other than behavioural test, pathological tests were carried out. Tyrosine hydroxylase (TH) immunofluorescence staining was used to assess the integrity of the nigrostriatal dopaminergic system. The enzyme rate-limiting in the catecholamine biosynthesis, TH is used as a marker for dopaminergic neurons and projections [9]. The 6-OHDA mice showed a severe decrease in the number of TH immunoreactive neurons in the substantia nigra and in the number of dopaminergic terminals in the striatum, suggesting that a large number of dopaminergic neurons and terminals were degenerated. This decrease was confirmed by quantitative analysis of the TH positive fluorescence. They are in line with the typical neuropathological signs observed in PD and confirm the induction of dopaminergic neurodegeneration in the experimental model [10].

To conclude, these results overall confirm that both lesioned areas have the ability to show salient PD motor symptoms. Therefore, the 6-OHDA model proves to be a valuable system to study PD motor dysfunction and to test potential treatment strategies. However, there were some caveats in this study. First, the sample sizes were small that can reduce statistical power and increase the individual differences. Second, behavioural measures were done for a limited time period, and may not cover the full range of symptom progression in PD. Thirdly, the open field test used predominantly to gauge non-motor symptoms, while other tests, such as the forced swim test, cognitive tests, etc., would provide a more comprehensive assessment of anxiety, depression and cognitive impairment. A larger number of animals and broader behavioural and histological evaluations are needed to further validate and characterize the model.

5. Conclusion

To conclude, Parkinson's disease (PD), the world's second common neurodegenerative disease, and the 6-OHDA mice model have been introduced. The study shows that the 6-OHDA model is a recapitulation of important pathological and behavioural features of PD, especially those involving the degeneration of the nigrostriatal dopaminergic pathway. 6-OHDA injection into either striatum

or substantia nigra led to evident motor deficits, such as changed gait parameters and extended 6-OHDA-injected pole test performance. These results are consistent with some of the classic motor features of PD, which include bradykinesia, coordination deficits, and diminished locomotor activity. In addition, TH immunofluorescence staining showed that there were significant decreases in TH-positive neurons and fibers in the substantia nigra and striatum, which further demonstrated effective dopaminergic neurodegeneration and the relevance of the pathological model.

Although it has some merits, the model was not very successful in simulating non-motor symptoms. Acute toxin-induced dopaminergic lesions alone were apparently not enough to induce significant changes in anxiety-related behavior in the open field, with anxiety being a core feature of PD in humans. This constraint is typical of the 6-OHDA model, which is not able to completely reproduce the multifactorial and progressive nature of the disease. The 6-OHDA lesion is unique in that it causes rapid and selective dopaminergic neuron loss, but unlike human PD, does not reproduce these critical pathological features: aggregation of α-synuclein, formation of lewy body structures, and widespread neural dysfunction.

However, the model is reproducible, the induction procedure is relatively simple, and the 6-OHDA produces a very robust motor phenotype, making it a valuable experimental tool for studying mechanisms of dopaminergic degeneration and therapeutic interventions. These results confirm its ongoing use in the field of PD research in the preclinical stage, especially in studies on motor dysfunction and neuroprotection. Larger sample sizes, extended follow-up periods, and additional behavioral and histological evaluations should be considered for future investigations. A combination of toxin-based and genetic or α-synuclein-based models could offer a more complete picture of PD pathology and enhance the translational value of experimental results for clinical use.

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